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Developing microbial inoculants for sustainable spinach cultivation: Soil and endophytic community insights

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Table S1. Phenotypic and differential growth characteristics of rhizobacterial isolates MP1–MP5 on selective media.

Characteristic	MP-1	MP-2	MP-3	MP-4	MP-5
Growth on Nutrient Agar	+	+	+	+	+
Growth on Burk Media	–	–	–	–	+
Growth on *Beijerinckia* Media	–	–	–	+	+
Growth on *Azospirillum* Media	–	+	–	–	–
Growth on Pikovskaya Media	+	+	+	+	+
Growth on Cetrimide agar Media	+	–	–	–	–
Growth of Tryptic Soy Agar	+	–	–	–	+

Table S2. Morphological Traits of Bacterial Isolates MP1–MP5.

Characteristic	MP-1	MP-2	MP-3	MP-4	MP-5
Cell Shape	Rod	Rod	Rod	Slightly curved rod	Rod
Pigment / Colony Colour	Green fluorescent	Very light yellow, slightly opalescent	Colourless, translucent	Brownish-white	Light yellow
Colony Morphology	Convex	Slightly convex	Raised with filaments	Circular, raised, mucoid (on N-free medium)	Circular,slightly convex, smooth appearance
Gram Stain	Negative	Negative	Positive	Negative	Negative
Motility	Motile	Motile	Motile	Motile	Motile

Table S3. Carbon source utilization by bacterial isolates (MP-1 to MP-5).

Bacterial isolate	Malic Acid	Glucose	Sucrose	Mannitol	Mannose
MP-1	–	+	+	–	–
MP-2	++ (fast)	+	+	–	–
MP-3	–	++ (early)	++ (early)	–	–
MP-4	–	+	+	+	–
MP-5	+	+	+	+	+

"+" = positive utilization, "–" = no utilization, "++ (fast/early)" = strong or early positive response

Table S4. Onset of growth and enzyme activities of bacterial isolates MP1–MP5.

Bacterial isolates	Growth onset in nutrient broth (28 °C, pH 7)	Amylase activity	Urease activity	Catalase activity
MP1	Within 4 hours	Strong	Absent	Present
MP2	Within 12 hours	Weak	Present	Present
MP3	Within 4 hours	Strong	Present	Present
MP4	Within 14 hours	Strong	Absent	Present
MP5	Within 4 hours	Strong	Present	Present

Table S5. Source of isolation and pairwise microbial interactions among bacterial isolates MP1–MP5: the potential interactions between the microbes when used together as a consortium. The notation "+/–" or "–/+" indicates that one microbe inhibits the other on nutrient agar, showing dominance; "+/+" indicates neutral growth; additional "+" signs reflect increasing dominance.

Microbe	Source	MP-1 (*Pseudomonas*), Rhizospheric and root endophytes	MP-2 (*Azospirillum*, Soil)	MP-3 (*Bacillus*, Rhizospheric and leaf endophytes)	MP-4 (*Beijerinckia*, Soil)	MP-5 (*Flavobacterium*, Leaf Endophyte)
MP-1	SOIL and Root Endophyte	0	+/-	+/+	+/-	+/++
MP-2	Soil	- /+	0	-/+	+/++	-/+++
MP-3	SOIL and Leaf Endophyte	+/++	+/-	0	+/-	+/++
MP-4	Soil	-/+	+/+	- /+	0	-/++
MP-5	Leaf Endophyte	+/++	+/-	+++/+	+/-	0

Table S6. Effects of different treatments on spinach growth parameters (mean ± SD). Treatments include CP (control soil), OCM (organic amendment), OCM+MI (organic amendment with microbial inoculants), and CP+MI (control soil with microbial inoculants).

Parameter	Treatment	Mean ± SD*	ANOVA F value	p-value
Area of Leaves (cm^2)	OCM+MI	238.07 ± 2.06 (a)	21725.56	1.14×10^{-22}
	OCM	212.00 ± 0.16 (b)		
	CP+MI	77.42 ± 0.65 (c)		
	CP	75.36 ± 0.90 (c)		
Plant Height (cm)	OCM+MI	36.58 ± 0.43 (a)	1134.07	5.52×10^{-15}
	OCM	36.00 ± 0.52 (a)		
	CP+MI	20.30 ± 0.15 (b)		
	CP	20.19 ± 0.86 (b)		
Root Breadth (mm)	OCM+MI	3.50 ± 0.09 (a)	53.62	3.18×10^{-7}
	OCM	3.18 ± 0.13 (a)		
	CP+MI	1.63 ± 0.30 (b)		
	CP	1.60 ± 0.43 (b)		
Root Length (cm)	OCM+MI	14.01 ± 0.28 (a)	377.40	3.88×10^{-12}
	OCM	14.00 ± 0.37 (a)		
	CP+MI	7.40 ± 0.27 (b)		
	CP	7.40 ± 0.57 (b)		

*Separately for each parameter, different letters (a, b, c) indicate statistically significant differences among treatments based on Tukey's HSD test ($p < 0.05$).

Table S7. Summary of Sequencing Data Quality and Processing for Soil Microbial Communities. This table shows the number of raw and merged reads, high-quality sequences, and non-chimeric sequences obtained from 16S rRNA gene sequencing of soil samples. It also includes total base pairs, average read length, quality scores (Q20 and Q30), and GC content (%) for each treatment group: CP, OCM, OCM+MI, and BP.

Sample name	Raw PE	Combined	Qualified	NoChime	Base (nt)	Avg length (nt)	Q20%	Q30%	GC%
CP	152979	151786	143655	130339	55438116	425.34	97.83	93.27	54.77
OCM	204661	202631	199316	76432	31736037	415.23	98.67	95.30	57.62
OCM+MI	204883	202742	199448	75583	31459384	416.22	98.60	95.23	57.65
BP	203671	203233	201210	96104	36033589	374.94	99.17	96.70	52.23

Table S8. Rank change of key bacterial species between OCM and OCM+MI. A lower rank in OCM+MI indicates a relative increase in rank position following inoculation.

Species	Rank in OCM	Rank in OCM+MI	Rank Change Direction
Bacillus aryabhattai	4	1	↑
Microbispora rosea	5	2	↑
Pseudomonas alcaligenes	7	4	↑
Bacillus cellulasensis	2	5	↓
Nocardioides aestuarii	9	6	↑
Lysobacter dokdonensis	15	10	↑
Lysobacter soli	14	12	↑
Bacillus marisflavi	18	16	↑
Agrobacterium radiobacter	19	18	↑
Paenibacillus lacus	36	20	↑
Devosia riboflavina	85	30	↑
Pseudoxanthomonas mexicana	28	31	↓
Nitrospira sp.	38	42	↓
Bacillus trypoxylicola	65	84	↓
Azotobacter salinestris	123	80	↑
Lysinibacillus sp. YS11	31	7	↑

Table S9. STREAMS- and MIxS-compliant 16S rRNA sequencing and analysis workflow showing standardized library preparation, bioinformatics processing, and public data deposition (NCBI SRA BioProject PRJNA1424381).

Section	Parameter	Description / Value
DNA preparation	Template DNA input	20–150 ng per reaction (150 ng before PCR; 20 ng per 25 µL reaction)
	DNA concentration before PCR	150 ng
	DNA quality assessment	1% agarose gel electrophoresis; NanoDrop
	DNA storage	−20 °C until use
PCR and library preparation	Primer sequences	Soil (V3–V4): 341F (5′-CCTACGGGNGGCWGCAG-3′), 806R (5′-GACTACHVGGGTATCTAATCC-3′); Endophytes (V5–V7): 799F (5′-AACMGGATTAGATACCCKG-3′), 1193R (5′-ACGTCATCCC-CACCTTCC-3′)
	PCR thermal cycling conditions	Initial denaturation: 95 °C, 3 min 25 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 30 s Final extension: 72 °C, 5 min
	Annealing temperature	55 °C
	Number of PCR cycles	25
	PCR reaction volume	25 µL
	PCR enzyme	KAPA HiFi HotStart ReadyMix (high-fidelity DNA polymerase)
	Buffer and Mg^{2+}	Manufacturer buffer; final Mg^{2+} ≈1.5 mM
	PCR technical replicates	Triplicate PCRs, pooled prior to purification
	PCR controls	Positive: Mock community DNA Negative: Nuclease-free water
	Indexing strategy	Dual indexing (8 bp indices via Nextera XT adapters; example: i7 ACTGGTCA, i5 TCGACGAT)
	Library quantification	NanoDrop (DNA purity) + TapeStation (fragment length)
Sequencing	Platform	Illumina MiSeq System
	Flow cell and kit	MiSeq Reagent Kit v3 (600-cycle)
	Flow cell type	MiSeq v3 flow cell
Bioinformatics pipeline	Software	QIIME2 (version 2023.x)
	Quality filtering	Phred score ≥30
	Minimum read length	≥200 bp after trimming
	Chimera removal	UCHIME (de novo)
	Rarefaction	Rarefied to minimum sequencing depth
	Low-abundance filtering	OTUs <0.01% abundance removed
	Taxonomic assignment database	SILVA v138
	Classifier	Naïve Bayesian classifier (QIIME2)
	Confidence threshold	70%
Reproducibility and data	Stochastic seed	Default stochastic seed generation (no manual fixed seed assigned)
	Data repository	NCBI Sequence Read Archive (SRA)
	BioProject	PRJNA1424381
	BioSamples	SAMN55383437, SAMN55383438, SAMN55383439, SAMN55383440
	Metadata standard	MIxS compliant
	Label standardization	Internal codes standardized for publication (e.g., 'CAM' → 'CP'; 'PR' → 'OCM+MI')

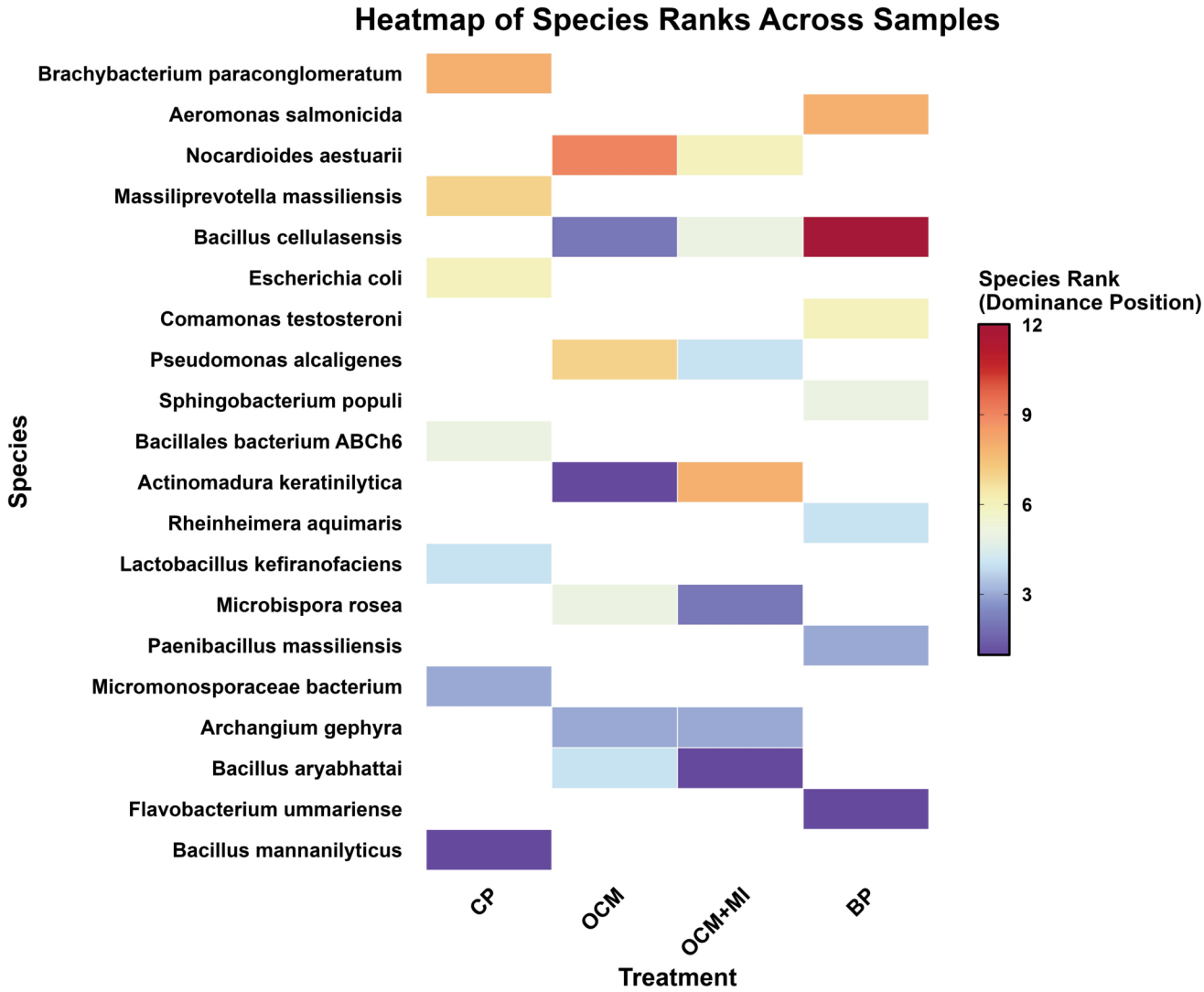


Figure S1. Heatmap showing species rank variation across soil treatments.The heatmap represents the ordinal rank positions of bacterial species across different soil treatments (CP, OCM, OCM+MI, BP), where ranks were calculated based on relative abundance values within each sample. Lower rank values indicate higher dominance, while higher rank values indicate lower dominance within the community. The color gradient represents rank magnitude (numerical rank scale shown in the legend), and does not represent quantitative abundance or log-transformed counts. This visualization highlights rank shifts and dominance restructuring of taxa across treatments rather than absolute abundance differences.

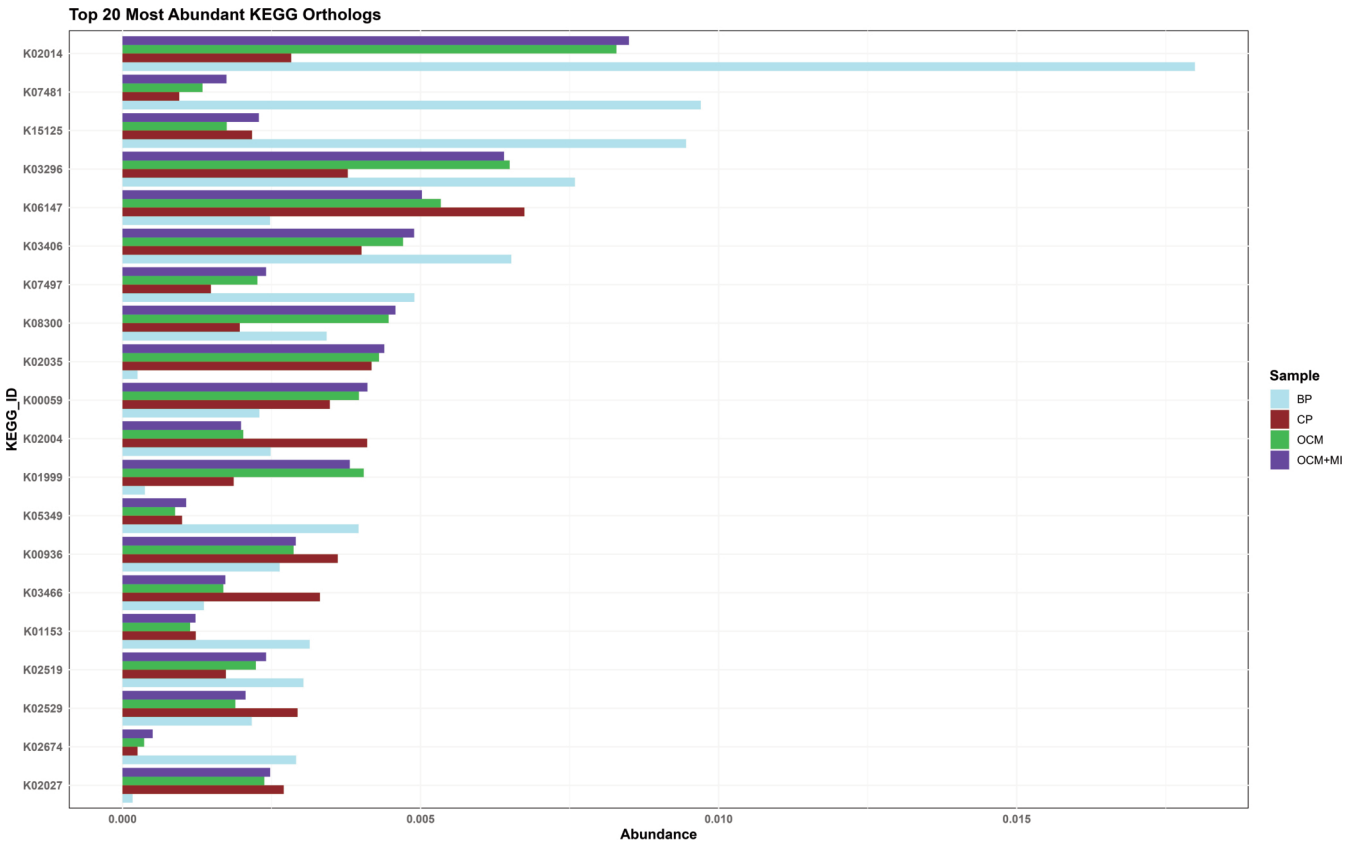


Figure S2. Comparative abundance of the top 20 KEGG orthologs across four soil treatments (BP, OCM, OCM+MI, and CP). Each bar represents the relative abundance of a specific ortholog (KO number) in each treatment.

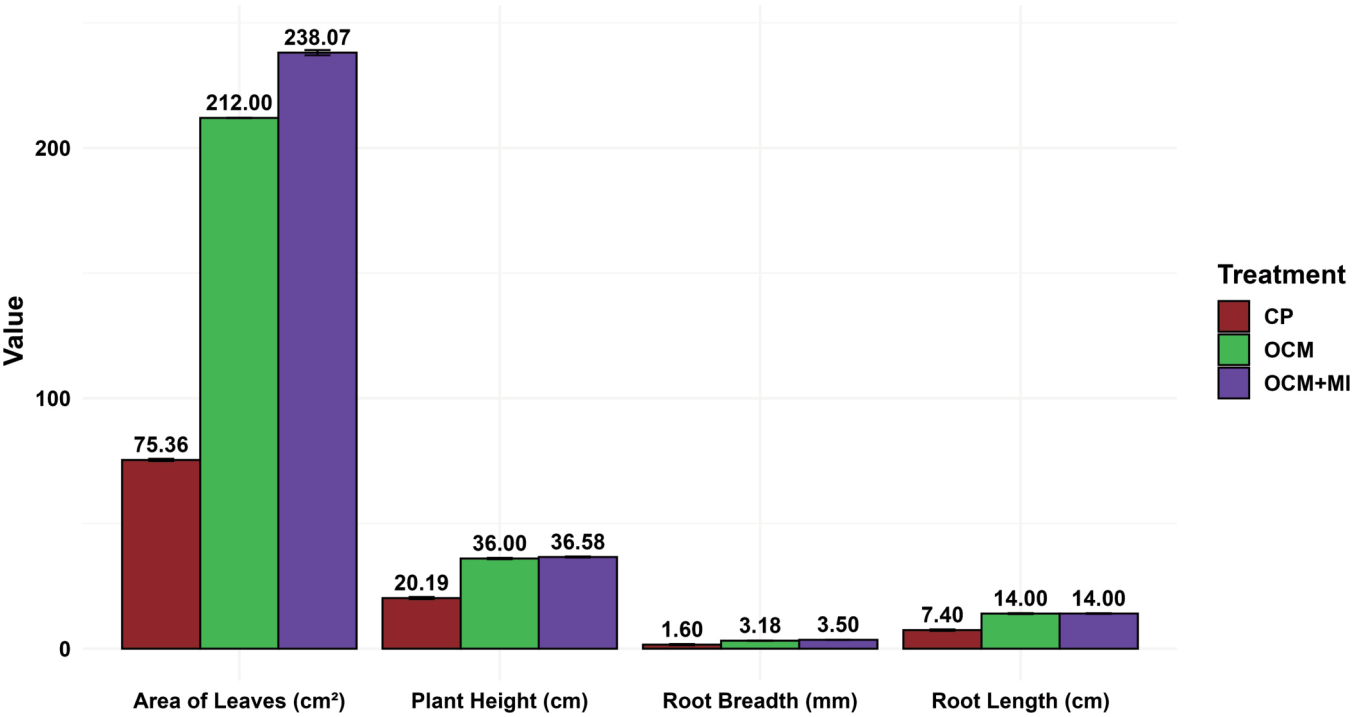


Figure S3. Comparison of biometric parameters across treatments.