

Article

Pakchoi Antioxidant Improvement and Differential Rhizobacterial Community Composition under Organic Fertilization

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Supplementary Information

Supplementary Methods

S1 Analysis of plant antioxidants

As for the antioxidants analysis, fresh leaf tissue (2.5 g) was flash-frozen in liquid nitrogen and homogenized in a mortar in 15 mL of 50% (v/v) acidified methanol (0.1 M HCl) extraction medium. The extraction was incubated for 20 min at room temperature and then centrifuged at 9000 g for 30 min. This extracted procedure was performed three times and the supernatants were pooled as a raw extract. The crude extract was dried under a vacuum at 40°C to remove acidified methanol and then rinsed with 100% methanol to a final volume of 10 ml for subsequent antioxidants analysis. Phenolic content in the extracts was determined colorimetrically according to the method suggested by Singleton, Orthofer and Lamuela-Raventós [1]. Shortly, 0.5 mL of the plant extract was mixed thoroughly with H₂O (0.5 mL) and 1M Folin-Ciocalteu reagent (2.0 mL) and incubated at room temperature for 3-5 min after which 10% (w/v) Na₂CO₃ (10 mL) was added to the mixture. Blue color was determined at 725 nm after 30 min of incubation. For phenolic acid determination, extract (1.0 mL) was well-mixed with a mixture combined with distilled water (5.0 mL), 0.5 M HCl (1.0 mL), Arnov reagent (1 mL, 10% (w/v) Na₂MoO₄ · 2H₂O and 10% (w/v) NaNO₂) and 1 M NaOH (1 mL), followed by adding distilled water to a final volume of 10 mL. Result of phenolic acid content was measured immediately by determination of the optical density (OD) at 590 nm [2]. Total flavonoids analysis was determined as described by Lamaison and Carnat [3]. Briefly, 1.0 mL of extract was reacted with 1.0 mL of 2% (w/v) AlCl₃ · 6H₂O (in methanol) and incubated at room temperature for 10 min. Total flavonoids content of the extract was calculated by measuring OD at 430 nm. To determine antioxidant activity 80 µL of extract and 1.92 mL of 0.06 mM 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in methanol was mixed and incubated for exactly 2.5 min. The mixture was measured by recording the absorbance at 515 nm [4]. A UV-Vis spectrophotometer (HITACHI, U-2900) was used for the determination of absorbance. Then the absorbance were evaluated against standard curves of gallic acid, caffeic acid, quercetin and trolox for phenolic content, phenolic acid, flavonoids and antioxidant activity, respectively.

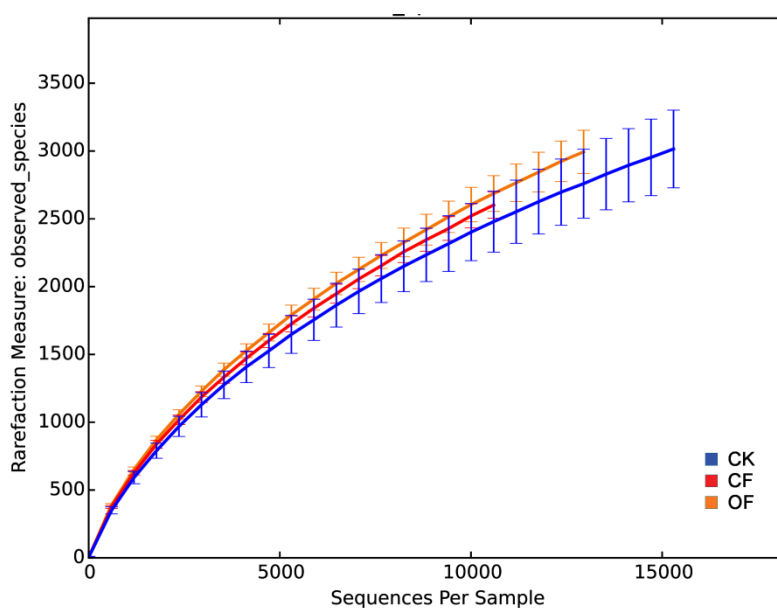
References

- [1] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, *Methods Enzymol.* 299 (1999) 152-178.
- [2] M. Szauffer-Hajdrych, Phenolic acids in leaves of species of the *Aquilegia L.* genus, *Herba Pol.* 50 (2004) 50-54.
- [3] J.L. Lamaison, A. Carnat, The amount of main flavonoids in flowers and leaves of *Crataegus monogyna* Jacq. and *Crataegus laevigata* (Poiret) DC. (Rosaceae), *Pharm. Acta Helv.* 65 (1990) 315-320.
- [4] U. Złotek, M. Świeca, A. Jakubczyk, Effect of abiotic elicitation on main health-promoting compounds, antioxidant activity and commercial quality of butter lettuce (*Lactuca sativa L.*), *Food Chem.* 148 (2014)

253-260.

Table S1. Chemical properties of soil and organic fertilizer used in this study.

Properties	Soil	Organic fertilizer
pH	7.32	7.9
EC (dS/m)	0.14	-
OM (%)	-	77.3
Water content (%)	-	12.7
Available N (mg/kg)	111.6	-
Available P (mg/kg)	181.7	-
Available K (mg/kg)	306.8	-
CEC (cmol ₍₊₎ /kg)	13.2	-
NH ₄ ⁺ -N (mg/kg)	7.86	-
NO ₃ ⁻ -N (mg/kg)	2.67	-
Total C (%)	1.92	27.84
Total N (%)	0.19	2.32
Total K (mg/kg)	2063	-
P ₂ O ₅ (%)	-	4.51
K ₂ O (%)	-	2.56

**Figure S1.** Rarefaction curve of Miseq pyrosequencing in the rhizosphere of pakchoi under different fertilizations. CK, control; CF, chemical fertilizer; OF, organic fertilizer.**Table S2.** Permutational multivariate analysis of variance (PERMANOVA) to investigate the effects of fertilization on the plant, soil and rhizobacterial community structure.

	Fertilization	
	F	P
Plant properties	75.33	0.016
Soil properties	35.53	0.004
Rhizobacterial community structure	1.918	0.005

Table 3. Pearson correlation among plant variables measured in this study.

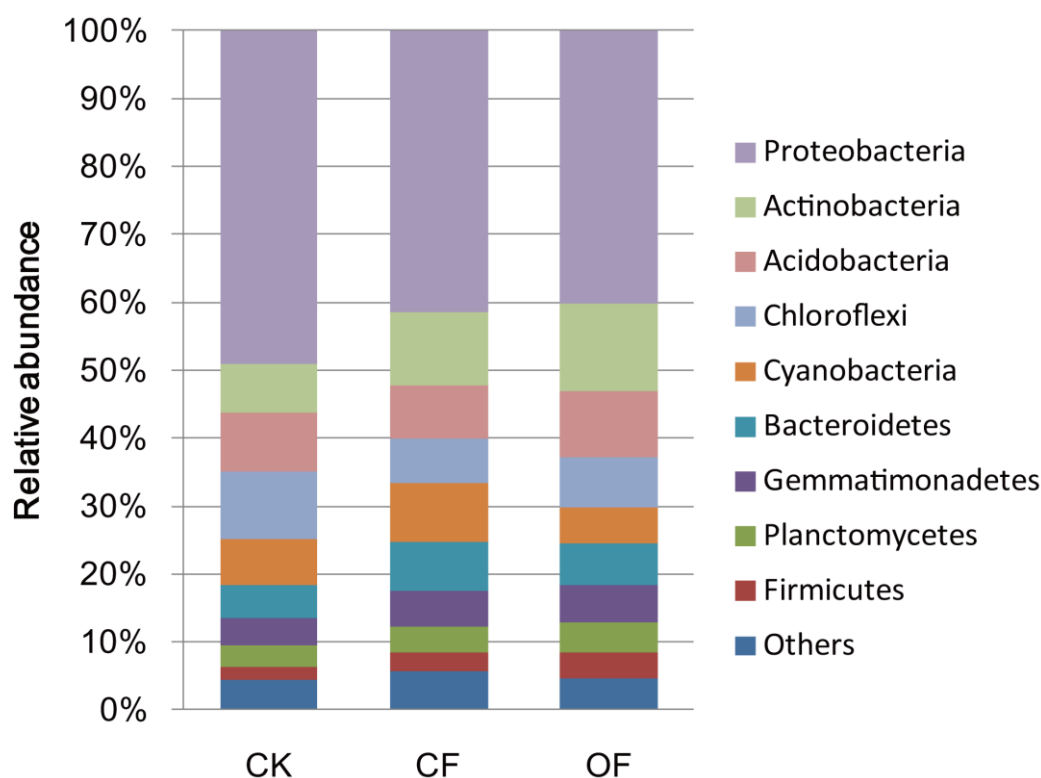
Parameter	Leaf number	Leaf area	Fresh weight	Dry weight	Phenolic content	Phenolic acid	Flavonoids	Antioxidant activity
Leaf number								
Leaf area	0.094							
Fresh weight	0.103	0.676 *						
Dry weight	0.717 *	0.090	0.559					
Phenolic content	0.201	-0.164	-0.086	0.350				
Phenolic acid	-0.365	-0.219	-0.497	-0.396	0.640			
Flavonoids	0.385	-0.059	0.171	0.619	0.916**	0.425		
Antioxidant activity	0.107	-0.103	-0.199	0.114	0.941**	0.742*	0.788*	

Correlation significant levels: * $P < 0.05$ level, ** $P < 0.01$.

Table S4. Reads, number of OTUs and α -diversity indices under different fertilizer treatments.

Treatment	Reads	OTU	Coverage	Chao 1	ACE	Shannon	Simpson
CK	39088	3876	0.91	1316±196 a	2246±338 a	8.44±0.68 a	0.98±0.02 a
CF	44480	3884	0.91	1342±140 a	2042±326 a	8.74±0.06 a	0.99±0.00 a
OF	39400	4067	0.90	1343±143 a	2075±558 a	8.96±0.29 a	0.99±0.00 a

Different letters indicate statistically significant differences ($p < 0.05$) according to Duncan's test ($n=3$). CK, control; CF, chemical fertilizer; OF, organic fertilizer.

**Figure 2.** Relative abundance of bacterial phyla in the rhizosphere of pakchoi under different fertilizations. CK, control; CF, chemical fertilizer; OF, organic fertilizer.

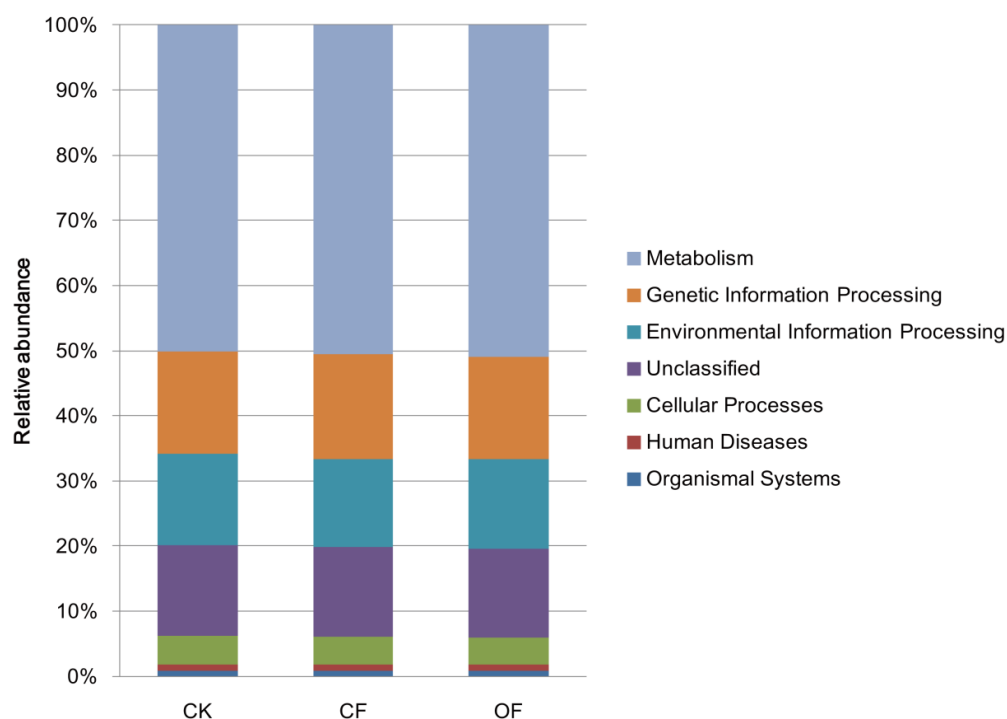


Figure 3. Predicted functional pathways of rhizobacterial communities under different fertilizations. CK, control; CF, chemical fertilizer; OF, organic fertilizer Table S5. The 40 most abundant rhizobacteria at genus level under different fertilizations (n=3).

Rank	Kingdom	Phylum	Class	Order	Family	Genus	Relative abundance (%)		
							CK	CF	OF
1	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	8.42	3.50	3.92
2	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<i>Methylibium</i>	3.62	2.45	1.69
3	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	<i>Bacillus</i>	1.17	1.92	2.74
4	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	<i>Pseudoxanthomonas</i>	1.91	1.43	1.16
5	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Kaistobacter</i>	1.81	1.40	1.28
6	Bacteria	Bacteroidetes	Saprosiriae	Saprosiriales	Chitinophagaceae	<i>Flavisolibacter</i>	1.45	1.59	1.26
7	Bacteria	Cyanobacteria	Synechococcophycideae	Pseudanabaenales	Pseudanabaenaceae	<i>Leptolyngbya</i>	1.60	1.19	1.32
8	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<i>Ramlibacter</i>	1.62	1.21	0.93
9	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	<i>Devosia</i>	0.64	1.29	1.77
10	Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	<i>Opitutus</i>	0.72	1.43	0.85
11	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	1.36	0.67	0.29
12	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	<i>Agrobacterium</i>	0.50	0.66	0.86
13	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	mitochondria	<i>Raphanus</i>	0.34	1.07	0.39
14	Bacteria	Chloroflexi	Anaerolineae	Ardenscatenales	Ardenscatenaceae	<i>Ardenscatena</i>	1.45	0.14	0.15
15	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	<i>Lysobacter</i>	0.45	0.67	0.49
16	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	<i>Arenimonas</i>	0.37	0.71	0.52
17	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<i>Hydrogenophaga</i>	0.24	0.55	0.78
18	Bacteria	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	<i>Planctomyces</i>	0.40	0.49	0.66
19	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	<i>Cellvibrio</i>	0.30	0.51	0.52
20	Archaea	Crenarchaeota	Thaumarchaeota	Nitrososphaerales	Nitrososphaeraceae	<i>Candidatus Nitrososphaera</i>	0.39	0.47	0.44
21	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioideaceae	<i>Aeromicrobium</i>	0.19	0.44	0.49
22	Bacteria	Actinobacteria	Acidimicrobia	Acidimicrobiales	Iamiaceae	<i>Iamia</i>	0.23	0.40	0.46
23	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	<i>Adhaeribacter</i>	0.19	0.27	0.58
24	Bacteria	Armatimonadetes	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae	<i>Fimbriimonas</i>	0.24	0.38	0.41
25	Bacteria	Cyanobacteria	Oscillatoriothycideae	Oscillatoriales	Phormidiaceae	<i>Arthrospira</i>	0.51	0.33	0.15
26	Bacteria	Chloroflexi	Chloroflexi	Chloroflexales	Oscillochloridaceae	<i>Oscillochloris</i>	0.44	0.26	0.22
27	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	<i>Microbacterium</i>	0.12	0.47	0.27
28	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	<i>Plesiocystis</i>	0.35	0.30	0.19
29	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<i>Limnohabitans</i>	0.27	0.26	0.29
30	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	<i>Luteimonas</i>	0.06	0.33	0.37
31	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Streptomyces</i>	0.16	0.35	0.25
32	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	<i>Rhodoplanes</i>	0.27	0.20	0.29
33	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	<i>Dyadobacter</i>	0.16	0.35	0.17
34	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	<i>Nitrospira</i>	0.23	0.22	0.22
35	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	<i>Balneimonas</i>	0.17	0.22	0.26
36	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacteriales	Solirubrobacteraceae	<i>Solirubrobacter</i>	0.23	0.19	0.23
37	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaeae	<i>Pseudonocardia</i>	0.15	0.18	0.32
38	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	<i>Agromyces</i>	0.10	0.26	0.23
39	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacteriales	Caulobacteraceae	<i>Phenylobacterium</i>	0.29	0.15	0.13
40	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<i>Azohydromonas</i>	0.17	0.18	0.18

control; CF, chemical fertilizer; OF, organic fertilizer