

Supplementary Materials

Combined effect of the potassium dose and plant biofertilization by *Acinetobacter calcoaceticus* on the growth, mineral content, nutritional quality, antioxidant activity and metabolomic features of tomatillo fruits (*Physalis ixocarpa* Brot.).

Plants

Heriberto F. Ramírez-Cariño, Carlos E. Ochoa-Velasco, José A. Guerrero-Analco, Juan L. Monribot-Villanueva, Concepción Calderón-García, Elizabeth González-Terreros, Cirenio Escamirosa-Tinoco, Isidro Morales*, Rogelio Valadez-Blanco*

* Correspondence:

rvaladez@mixteco.utm.mx (R. Valadez-Blanco), Instituto de Agroindustrias, Universidad Tecnológica de la Mixteca, Carretera a Acatlma km. 2.5, Huajuapan de León, Oaxaca 69000, México

imoralesg@ipn.mx (I. Morales), Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional - Unidad Oaxaca, Hornos 1003, Santa Cruz Xoxocotlán, Oaxaca 71230, México

SUPPLEMENTARY TABLES

Table S1. Color parameters (CIELab) for the tomatillo fruits (*Physalis ixocarpa* Brot. cv. "cáscara morada") in the evaluation of the biofertilizing effect of *Acinetobacter calcoaceticus* UTMR2 at different doses of potassium.

Treatment ¹	L*	a*	b*
KF100	55.5 ± 1.34 ^{ab}	-5.9 ± 0.91 ^{ab}	24.3 ± 2.16 ^a
KF75	55.9 ± 2.88 ^{ab}	-5.6 ± 1.05 ^a	24.1 ± 2.62 ^a
KF50	56.3 ± 1.59 ^a	-6.9 ± 1.21 ^{ab}	27.8 ± 3.08 ^a
KF0	53.4 ± 2.73 ^{ab}	-7.2 ± 0.78 ^{ab}	25.8 ± 3.67 ^a
KB100	54.2 ± 3.10 ^{ab}	-7.2 ± 0.56 ^{ab}	26.9 ± 2.10 ^a
KB75	53.8 ± 2.84 ^{ab}	-6.7 ± 1.09 ^{ab}	24.6 ± 1.26 ^a
KB50	51.1 ± 1.44 ^b	-7.7 ± 0.78 ^b	25.9 ± 2.07 ^a
KB0	54.7 ± 3.86 ^{ab}	-6.7 ± 1.63 ^{ab}	24.8 ± 3.60 ^a

¹KF#: Treatments with chemical fertilization only; KB#: Treatments with chemical fertilization and inoculation with *A. calcoaceticus* UTMR2; #: potassium fertilization dose (%) based on the recommended dose for tomatillo cultivation [19]. Different superscript letters indicate a significant difference between the treatments according to the HSD Tukey test (p < 0.05, n = 3).

Table S2. Mineral-element content (g/kg dw) in tomatillo fruits (*Physalis ixocarpa* Brot. cv. “cáscara morada”) in the evaluation of the biofertilizing effect of *Acinetobacter calcoaceticus* UTMR2 at different doses of potassium.

Treatment ¹	K	P	Ca	Na	Mg	Mn
KF100	17.1 ± 5.21 ^a	1.43 ± 0.10 ^a	0.03 ± 0.03 ^b	0.83 ± 0.10 ^d	2.6 ± 0.15 ^a	0.018 ± 0.003 ^a
KF75	18.6 ± 38 ^a	1.40 ± 0.08 ^a	0.09 ± 0.11 ^{ab}	1.33 ± 0.04 ^{bc}	2.7 ± 0.09 ^a	0.020 ± 0.003 ^a
KF50	24.2 ± 3.90 ^a	1.34 ± 0.28 ^a	0.16 ± 0.08 ^{ab}	1.32 ± 0.03 ^{bc}	2.5 ± 0.25 ^a	0.017 ± 0.002 ^a
KF0	18.8 ± 2.57 ^a	1.38 ± 0.22 ^a	0.28 ± 0.08 ^a	1.52 ± 0.33 ^b	2.8 ± 0.22 ^a	0.017 ± 0.003 ^a
KB100	18.4 ± 1.68 ^a	1.48 ± 0.25 ^a	0.04 ± 0.08 ^b	1.22 ± 0.11 ^{bcd}	2.6 ± 0.36 ^a	0.020 ± 0.002 ^a
KB75	17.4 ± 1.42 ^a	1.45 ± 0.05 ^a	0.19 ± 0.07 ^{ab}	0.90 ± 0.17 ^{cd}	2.7 ± 0.37 ^a	0.011 ± 0.010 ^a
KB50	19.9 ± 1.29 ^a	1.76 ± 0.45 ^a	0.23 ± 0.05 ^{ab}	2.26 ± 0.18 ^a	3.4 ± 0.54 ^a	0.023 ± 0.005 ^a
KB0	17.0 ± 1.55 ^a	1.65 ± 0.18 ^a	0.04 ± 0.03 ^b	0.87 ± 0.08 ^d	2.9 ± 0.18 ^a	0.016 ± 0.001 ^a

¹KF#: Treatments with chemical fertilization only; KB#: Treatments with chemical fertilization and inoculation with *A. calcoaceticus* UTMR2; #: potassium fertilization dose (%) based on the recommended dose for tomatillo cultivation [19]. Different superscript letters indicate a significant difference between the treatments according to the HSD Tukey test (p < 0.05, n = 3).

Table S3. Two-way analysis of variance (ANOVA) for the relevant parameters of tomatillo plants (*Physalis ixocarpa* Brot. cv. “cáscara morada”) in the evaluation of the biofertilizing effect of *Acinetobacter calcoaceticus* UTMR2 at different doses of potassium.

SV	DF	Mean squares						
		[K]	[P]	[Ca]	[Na]	[Mg]	Chlorophyll	Dry weight
K dose	3	16.61ns (0.07)	1.89*** (0.0001)	13.33*** (0.0001)	6.20*** (0.0001)	6.56*** (0.0001)	470.84* (0.0112)	329.60*** (0.0001)
Inoculation	1	7.62ns (0.27)	0.13*** (0.0008)	8.02** (0.0025)	0.03ns (0.7426)	2.50*** (0.0001)	4108.16*** (0.0001)	766.14*** (0.0001)
K dose*Inoculation	3	53.47** (0.001)	0.29*** (0.0001)	4.88** (0.0020)	11.02*** (0.0001)	2.21*** (0.0001)	615.87*** (0.0038)	199.17*** (0.0001)
Error	16							
Total	23							

SV: sources of variation; DF: degrees of freedom; value in parenthesis, level of significance; ns: non-significant value of F ($P > 0.05$), *: significant F value ($P \leq 0.05$), **: highly significant value of F ($P \leq 0.01$), ***: very highly significant value of F ($P \leq 0.001$).

Table S4. Two-way analysis of variance (ANOVA) for the relevant parameters of tomatillo fruits (*Physalis ixocarpa* Brot. cv. “cáscara morada”) in the evaluation of the biofertilizing effect of *Acinetobacter calcoaceticus* UTMR2 at different doses of potassium.

SV	DF	Mean squares					
		Yield index	Maturity index	Protein	Fiber	Fat	FRAP
K dose	3	0.088*** (0.0001)	7.46*** (0.0001)	1.15*** (0.0001)	0.52*** (0.0001)	0.13*** (0.0001)	106770.47*** (0.0001)
Inoculation	1	0.00003ns (0.911)	0.26*** (0.0036)	0.19*** (0.0061)	1.11*** (0.0001)	0.13*** (0.0001)	25200.72*** (0.0001)
K dose*Inoculation	2	0.213*** (0.0001)	8.91*** (0.0001)	0.63*** (0.0001)	0.29*** (0.0014)	0.04*** (0.0027)	414287.64*** (0.0001)
Error	16						
Total	23						

SV: sources of variation; DF: degrees of freedom; value in parenthesis: level of significance; ns: non-significant value of F ($P > 0.05$), *: significant F value ($P \leq 0.05$), **: highly significant value of F ($P \leq 0.01$), ***: very highly significant value of F ($P \leq 0.001$).

Table S5. dMRM, mass spectrometric and quantification conditions for each phenolic compound in the evaluation of the biofertilizing effect of *Acinetobacter calcoaceticus* UTMR2 in tomatillo fruits (*Physalis ixocarpa* Brot. cv. “cáscara morada”) at 75% of the potassium recommended dose [19].

Compound	dMRM transition			Mass spectrometric conditions			Quantification conditions		
	Precursor ion	Production	Retention time	Collision energy	Fragmentor	Polarity	Quantification range (μM)	Regression type	R ²
Shikimic acid	173.1	111.1	0.49	10	100	Negative	0.5 - 19	Quadratic	0.99
Gallic acid	169.0	125.2	1.4	10	100	Negative	1 - 19	Quadratic	0.99
L-Phenylalanine	166.1	131.0	1.92	10	100	Positive	0.25 - 19	Quadratic	0.99
Protocatechuic acid	153.0	109.1	2.5	10	100	Negative	0.25 - 19	Quadratic	0.99
4-Hydroxybenzoic acid	137.1	92.8	3.76	10	100	Negative	0.25 - 19	Quadratic	0.99
Gentisic acid	153.0	109.0	3.83	10	100	Negative	0.25 - 19	Quadratic	0.99
4-Hydroxyphenylacetic acid	107.1	77.0	4.72	20	140	Positive	0.25 - 19	Quadratic	0.99
(-)Epigallocatechin	305.1	125.0	4.83	20	140	Negative	1 - 17	Quadratic	0.99
(+)-Catechin	291.0	138.9	5.07	10	100	Positive	0.5 - 19	Quadratic	0.99
Vanillic acid	169.0	93.0	5.12	10	100	Positive	0.25 - 19	Quadratic	0.99
Scopolin	355.1	193.0	5.25	20	100	Positive	0.25 - 19	Quadratic	0.99
Chlorogenic acid	355.1	163.0	5.34	10	100	Positive	0.25 - 19	Quadratic	0.99
Caffeic acid	181.0	163.	5.38	10	100	Positive	0.5 - 19	Quadratic	0.99
Malvin	655.1	331.1	5.82	40	100	Positive	0.5 - 19	Quadratic	0.99
Kuromanin	449.0	286.9	6.34	30	100	Positive	0.5 - 19	Quadratic	0.99
Procyanidin B2	577.1	425.1	6.4	10	100	Negative	1 - 19	Quadratic	0.99
Vanillin	153.0	124.9	6.52	10	100	Positive	0.25 - 19	Quadratic	0.99
Keracyanin	595.2	287.1	6.88	20	100	Positive	0.5 - 19	Quadratic	0.99
(-)Epicatechin	291.0	138.8	6.96	10	100	Positive	0.5 - 19	Quadratic	0.99
4-Coumaric acid	165.0	147.0	7.21	10	100	Positive	0.25 - 19	Quadratic	0.99
Mangiferin	423.0	302.8	7.32	10	100	Positive	0.5 - 19	Quadratic	0.99

Umbelliferone	163.0	107.0	7.64	30	100	Positive	0.25 - 19	Quadratic	0.99
(-)Gallocatechin gallate	458.9	139.0	7.95	20	80	Positive	1 - 19	Quadratic	0.99
Scopoletin	193.0	133.0	8.4	10	100	Positive	0.25 - 19	Quadratic	0.99
Ferulic acid	195.1	145.0	8.6	20	100	Positive	0.25 - 19	Quadratic	0.99
Quercetin 3,4-di-O-glucoside	627.0	302.9	8.77	10	100	Positive	0.5 - 19	Quadratic	0.99
3-Coumaric acid	165.05	147.04	8.81	10	100	Positive	0.5 - 19	Quadratic	0.99
Salicylic acid	137.0	93	9.15	10	100	Negative	0.5 - 19	Quadratic	0.99
Sinapic acid	225.1	207.1	9.16	10	100	Positive	0.25 - 19	Quadratic	0.99
Epicatechin gallate	443.1	123.0	9.83	10	100	Positive	1 - 19	Quadratic	0.99
Ellagic acid	300.5	145.0	9.98	30	170	Negative	1 - 19	Quadratic	0.99
Myricitrin	465.0	318.9	10.03	10	100	Positive	1 - 19	Quadratic	0.99
Pelargonidin	271.1	121	10.22	20	10	Positive	1 - 19	Quadratic	0.97
Quercetin 3-D-galactoside	465.0	302.9	10.26	10	100	Positive	0.25 - 19	Quadratic	0.99
Rutin	611.0	302.9	10.35	10	100	Positive	0.25 - 19	Quadratic	0.99
p-Anisic acid	153.1	109.0	10.45	5	120	Positive	0.25 - 19	Quadratic	0.99
Quercetin 3-glucoside	465.0	303.0	10.57	10	100	Positive	0.25 - 19	Quadratic	0.99
Luteolin 7-O-glucoside	449.0	287.0	10.77	10	100	Positive	0.5 - 19	Quadratic	0.99
Malvidin	331.1	287.1	11.14	20	100	Positive	1 - 17	Quadratic	0.96
2,4-Dimethoxy-6-methylbenzoic acid	197.0	179.0	11.41	5	80	Positive	0.25 - 19	Quadratic	0.99
Penta-O-galloyl-B-D-glucose	771.1	153.0	11.68	20	100	Positive	0.5 - 19	Quadratic	0.99
Kaemperol 3-O-glucoside	449.0	286.9	11.91	10	100	Positive	0.25 - 19	Quadratic	0.99
Quercitrin	449.1	303.1	11.95	10	100	Positive	0.5 - 19	Quadratic	0.99
Naringin	273.0	153.0	12.13	10	120	Positive	0.25 - 19	Quadratic	0.99
Myricetin	317.0	179.0	12.29	10	100	Negative	0.5 - 15	Quadratic	0.99
Hesperidin	609.1	301.1	12.68	20	100	Negative	0.5 - 19	Quadratic	0.99
trans-Resveratrol	229.1	135.0	12.69	10	100	Positive	0.5 - 19	Quadratic	0.99
Rosmarinic acid	361.1	163.0	12.8	10	100	Positive	0.5 - 19	Quadratic	0.99

Secoisolariciresinol	363.2	137.1	13.02	20	100	Positive	0.5 - 19	Quadratic	0.99
Phloridzin	435.0	272.9	13.04	10	100	Negative	0.25 - 19	Quadratic	0.99
<i>trans</i> -Cinnamic acid	149.1	131.0	14.08	10	100	Positive	0.25 - 19	Quadratic	0.99
Psoralen	187.0	131.1	14.99	20	100	Positive	0.25 - 19	Quadratic	0.99
Quercetin	302.9	153.1	15.18	35	100	Positive	1 - 19	Quadratic	0.99
Luteolin	287.1	153.0	15.28	30	100	Positive	0.5 - 19	Quadratic	0.99
Angelicin	187.0	131.1	15.75	20	100	Positive	0.5 - 19	Quadratic	0.99
Naringenin	271.0	151	16.79	10	100	Negative	0.5 - 19	Quadratic	0.99
Apigenin	271.0	153.0	17.45	30	100	Positive	0.5 - 19	Quadratic	0.99
Matairesinol	359.2	137.1	17.55	10	100	Positive	0.25 - 19	Quadratic	0.99
Kaempferol	287.1	153.0	17.81	30	100	Positive	0.25 - 19	Quadratic	0.99
Hesperetin	303.1	177.1	18.06	20	100	Positive	0.25 - 19	Quadratic	0.99
Podophyllotoxin	415.1	397.1	19.01	10	100	Positive	0.25 - 19	Quadratic	0.99
Methyl cinnamate	163.1	131.0	21.46	6	100	Positive	0.25 - 1	Quadratic	0.99
Nordihydroguaiaretic acid	303.0	193.1	22.72	10	100	Positive	0.5 - 19	Quadratic	0.99
Chrysin	255.1	153.0	22.89	40	100	Positive	0.25 - 19	Quadratic	0.99
Kaempferide	301.0	258.2	24.38	20	100	Positive	0.5 - 19	Quadratic	0.99
Emodin	269.0	225.0	27.45	20	150	Negative	1 - 17	Quadratic	0.99
Chrysophanol	255.1	153.0	31.34	40	100	Positive	0.25 - 19	Quadratic	0.99

The retention time variation allowed for the search of the compounds were 2 min in each case. The cell accelerator voltage was 7 V for each compound. Dilutions were made if the concentration of some compounds were higher than the linearity range.

SUPPLEMENTARY FIGURES

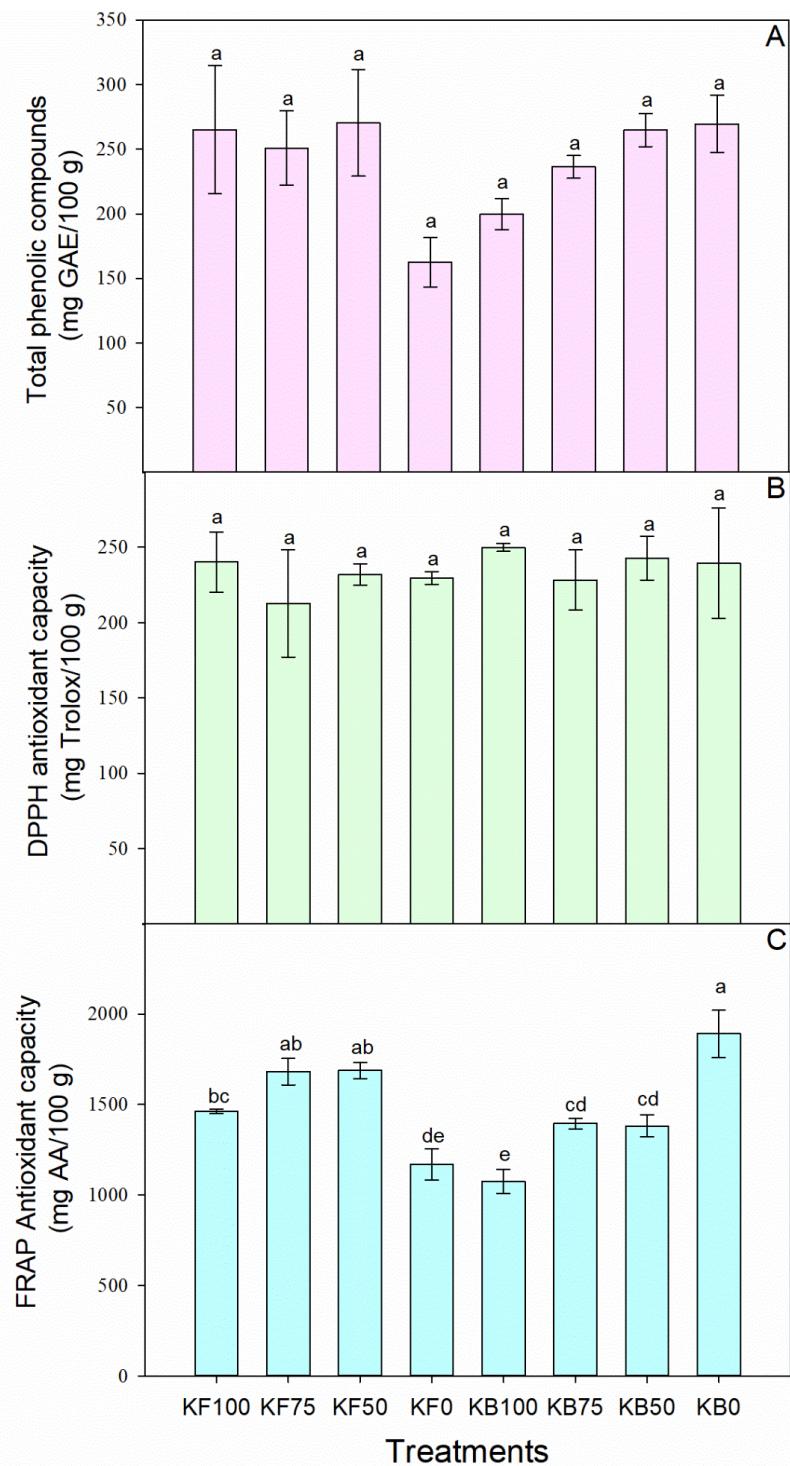


Figure S1. Phenols content and antioxidant capacity in tomatillo fruits (*Physalis ixocarpa* Brot. cv. "cáscara morada") in the evaluation of the biofertilizing effect of *Acinetobacter calcoaceticus* UTMR2 at different doses of potassium. KF#: Treatments with chemical fertilization only; KB#: Treatments with chemical fertilization and inoculation with *Acinetobacter calcoaceticus* UTMR2; #: potassium fertilization dose (%) based on the recommended dose for tomatillo cultivation [19]. Different superscript letters indicate a significant difference between the treatments. GAE: gallic acid equivalent; AA: ascorbic acid.

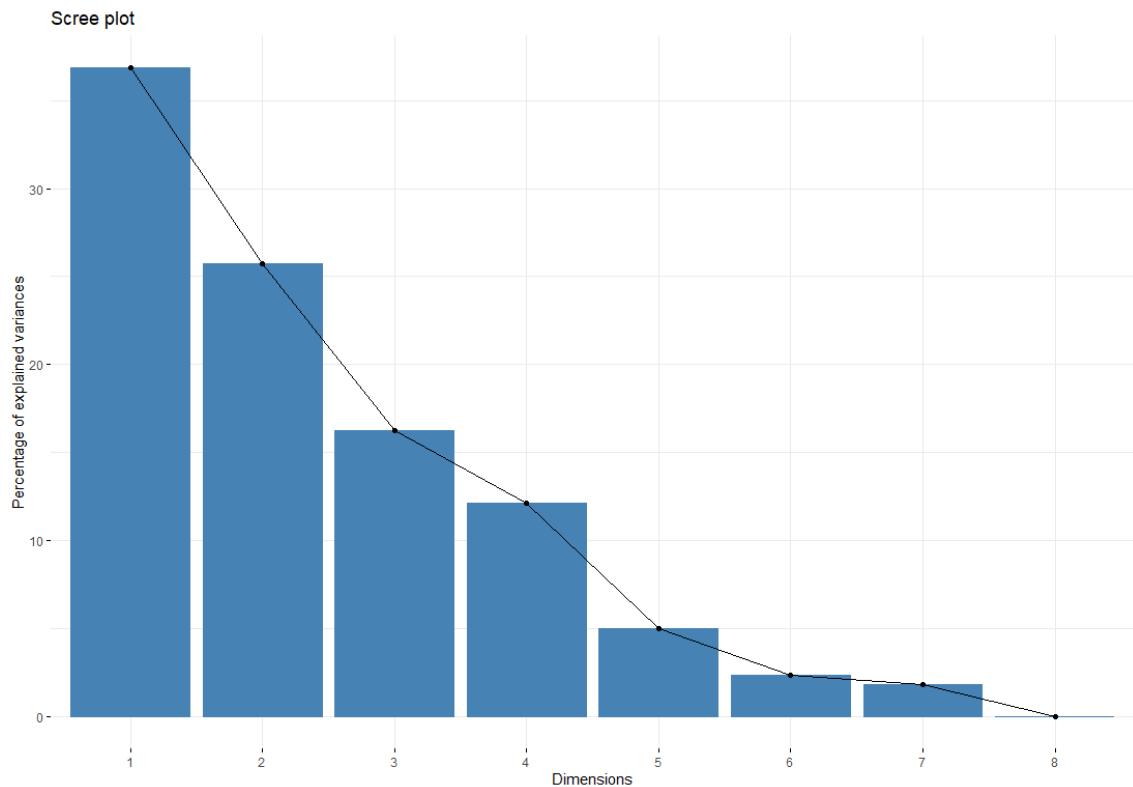


Figure S2. Scree plot for the percentage of coverage of the PCA analysis in the evaluation of the biofertilizing effect of *Acinetobacter calcoaceticus* UTMR2 in tomatillo plants (*Physalis ixocarpa* Brot. cv. "cáscara morada") at different doses of potassium.