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Abstract

Beneficial Microbes Application on Tomato Significantly Improves Accumulation of Metabolites with Nutraceutical Value †

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Abstract: Tomato (*Solanum lycopersicum*) is an important crop and is consumed worldwide. This vegetable is an excellent source of natural compounds (i.e., antioxidants including vitamins C and E, lycopene, b-carotene, lutein and flavonoids) and minerals useful for human health. Several studies have shown the correlation between tomato consumption and the prevention of some types of cancer and chronic cardiovascular diseases. In this study, the improvement of nutritional value of tomato, by using beneficial microorganisms, including selected strains of *Streptomyces*, *Bacillus* and *Trichoderma*, has been investigated. These microbes were applied on tomato plants in a field trial either as single inoculants or as microbial consortia. After the treatments, plants were subjected to a metabolomic analysis by LC-MS qTOF and led to the identification of sixteen metabolites, including tomatine and its derivatives, solafloridine, apo-13-zeaxanthinone, deoxy phytoprostane and L-phenylalanine. Results showed a significant difference in relative abundance of these metabolites among treatments. *Bacillus* application, alone or in combination with T22, induced the production of tomatine, while *Trichoderma* alone or in combination with *Streptomyces* or *Bacillus* and combination between *Streptomyces* and *Bacillus*, induced the production of solafloridine. The combination of *Streptomyces* and *Trichoderma* increased the accumulation of solafloridine, apo-13-zeaxanthinone, deoxy phytoprostane and L-phenylalanine, compared with the single treatments. In conclusion, field applications of *Streptomyces*, *Bacillus* and *Trichoderma* significantly induced metabolic profile change of tomato and the accumulation of metabolites with nutraceutical value.

Keywords: metabolomics; antioxidants; *Streptomyces*; *Bacillus*; *Trichoderma*

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Beneficial microbes application on tomato significantly improves accumulation of metabolites with nutraceutical value

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Tomato (*Solanum lycopersicum*) is an important crop and is consumed worldwide. This vegetable is an excellent source of natural compounds (i.e antioxidants including vitamins C and E, lycopene, b-carotene, lutein and flavonoids) and minerals useful for human health.

AIM OF THE WORK:

Investigate the impact of beneficial microorganisms application on tomato metabolome.

MATERIALS AND METHODS:

Streptomyces fulvissimus, Bacillus subtilis and *Trichoderma afroharzianum* were applied on tomato plants in a field trial either as single inoculants or as microbial consortia. After the treatments, plants were harvested and subjected to organic extraction (Figure 1) followed by metabolomic analysis (LC-MS qTOF).

Figure 1. Extraction of metabolites from tomato leaves.

IDENTIFICATION OF PLANT METABOLITES:

Spectrometric analysis led to the identification of several secondary metabolites (i.e. tomatine, solasodine, etc.) belonging to different classes of natural compounds (Table 1).

METABOLOMIC ANALYSIS:

Metabolomic analysis highlighted several differentially accumulated compounds, whose abundance is dependent on the treatment (PCA on Figure 2).

Compound	RT (min)	Experimental Mass (Da)	Regulation					
			B vs C	S vs C	T vs C	B+T vs C	B+S vs C	S+T vs C
Tomatine	5.270	1033.55	↑	↓	↑	↑	↑	↓
Solafloridine	6.693	415.3462	↓	↓	↑	↑	↑	↑
Apo-13-zeaxanthinone	8.855	274.1939	↓	↓	↓	↓	↑	↓
Deoxy phytoprostane J1	6.813	290.1891	↓	↓	↓	↓	↓	↓
Colneleic acid	9.726002	294.2206	↓	↓	↓	↓	↓	↓
Solasodine	5.232685	413.3294	↑	↓	↑	↓	↑	↓
Isoorientin 2"-O-glucopyranoside	5.007	610.1537	↓	↓	↓	↓	↓	↓
Quinic acid	1.291	192.0632	↓	↓	↓	↓	↓	↓
Quercetin 3-(2G-apiosylrutinoside)	4.842	742.1949	↑	↑	↑	↑	↑	↑
beta1-Tomatine	5.322	901.5028	↑	↑	↑	↑	↑	↑
Kaempferol 3-galactoside-7-rhamnoside	5.166999	594.1582	↓	↓	↓	↓	↓	↓

Table 1. Putatively identified metabolites differentially accumulated in plants treated with *Streptomyces fulvissimus* (S), *Trichoderma afroharzianum* (T) and *Bacillus subtilis* (B) or as mix (B+S, B+T, S+T) compared to control (group C, untreated plants).
In black = putatively identified molecule from LC-MS analysis performed in positive mode
In red = putatively identified molecule from LC-MS analysis performed in negative mode
↑ Up-regulated *vs* control (C). ↓ Down-regulated vs control (C).

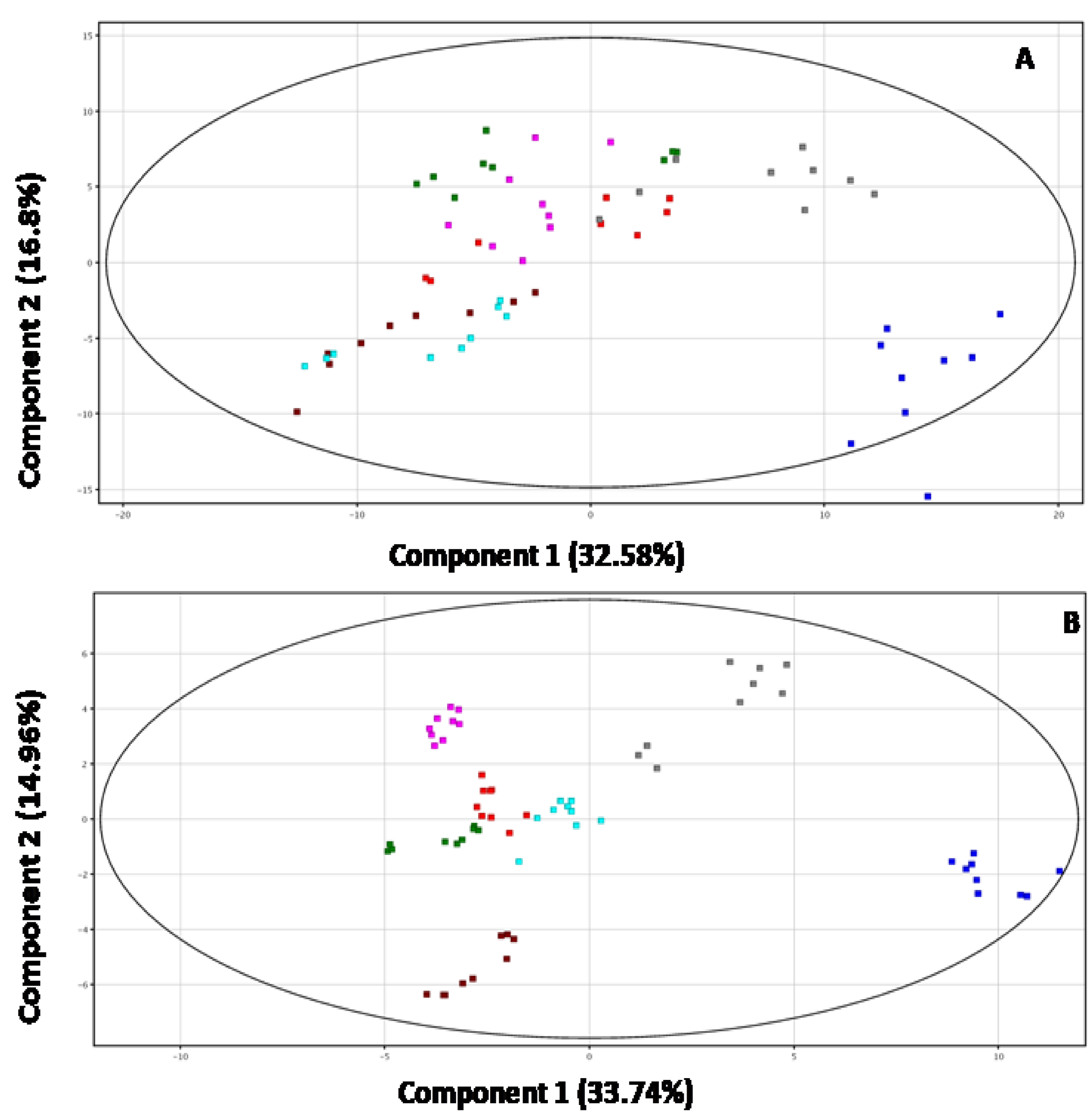

Figure 2. Principal components analysis (PCA) score plots of the LC–MS data acquired in positive (B) and negative (A) mode. Each group of replicates subjected to different treatments is depicted with a different color: control group (C) in blu; *Streptomyces fulvissimus* group (S) in brown; *Trichoderma afroharzianum* group (T) in pink; microbial consortium *Streptomyces fulvissimus* and *Bacillus subtilis* group (S+B) in grey; *Bacillus subtilis* group (B) in red; microbial consortium *Streptomyces fulvissimus* and *Trichoderma afroharzianum* group (S+T) in green; microbial consortium *Trichoderma afroharzianum* and *Bacillus subtilis* group (T+B) in light blue.

Conclusion:

Field applications of *Streptomyces fulvissimus, Bacillus subtilis* and *Trichoderma afroharzianum* induced changes in the metabolic profile of tomato. In particular, a certain accumulation of molecules has been observed, such as tomatin, with demonstrated antioxidant, antitumoral and fungicidal properties. The accumulation of these metabolites with nutraceutical value represents a starting point for further future studies to investigate the efficacy of these beneficial strains.

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