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Research Article

Effects of Endophytic and Rhizosphere Bacteria on Antioxidant Capacity and Fruit Phytochemical Composition of Muna Local Tomato (*Solanum lycopersicum* L.)

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Abstract

Background and Objective: Muna local tomato (*Solanum lycopersicum* L.) is a valuable local genetic resource with potential as a functional food due to its bioactive compounds and antioxidant activity. However, the use of endophytic-rhizosphere bacterial consortia to improve the phytochemical quality and antioxidant capacity of local tomatoes remains limited. This study aimed to evaluate the effects of single isolates and consortia of endophytic-rhizosphere bacteria on the antioxidant activity and phytochemical profile of Muna local tomato fruits. **Materials and Methods:** The experiment was arranged in a randomized block design with eight treatments, consisting of a control, *Pseudomonas* sp. SWRII B02, *Bacillus* sp. CKD061, *Pseudomonas* sp. Ke03 and combinations of two and three bacterial isolates. Antioxidant activity was determined using the DPPH assay, while phytochemical profiling was performed using LC-MS/MS. Quantitative data, including DPPH inhibition and IC₅₀ values, were analyzed by ANOVA followed by DMRT at $\alpha = 0.05$. **Results:** All isolate combinations were compatible *in vitro*. Biological agent treatments enhanced antioxidant activity compared with the control. *Bacillus* sp. CKD061 exhibited the strongest antioxidant activity, with the lowest IC₅₀ value of 41.15 ppm, while the consortium of *Pseudomonas* sp. SWRII B02 and *Bacillus* sp. CKD061 was the most effective bacterial combination, with an IC₅₀ value of 47.14 ppm. The LC-MS/MS analysis revealed differences in phytochemical compounds among treatments. Fucosylthreonine, hupeheninoside and triacetoxypiperidinone were detected in all treatments. Solasodine glycoside was detected in the control and *Bacillus* sp. CKD061 treatments, whereas tomatidine was detected only in the consortium of *Pseudomonas* sp. SWRII B02 and *Bacillus* sp. CKD061. After one week of storage, the metabolite profile changed, as indicated by the presence of N-fructosylvaline, N-succinyl-2-amino-6-oxo-L-pimelic acid, tetrodamine and epivolkenin. **Conclusion:** *Bacillus* sp. CKD061 and its consortium with *Pseudomonas* sp. SWRII B02 have potential as bioinoculants to improve the functional quality of Muna local tomato fruits.

Key words: Antioxidant activity, bioinoculant, endophytic bacteria, LC-MS/MS, Muna local tomato, rhizosphere bacteria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important horticultural commodity with high economic, nutritional and functional value¹. Tomato fruit is known as a source of bioactive compounds, such as lycopene, β -carotene, ascorbic acid, phenolics, flavonoids, alkaloids and various other secondary metabolites that contribute to antioxidant activity. These compounds play a role in scavenging free radicals, reducing oxidative stress and enhancing the functional value of tomato fruit as a healthy food source². However, the accumulation of phytochemical compounds in tomato fruit is not constant; rather, it is influenced by genetic factors, environmental conditions, cultivation practices, nutrient status and plant interactions with microorganisms living around or within plant tissues³.

One biological approach that has been increasingly developed to improve plant quality is the use of Plant Growth-Promoting Bacteria (PGPB). These bacteria can enhance plant growth and quality through several mechanisms, including phosphate solubilization, nitrogen fixation, the production of growth hormones such as indole-3-acetic acid (IAA) and cytokinins, siderophore production and the induction of systemic resistance in plants⁴. Among PGPB groups, endophytic and rhizosphere bacteria occupy important ecological positions because they interact with plants in different compartments. Endophytic bacteria live within plant tissues without causing disease symptoms, whereas rhizosphere bacteria inhabit the root zone and interact directly with root exudates⁵. Endophytic bacteria have the potential to modulate plant secondary metabolism, including the biosynthesis of phenolic compounds, terpenoids, alkaloids and other antioxidant compounds. Meanwhile, rhizosphere bacteria play a role in increasing nutrient availability, improving the root environment and inducing plant physiological responses related to growth and defence⁶. Thus, these two bacterial groups not only function as growth promoters but also have the potential to influence the biochemical quality and functional value of fruits.

Recent approaches in the development of agricultural bioinoculants are no longer focused solely on the use of single strains but have increasingly shifted toward the application of bacterial consortia. Bacterial consortia allow multiple strains to act synergistically through complementary mechanisms. The combination of endophytic and rhizosphere bacteria can theoretically broaden the scope of microbial colonization, from the root zone to internal plant tissues, thereby enabling a more comprehensive plant response⁷. Although various studies have reported the roles of endophytic and rhizosphere

bacteria in tomato growth and quality, studies specifically evaluating the effects of combined endophytic-rhizosphere bacterial consortia on the antioxidant activity and phytochemical profile of tomato fruit remain limited. Most previous studies have emphasized growth, yield or specific quality parameters, such as lycopene, total phenolics or vitamin C⁸⁻¹⁰. However, studies integrating antioxidant assays with LC-MS/MS-based metabolite profiling remain limited, particularly in local tomato genotypes adapted to specific agroecological conditions. Therefore, research is needed to explain how the application of single strains and endophytic-rhizosphere bacterial consortia affects antioxidant activity and phytochemical composition in tomato fruit.

Muna local tomato is a local genetic resource with potential for development as a functional food. However, the use of microbiological approaches to enhance the phytochemical quality and antioxidant activity of this local tomato has not been widely investigated. This study is important because the application of bacterial consortia is not only directed toward improving plant growth but also toward enhancing the functional quality of fruit through increased antioxidant activity and changes in secondary metabolite profiles.

Based on this background, this study aimed to evaluate the effects of single strains and endophytic-rhizosphere bacterial consortia on the antioxidant activity and phytochemical profile of Muna local tomato (*Solanum lycopersicum* L.) fruits, as well as to identify the most promising isolate or isolate combination as a bioinoculant for improving fruit functional quality. This study is expected to provide a scientific basis for the development of bioinoculants based on beneficial microbial isolates that not only support sustainable agricultural systems but also enhance the functional value and biochemical quality of local tomato fruits.

MATERIALS AND METHODS

Research location and materials: This study was conducted at the Biology Laboratory and Greenhouse of Universitas Islam Negeri Kendari from July to December 2025. The plant material used was Muna local tomato (*Solanum lycopersicum* L.). The bacterial isolates used consisted of the endophytic bacterial isolate *Pseudomonas* sp. SWRII B02, obtained from the collection of La Fua *et al.*¹ and the rhizosphere bacterial isolate *Bacillus* sp. CKD061, as well as the endophytic bacterial isolate *Pseudomonas* sp. Ke03, obtained from the collection of Sutariati *et al.*¹¹. The chemicals used for antioxidant analysis and phytochemical profiling included methanol, DPPH, vitamin C

as a reference standard and supporting materials for bacterial culture, such as Tryptic Soy Broth (TSB), agar, sterile distilled water, 70% alcohol and other microbiological laboratory materials. The main instruments used included a laminar air flow cabinet, autoclave, incubator, UV-Vis spectrophotometer, rotary evaporator and LC-MS/MS.

Research design: This study was arranged in a Randomized Block Design (RBD) with eight biological agent treatments consisting of single-isolate applications and combinations of endophytic-rhizosphere bacterial isolates. The bacterial isolates used included *Pseudomonas* sp. SWRII B02, *Bacillus* sp. CKD061 and *Pseudomonas* sp. Ke03. The control treatment was used as a comparison without bacterial inoculation, whereas the other treatments consisted of the application of each isolate individually or as consortia of two and three isolates. The treatment arrangements were as follows: A0 = control without bacterial inoculation, A1 = inoculation with *Pseudomonas* sp. SWRII B02, A2 = inoculation with *Bacillus* sp. CKD061, A3 = inoculation with *Pseudomonas* sp. Ke03, A4 = combination of *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061, A5 = combination of *Pseudomonas* sp. SWRII B02+*Pseudomonas* sp. Ke03, A6 = combination of *Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03 and A7 = combination of *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03. Each treatment was replicated three times, resulting in 24 experimental units. Each experimental unit consisted of eight Muna local tomato plants grown in polybags; therefore, the total number of plants used was 192. Blocking was performed to minimize the effects of environmental variability in the greenhouse, while treatments within each block were assigned randomly. Tomato fruits used for antioxidant activity analysis and phytochemical profiling were collected from plants in each treatment and replication at a uniform maturity stage.

Preparation and application of bacterial inoculum: Each bacterial isolate, namely *Pseudomonas* sp. SWRII B02, *Bacillus* sp. CKD061 and *Pseudomonas* sp. Ke03, was aseptically refreshed on TSA medium and incubated at 28-30°C for 24-48 hrs. Active colonies of each isolate were transferred into sterile TSB medium and incubated at 30°C for approximately 24 hrs with shaking at 150-180 rpm. The bacterial suspension was then adjusted to a density of 1×10^8 CFU/mL. Inoculum density was determined based on OD₆₀₀ and confirmed by serial dilution and colony counting on TSA medium. For the two-isolate consortium treatments, suspensions of each isolate at a density of 1×10^8 CFU/mL were mixed at a 1:1 ratio, whereas the three-isolate consortium was prepared at a 1:1:1 ratio. Mixing was

performed aseptically immediately before application. The inoculum was applied to the root zone by watering the growing medium around the base of the stem. The first application was carried out at 7 days after transplanting at a dose of 20 mL per plant, while the second application was conducted at the early flowering stage at a dose of 40 mL per plant¹². Control plants received a sterile carrier solution at the same volume and application time without bacterial addition. All plants were maintained under the same greenhouse conditions until the fruits reached a uniform red-ripe stage for antioxidant activity analysis and phytochemical profiling.

Compatibility test of bacterial isolates: Before being applied as consortia, the bacterial isolates were tested for their compatibility *in vitro*. The biological agent isolates were grown on solid TSA medium. One loopful of an isolate was streaked onto a Petri dish and incubated for 24 hrs. Subsequently, the suspension of another isolate was applied onto a sterile filter paper measuring 1 × 1 cm and placed on the surface of the medium previously grown with the first isolate. The Petri dishes were then incubated for 48 hrs¹². The isolates were considered compatible when no clear zone was formed around the filter paper. Conversely, the formation of a clear zone indicated antagonistic activity between isolates.

Harvesting and preparation of tomato fruit samples: Muna local tomato (*Solanum lycopersicum* L.) fruits were harvested from plants treated with biological agents at a uniform red-ripe stage. The fruits used were healthy, free from disease symptoms, mechanically undamaged, uncracked, non-rotten and relatively uniform in size, shape and color¹³. After harvest, tomato fruits from each treatment and replication were divided into two sample groups. The first group was processed immediately under fresh conditions for antioxidant activity analysis and LC-MS/MS-based phytochemical profiling. The second group was stored for one week to evaluate changes in the phytochemical compound profile during postharvest storage. Samples from each treatment and replication were labeled according to treatment code, cleaned with sterile distilled water, air-dried at room temperature and weighed to determine their initial weight. For antioxidant activity analysis and phytochemical profiling, fruits from the same experimental unit were cut using a sterile knife and homogenized to obtain a representative composite sample. During preparation, the samples were protected from direct light and excessive heat to prevent the degradation of bioactive compounds. The homogenized samples were used for methanol extract preparation. When extraction was not performed immediately, the samples were stored in closed sterile containers at -20°C for short-term storage or at -80°C before LC-MS/MS analysis.

Preparation of tomato fruit extract: Tomato fruit extract was prepared using the maceration method. Muna local tomato (*Solanum lycopersicum* L.) fruits at the red-ripe stage were cleaned, air-dried, cut into small pieces and homogenized. A total of 100 g of tomato fruit homogenate from each sample group was placed in a closed maceration container and 750 mL of methanol was added at a sample-to-solvent ratio of 1:7.5 (w/v). Maceration was carried out for 3×24 hrs at room temperature, protected from light and with occasional stirring. The mixture was then filtered using Whatman No. 1 filter paper and the filtrate was concentrated using a rotary evaporator at approximately 40°C until a thick extract was obtained. The concentrated extract was transferred into dark-colored vial bottles, labeled according to treatment code and stored at -20°C until further analysis. For the DPPH assay, the extract was reconstituted in methanol to obtain a 1000 ppm stock solution and subsequently diluted into a concentration series of 25, 50, 75 and 100 ppm. For LC-MS/MS analysis, the extract was reconstituted in methanol or 80% methanol and filtered through a 0.22 µm membrane filter before injection into the LC-MS/MS system.

Antioxidant activity assay using the DPPH method: The antioxidant activity of tomato fruit extracts was evaluated using the DPPH free radical scavenging method. The DPPH solution was prepared by dissolving 5 mg of DPPH crystals in methanol to a final volume of 50 mL, resulting in a concentration of 100 mg/L. The stock solution of tomato fruit extract was prepared by dissolving 10 mg of extract in 10 mL of methanol to obtain a concentration of 1000 ppm. From this stock solution, a series of concentrations of 25, 50, 75 and 100 ppm was prepared. Vitamin C was used as a reference standard following the same procedure. The vitamin C stock solution was prepared at a concentration of 1000 ppm and then diluted to obtain concentration series of 25, 50, 75 and 100 ppm. The blank solution was prepared by mixing 1 mL of 100 mg/L DPPH solution with 3 mL of methanol and incubating it for 30 min at 37°C. The maximum wavelength was determined within the range of 400-800 nm and sample absorbance was measured at 517 nm. The extract assay was performed by mixing 1 mL of test solution from each concentration with 1 mL of 100 mg/L DPPH solution and 2 mL of methanol. The mixture was placed in a test tube, incubated for 30 min at 37°C and its absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Antioxidant activity was determined based on the color

change of DPPH from purple to yellow, indicating the ability of the extract to reduce DPPH free radicals. The percentage of inhibition was calculated using the following formula¹⁴:

$$\text{DPPH radical inhibition (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100\%$$

Phytochemical compound profile analysis: The phytochemical compound profile of Muna local tomato fruit extracts was analyzed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)¹⁵. This method was used to detect metabolite features based on m/z values, molecular mass, retention time, fragmentation patterns and agreement with databases or literature on tomato metabolites. A total of 1 mg of concentrated extract was dissolved in 1 mL of HPLC-grade methanol. The solution was then homogenized using a vortex, sonicated for 10 min and centrifuged at 10,000 rpm for 10 min at 4°C. The resulting supernatant was filtered through a 0.22 µm membrane filter and transferred into an LC-MS/MS vial for analysis. A 10 µL aliquot of the sample was injected into the LC-MS/MS system using a C18 column measuring 2×150 mm at a flow rate of 0.3 mL/min. Compound separation was performed using two mobile phases: Mobile phase A consisted of ultrapure water containing 0.1% formic acid, while mobile phase B consisted of methanol containing 0.1% formic acid. The elution system was run in gradient mode, with mobile phase B increased from 5% to 95%, followed by a return to the initial condition for column re-equilibration. The column temperature was maintained at 30-40°C. Mass detection was performed using an electrospray ionization source in positive and/or negative ion mode over an m/z scanning range of 50-1,200. Data acquisition was performed in full-scan and MS/MS modes to obtain information on molecular mass and compound fragmentation patterns. Metabolite annotation was performed based on the observed accurate m/z values, retention times, inferred ion or adduct types, MS/MS fragmentation information and comparison with available databases. Features that could not be confidently matched with reference spectra or authentic standards were not reported as confirmed compounds. Instead, these features were reported as unidentified LC-MS/MS features

Data analysis: Quantitative data, including DPPH inhibition percentages and IC₅₀ values, were analyzed using One-way Analysis of Variance (ANOVA). When significant differences were detected at the 95% confidence level, Duncan's Multiple Range Test (DMRT) was performed at α = 0.05.

RESULTS

Compatibility of endophytic and rhizosphere bacterial isolates:

The compatibility test of bacterial isolates was conducted to determine the possible antagonistic activity among isolates before their use in consortium treatments. This test was intended to determine whether antagonistic interactions occurred among the bacterial isolates to be combined. The results of the compatibility test of endophytic and rhizosphere bacterial isolates are presented in Table 1.

Table 1 shows that, in the compatibility test of endophytic and rhizosphere bacterial isolates, all tested isolate combinations did not form clear zones on the test medium. The absence of clear zones indicated that the isolates did not inhibit each other's growth *in vitro*. Therefore, the combinations of *Bacillus* sp. CKD061+*Pseudomonas* sp. SWRII B02, *Pseudomonas* sp. SWRII B02+*Pseudomonas* sp. Ke03, *Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03 and the three-isolate combination of *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03 were considered compatible.

Antioxidant activity of Muna local tomato fruit extracts: The antioxidant activity of Muna local tomato fruit extracts was analyzed based on the percentage of DPPH radical inhibition and IC_{50} values, as presented in Table 2.

The results showed that biological agent treatments produced different responses in the antioxidant activity of tomato fruits. Based on the percentage of DPPH inhibition, the combination of *Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03 produced the highest value of 18.02%, followed by the combination of *Pseudomonas* sp. SWRII B02+*Pseudomonas* sp. Ke03, with a value of 17.77%. The uninoculated control produced the lowest inhibition value of 12.20%.

Based on the IC_{50} value, the single-isolate treatment of *Bacillus* sp. CKD061 showed the strongest antioxidant activity, with an IC_{50} value of 41.15 ppm. The combination of *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061 showed an IC value of 47.14 ppm and was the best consortium treatment based on the IC_{50} parameter. In contrast, the control and *Pseudomonas* sp. Ke03 treatments showed high IC_{50} values of 469.05 and 372.65 ppm, respectively, indicating weaker antioxidant activity than the other biological agent treatments.

These results indicate that the increase in DPPH inhibition percentage was not always directly associated with a decrease in IC_{50} value. The combination treatment of *Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03 produced the highest inhibition percentage, but its IC_{50} value was higher than that

of the single-isolate treatment of *Bacillus* sp. CKD061. Overall, *Bacillus* sp. CKD061 showed an important contribution to enhancing the antioxidant activity of Muna local tomato fruits, both as a single isolate and in combination with *Pseudomonas* sp. SWRII B02.

Phytochemical compound profiles in Muna local tomato

fruits: LC-MS/MS analysis was performed to determine the phytochemical compound profiles of Muna local tomato fruit extracts treated with biological agents. The data are presented under two conditions: Fresh fruit and fruit after one week of storage. The phytochemical compounds identified in fresh Muna local tomato fruit extracts based on LC-MS/MS analysis are presented in Table 3.

The LC-MS/MS results presented in Table 3 showed the presence of several phytochemical compounds in the control, single-isolate and bacterial consortium treatments. Several compounds were repeatedly detected in almost all treatments, particularly fucosylthreonine, hupeheninoside and triacetoxypiperidinone, with neutral masses of 265.11615 Da, 577.39785 Da and 273.08485 Da, respectively. In addition to the compounds commonly detected across nearly all treatments, several metabolites were observed only in specific treatments. Solasodine glucoside was detected in the control and *Bacillus* sp. CKD061 treatments, with a neutral mass of 575.38220 Da. 2-Hbhm was detected in the *Bacillus* sp. CKD061 and *Pseudomonas* sp. Ke03 treatments, with a neutral mass of 393.36068 Da. Meanwhile, tomatidine was detected in the combined treatment of *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061, with a neutral mass of 415.34503 Da.

In addition to these metabolites, one unidentified phytochemical LC-MS feature was detected only in the A0 treatment. This feature was tentatively assigned the molecular formula $C_8H_{19}NO_9$ and exhibited an observed m/z of 274.1126, a neutral mass of 273.10598 Da, a retention time of 1.36 min, a detector count of 86,318 and a mass error of -0.7 mDa. The mass difference between the observed m/z and the neutral mass was consistent with the formation of a protonated ion, $[M+H]^+$. However, because this feature could not be definitively confirmed through MS/MS fragmentation matching or comparison with an authentic reference standard, it was classified as an unidentified LC-MS feature rather than a confirmed compound.

After characterizing the phytochemical profile of fresh tomato fruits, postharvest changes in metabolite composition were further evaluated by storing tomato fruits from each treatment for one week. LC-MS/MS analysis showed clear differences between the phytochemical profiles of fresh and stored fruits, as presented in Table 4.

Table 1: Compatibility test results of endophytic and rhizosphere bacterial isolates

Isolate combination	Compatibility test result	Interpretation
<i>Pseudomonas</i> sp. SWRII B02+ <i>Pseudomonas</i> sp. Ke03	No clear zone formed	Compatible
<i>Pseudomonas</i> sp. SWRII B02+ <i>Bacillus</i> sp. CKD061	No clear zone formed	Compatible
<i>Bacillus</i> sp. CKD061+ <i>Pseudomonas</i> sp. Ke03	No clear zone formed	Compatible
<i>Pseudomonas</i> sp. SWRII B02+ <i>Bacillus</i> sp. CKD061+ <i>Pseudomonas</i> sp. Ke03.	No clear zone formed	Compatible

Table 2: Antioxidant activity of Muna local tomato fruit extracts under different biological agent treatments

Treatment	Biological agent	Mean DPPH inhibition (%)	IC ₅₀ (ppm)	Antioxidant activity
A0	Control	12.20 ± 1.39 ^b	469.05 ± 166.11 ^a	Very weak
A1	<i>Pseudomonas</i> sp. SWRII B02	15.54 ± 3.94 ^a	50.22 ± 47.07 ^b	Strong
A2	<i>Bacillus</i> sp. CKD061	15.06 ± 2.26 ^a	41.15 ± 20.37 ^b	Very strong
A3	<i>Pseudomonas</i> sp. Ke03	13.33 ± 2.68 ^{ab}	372.65 ± 80.83 ^a	Very weak
A4	<i>Pseudomonas</i> sp. SWRII B02+ <i>Bacillus</i> sp. CKD061	13.34 ± 2.75 ^{ab}	47.14 ± 1.92 ^b	Very strong
A5	<i>Pseudomonas</i> sp. SWRII B02+ <i>Pseudomonas</i> sp. Ke03	17.77 ± 7.82 ^a	62.74 ± 17.83 ^b	Strong
A6	<i>Bacillus</i> sp. CKD061+ <i>Pseudomonas</i> sp. Ke03	18.02 ± 6.85 ^a	74.87 ± 10.34 ^b	Strong
A7	<i>Pseudomonas</i> sp. SWRII B02+ <i>Bacillus</i> sp. CKD061+ <i>Pseudomonas</i> sp. Ke03	15.47 ± 5.78 ^a	51.31 ± 15.61 ^b	Strong

Values are presented as Mean ± standard deviation from three replications (n = 3). Different lowercase letters in the same column indicate significant differences based on DMRT at $\alpha = 0.05$. Antioxidant activity was classified based on IC₅₀ values: IC₅₀ < 50 ppm = Very strong, 50-100 ppm = Strong, 100-150 ppm = Moderate, 150-200 ppm = Weak and >200 ppm = Very weak

Table 3: Phytochemical compounds in fresh Muna local tomato fruit extracts

Compound	Formula	m/z	Molecular mass (Da)	Treatment
Fucosylthreonine	C ₁₀ H ₁₉ NO ₇	266.1233-266.1236	265.11615	A0-A7
Hupeheninoside	C ₃₃ H ₅₅ NO ₇	578.4049-578.4057	577.39785	A0-A7
Triacetoxypiperidinone	C ₁₁ H ₁₅ NO ₇	274.0919-274.0924	273.08485	A0-A7
Solasodine glucoside	C ₃₃ H ₅₃ NO ₇	576.3899-576.3903	575.38220	A0, A2
2-Hbhm	C ₂₅ H ₄₇ NO ₂	416.3511-416.3519	393.36068	A2, A3
Tomatidine	C ₂₇ H ₄₅ NO ₂	416.3522	415.34503	A4
Unidentified LC-MS feature	C ₈ H ₁₈ NO ₉	274.1126	273.10598	A0

A0: Control, A1: *Pseudomonas* sp. SWRII B02, A2: *Bacillus* sp. CKD061, A3: *Pseudomonas* sp. Ke03, A4: *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061, A5: *Pseudomonas* sp. SWRII B02+*Pseudomonas* sp. Ke03, A6: *Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03, A7: *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03. 2-Hbhm = 2-hexadecylamino-2,3-bis (hydroxymethyl)norbornene. Unidentified LC-MS feature indicates a detected LC-MS signal that could not be confidently assigned to a specific compound based on the available analytical evidence

Table 4: Phytochemical compounds in Muna local tomato fruit extracts after one week of storage

Compound	Formula	m/z	Molecular mass (Da)	Treatment
Fucosylthreonine	C ₁₀ H ₁₉ NO ₇	266.1232-266.1237	265.11615	A0-A7
Hupeheninoside	C ₃₃ H ₅₅ NO ₇	578.4050-578.4058	577.39785	A0-A7
N-fructosylvaline	C ₁₁ H ₂₁ NO ₇	280.1386-280.1391	279.13180	A0, A2, A3, A4, A5, A6, A7
N-succinyl-2-amino-6-oxo-L-pimelic acid	C ₁₁ H ₁₅ NO ₇	274.0919-278.0879	273.08485/	A0-A7
Triacetoxypiperidinone	C ₁₁ H ₁₅ NO ₇	274.0921	273.08485	A0
Acryloyl glucosamine	C ₉ H ₁₅ NO ₆	234.0969	233.08994	A1
Tetradamine	C ₁₀ H ₁₅ NO ₈	278.0876-278.0878	277.07977	A2, A3
Epivolkenin	C ₁₂ H ₁₇ NO ₇	288.1075-288.1078	287.10050	A5, A6, A7

A0: Control, A1: *Pseudomonas* sp. SWRII B02; A2: *Bacillus* sp. CKD061, A3: *Pseudomonas* sp. Ke03; A4: *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061, A5: *Pseudomonas* sp. SWRII B02+*Pseudomonas* sp. Ke03, A6: *Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03, A7: *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03

Table 4 shows that, after one week of storage, the phytochemical profile contained several active compounds with prominent spectra in tomato fruit extracts, including N-fructosylvaline, hupeheninoside, tetradamine, N-succinyl-2-amino-6-oxo-L-pimelic acid, fucosylthreonine and epivolkenin. N-succinyl-2-amino-6-oxo-L-pimelic acid and N-fructosylvaline were identified as compounds metabolized during storage, with molecular masses of 273.08485 and 279.13180 Da, respectively. Tetradamine, with a molecular mass of 277.07977 Da, was detected in the *Bacillus* sp. CKD061 and *Pseudomonas* sp. Ke03 treatments. Meanwhile, epivolkenin, with a molecular

mass of 287.10050 Da, was detected in the consortium treatments of *Pseudomonas* sp. SWRII B02+*Pseudomonas* sp. Ke03, *Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03 and *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061+*Pseudomonas* sp. Ke03.

DISCUSSION

The results of this study showed that the application of endophytic and rhizosphere bacteria modulated the antioxidant capacity and phytochemical profile of Muna local

tomato fruits. This was reflected in differences in DPPH inhibition values, IC_{50} values and the distribution of phytochemical compounds among treatments. In general, most bacterial treatments produced stronger antioxidant activity than the control, although the responses varied between single isolates and consortia. These findings indicate that plant-microbe interactions are not only associated with plant growth but may also influence fruit biochemical quality, particularly through changes in antioxidant capacity and secondary metabolite profiles¹⁶.

The absence of inhibition zones in all isolate combinations indicated that *Pseudomonas* sp. SWRII B02, *Bacillus* sp. CKD061 and *Pseudomonas* sp. Ke03 were compatible *in vitro*. This compatibility provides an important basis for consortium application because the success of multistrain bioinoculants depends on the ability of each isolate to coexist, maintain viability and perform its biological functions without inhibiting other consortium members¹⁷. Therefore, the isolate combinations used in this study have initial feasibility for development as endophytic-rhizosphere bacterial consortia in Muna local tomato.

Among all treatments, *Bacillus* sp. CKD061 showed the strongest antioxidant performance, as indicated by the lowest IC_{50} value of 41.15 ppm. Meanwhile, the consortium of *Pseudomonas* sp. SWRII B02 + *Bacillus* sp. CKD061 produced an IC_{50} value of 47.14 ppm and was the best consortium treatment. In contrast, the control and *Pseudomonas* sp. Ke03 treatments showed higher IC_{50} values, indicating relatively weaker antioxidant activity. These results suggest that *Bacillus* sp. CKD061 played an important role in enhancing the antioxidant capacity of Muna local tomato fruits, both as a single isolate and in combination with *Pseudomonas* sp. SWRII B02.

The strong effect of *Bacillus* sp. CKD061 may be related to the ability of *Bacillus* spp. to influence plant physiology. These bacteria are known to produce bioactive metabolites, enzymes, phytohormones and volatile compounds, as well as to induce systemic resistance in plants¹⁸. Activation of these physiological responses may promote changes in secondary metabolism related to the formation of antioxidant compounds. In tomato fruits, antioxidant activity is commonly associated with bioactive compounds such as carotenoids, lycopene, β -carotene, phenolics, flavonoids, vitamin C and certain alkaloids^{19,20}. Therefore, the low IC_{50} value observed in the *Bacillus* sp. CKD061 treatment may indicate that this isolate stimulated biochemical responses that supported increased fruit antioxidant capacity.

Nevertheless, the increase in DPPH inhibition percentage was not always parallel to a decrease in IC_{50} value. The *Bacillus* sp. CKD061 + *Pseudomonas* sp. Ke03 treatment produced the highest DPPH inhibition percentage but not the lowest IC_{50} value. Conversely, *Bacillus* sp. CKD061 produced the lowest IC_{50} value, although its inhibition percentage was not the highest. This difference indicates that antioxidant activity should not be interpreted solely based on inhibition percentage at a single concentration²¹. The IC_{50} value is more representative because it reflects the extract concentration required to scavenge 50% of DPPH free radicals. Thus, in this study, IC_{50} was used as the main indicator of antioxidant strength, while inhibition percentage served as supporting data.

The response pattern of the consortia also showed that increasing the number of isolates did not necessarily result in a stronger biological effect²². The three-isolate consortium showed strong antioxidant activity but did not exceed the single isolate *Bacillus* sp. CKD061 or the two-isolate consortium *Pseudomonas* sp. SWRII B02 + *Bacillus* sp. CKD061. This indicates that consortium effectiveness is determined more by functional compatibility, interaction stability, colonization ability and minimal competition among isolates than by the number of strains used. Therefore, the design of microbial consortia should be based on actual plant responses and biological compatibility rather than compositional complexity alone^{23,24}.

The LC-MS/MS analysis provided further insight into possible metabolite changes associated with antioxidant activity. In fresh fruits, *Fucosyl threonine*, *hupeheninoside* and *Triacetyl piperidinone* were detected in all treatments. The presence of these compounds in both the control and bacterial treatments suggests that they may be part of the basal metabolite profile of Muna local tomato. However, several compounds appeared specifically in certain treatments, such as solasodine glucoside in the *Bacillus* sp. CKD061 treatment and tomatidine in the consortium of *Pseudomonas* sp. SWRII B02 + *Bacillus* sp. CKD061. This pattern indicates that bacterial inoculation can modulate the distribution of phytochemical compounds, although some basal metabolites are maintained by the plant²⁵.

The detection of tomatidine in the *Pseudomonas* sp. SWRII B02 + *Bacillus* sp. CKD061 treatment is one of the important findings of this study. Tomatidine is the aglycone of tomatine, a steroidal glycoalkaloid naturally found in tomato plants and associated with plant defense systems²⁶. Its presence in the consortium treatment, which also showed a

low IC_{50} value, provides preliminary evidence that the *Pseudomonas-Bacillus* combination is associated with the modulation of secondary metabolism, particularly pathways related to plant defense compounds. Thus, this finding opens opportunities for further research on the relationship among bacterial inoculation, glycoalkaloid biosynthesis and antioxidant capacity in tomato fruit.

In addition to tomatidine, the detection of solasodine glucoside in the *Bacillus* sp. CKD061 treatment further supports the possibility that this isolate may influence alkaloid metabolism or plant defense-related compounds. Because the *Bacillus* sp. CKD061 treatment had the lowest IC_{50} value, solasodine glucoside may serve as a relevant metabolic marker for further investigation. However, as with tomatidine, a direct relationship between this compound and antioxidant activity cannot be concluded without targeted quantitative analysis. Therefore, the LC-MS/MS results in this study should be interpreted as an exploratory basis for identifying candidate metabolites that may contribute to enhanced antioxidant activity.

Changes in compound profiles after one week of storage provide an additional dimension for understanding the effects of microbial treatments. Compounds such as N-fructosylvaline, N-succinyl-2-amino-6-oxo-L-pimelic acid, tetradamine and epivolkenin were detected in specific treatments after storage. These changes indicate that tomato fruits continue to undergo postharvest metabolic dynamics, including further ripening, metabolite transformation, degradation of primary compounds and responses to oxidative stress during storage²⁷. Thus, preharvest bacterial treatment may leave residual physiological effects that influence metabolite composition after harvest.

This postharvest aspect is important because most studies on PGPB in tomato have focused mainly on plant growth, fruit production or the content of specific compounds at harvest²⁸⁻³⁰. The present study expands the analytical scope by showing that bacterial treatments are also associated with changes in metabolite profiles after storage. This is relevant to the development of tomato as a functional food because fruit bioactive quality is determined not only at harvest but also during distribution, storage and consumption. Therefore, microbial effects on postharvest metabolite stability or transformation deserve further investigation.

Overall, this study demonstrated that endophytic and rhizosphere bacteria can modulate the antioxidant activity and phytochemical profile of Muna local tomato fruits. *Bacillus* sp. CKD061 emerged as the most effective isolate based on IC_{50} values, whereas the consortium of

Pseudomonas sp. SWRII B02+*Bacillus* sp. CKD061 was the most promising bacterial combination because it produced strong antioxidant activity and a distinctive metabolite profile characterized by tomatidine. The main novelty of this study lies in the evidence that endophytic-rhizosphere bioinoculants can be directed not only to support plant growth but also to enhance the functional quality of local fruits. These findings provide a scientific basis for developing microbial consortia as a natural and sustainable biofortification strategy for Muna local tomato.

CONCLUSION

This study demonstrated that the application of endophytic and rhizosphere bacteria can modulate the antioxidant capacity and phytochemical profile of Muna local tomato fruits. *Bacillus* sp. CKD061 was the most effective isolate, as indicated by the lowest IC_{50} value of 41.15 ppm, whereas the consortium of *Pseudomonas* sp. SWRII B02 and *Bacillus* sp. CKD061 was the most promising bacterial combination, with an IC_{50} value of 47.14 ppm. LC-MS/MS analysis indicated that bacterial treatments influenced the distribution of putatively identified phytochemical compounds, including solasodine glucoside in the *Bacillus* sp. CKD061 treatment and tomatidine in the *Pseudomonas* sp. SWRII B02+*Bacillus* sp. CKD061 consortium. Changes in metabolite profiles after one week of storage further suggest that microbial treatments may affect postharvest biochemical dynamics. These findings provide preliminary evidence for the development of bioinoculants to improve the functional quality of Muna local tomato fruits; however, further validation under field conditions is required.

SIGNIFICANCE STATEMENT

The present research highlights the potential use of beneficial endophytic and rhizosphere bacteria as natural bioinoculants to improve the functional quality of Muna local tomato (*Solanum lycopersicum* L.). By integrating antioxidant activity assays with LC-MS/MS-based phytochemical profiling, this research provides evidence that bacterial treatments can influence antioxidant capacity and metabolite composition in tomato fruits. Among the tested isolates, *Bacillus* sp. CKD061 showed the most prominent effect, producing the lowest IC_{50} value of 41.15 ppm, whereas the consortium of *Pseudomonas* sp. SWRII B02 and *Bacillus* sp. CKD061 emerged as the most promising bacterial combination. The detection of treatment-

specific compounds, including solasodine glucoside and tomatidine, further supports the role of microbial inoculation in modulating fruit phytochemical profiles. These findings provide a scientific basis for developing sustainable microbial-based strategies to enhance the functional value of horticultural crops.

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