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Potential of *Pseudarthrobacter chlorophenolicus* BF2P4-5 as a Biofertilizer for the Growth Promotion of Tomato Plants

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Abstract: BF2P4-5 was isolated from the rhizosphere soil of tomato plants, and its potential to promote plant growth was investigated in tomato plants. An in vitro test revealed that the strain could fix nitrogen, solubilize phosphate and potassium, and synthesize indole acetic acid. The bacterial strain was identified and characterized as a kind of *Pseudarthrobacter chlorophenolicus* based on the analysis of culture characteristics, physiological and biochemical characteristics, and 16S rRNA gene sequence (GenBank accession number OP135548.1). pH 7.0, 15% NaCl, and 35 °C temperature were ideal for optimal strain growth under culture conditions. Tomato plants grown on a cocopeat substrate were inoculated with BF2P4-5 suspension (OD_{600} 2.0). Positive control plants were inoculated with Nitrogen Phosphorus Potassium (NPK) fertilizer. This BF2P4-5 strain and NPK treatments were complemented with a negative control, in which only tap water was applied to tomato roots, thus, establishing three distinct treatment modalities with five replications each. Two months of greenhouse trials of inoculated tomato plants improved growth parameters. Interestingly, in most of the growth metrics evaluated, tomato plants treated with strain BF2P4-5 showed little to no variation with NPK fertilizer treatment, including plant height, stem length, girth, leaf number per plant, and chlorophyll content, when compared to uninoculated plants. Furthermore, the conditions for the cocopeat plants, including pH, EC, and moisture, were within acceptable limits. Furthermore, inoculation with BF2P4-5 increased the nitrogen, phosphorus, and potassium content available in the cocopeat medium. The results showed that the strain exhibited traits for the promotion of plant growth and could be deployed as an eco-friendly microbial biofertilizer for tomatoes and probably other essential crops.

Keywords: *Pseudarthrobacter chlorophenolicus*; NPK fertilizer; microbial biofertilizer; cocopeat; tomatoes

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1. Introduction

Modern intensive agriculture production over the years has relied heavily on chemical fertilizers for high-yield production to feed the growing population. Nonetheless, chemical fertilizers have become abundant and are used indiscriminately in agriculture to increase crop production. However, unfortunately, it is considered a non-sustainable approach that negatively impacts soil, the environment, and human health [1]. The extensive use of fertilizers and pesticides not only destroys beneficial microbes in the soil but also disrupts agroecology. Deterioration of soil productivity leads to impaired beneficial microbial diversity and can threaten ecosystem function and environmental quality. Using conventional and environmentally friendly agricultural practices is vital for sustainable agricultural development and food security [2]. Plant-growth-promoting bacteria (PGPB) are bacteria that

colonize the rhizospheres of plants that enhance plant growth through various mechanisms, such as nitrogen fixation, phosphate solubilization, potassium quorum sensing, etc. [3]. PGPB offers multiple ways to replace chemical fertilizers, pesticides, etc. Therefore, this quality has led to their significant increased demand and has shown considerable promise in achieving the goal of sustainability.

Tomatoes (*Lycopersicum esculentum*), a member of the nightshade family Solanaceae, are primary crop plants and a model system for fruit development that can be grown in various geographical zones in open fields or under greenhouse conditions. They are considered one of the world's most well-known and extensively cultivated horticultural crop vegetables [4]. Due to increasing demand, tomatoes have excellent potential for increased commercialization [5]. Tomatoes are high in vitamins and minerals. They have become a valuable source of lycopene, a potent antioxidant with anticarcinogenic properties. They also provide the diet with vitamins (A, B, and C), minerals, potassium, iron, and calcium [6]. The tomato plant requires a considerable amount of nitrogen, phosphorus, and potassium during its growth to maintain good growth and high yield and this is supplied mainly through inorganic chemical fertilizers. To avoid the residual toxicity of chemical fertilizers, biofertilizer-microbial inoculants, which can promote plant growth and productivity, are accepted worldwide as an alternative eco-friendly NPK fertilizer [7].

The genus *Arthrobacter* is a group of extensive pleomorphic bacteria that includes many species that are widespread in nature [2]. As a result of their metabolic versatility, they are reported to exist in a diverse range of environments, such as soils, aerial surfaces of plants, phyllosphere, and ubiquitous origin wastewater sediments, with influential roles in agriculture [8]. These bacteria can effectively use organic and inorganic compounds as a metabolism substrate, acting as a tool for bioremediation in agriculture [8]. Recent classification based on 16S rRNA phylogeny revealed that the genus *Arthrobacter* was dissected into eleven main groups, such as *Arthrobacter aurescens*, *Arthrobacter globiformis*, *Arthrobacter oryzae*, *Arthrobacter pascens*, *Arthrobacter humicola*, *Arthrobacter oxydans*, *Arthrobacter protophormiae*, *Arthrobacter sulfureus*, *Arthrobacter citreus*, *Arthrobacter agilis*, *Arthrobacter psychrolactophilus*, *Arthrobacter pigmenti*, *Arthrobacter albuscumminsii*, and *Arthrobacter soli* [8].

Certain *Arthrobacter* species, predominantly soil-dwelling rhizobacteria, have been identified as plant growth promoters due to their multiple growth-promoting activities, such as nitrogen fixation, phosphate, and potassium solubilization, as well as Indole acetic acid synthesis [2,9–12]. Members of the genus *Arthrobacter* are very significant as they are the most extensively isolated bacteria and mostly from soils and wastewater [13]. Currently, more than 70 identified species of Arthrobacter with validly published names have been isolated from various sources, particularly from the rhizosphere of plants, including *A. cupresi* [14], *A. nicotinovorans* [9], *A. chlorophenolicus* [15,16], *A. oryzae* [17], *A. pokkalii* [11] and *A. nitroguajacolicus* [18], and *Arthrobacter ginkgonis* [19]. The association of various strains of *Arthrobacter* with different plants reveals beneficial implications for plant growth and yield. In plants grown in saline, drought-prone, and low-nutritive agricultural soils, *Arthrobacter* species have played essential roles in protecting plants from abiotic stresses and have also helped to improve plant health and yield, thus, proving themselves as a notable member of the rhizosphere microflora [2,11].

As a result, the current study sought to detect nitrogen-fixing, potassium- and phosphate-solubilizing *Pseudarthrobacter chlorophenolicus* BF2P4-5 isolated from the rhizosphere of tomato plants and to evaluate its potential to promote plant growth in tomato plants.

2. Results

2.1. Morphological and Biochemical Identification of the Bacterium Strain BF2P4-5

The colony of strain BF2P4-5 was circular, with raised elevation, the margin being entire, punctiform in size, cream pigmentation, smooth texture, glistening surface, and translucent. Under the transmission electron microscope, the cells of strain BF2P4-5 were found to be Staphylococci in shape and Gram-stain positive (Table 1). In addition, Table 1 outlines the major biochemical characteristics of the bacterial strain BF2P4-5. This strain

is positive for oxidase test, catalase, citrate utilization, starch hydrolysis, motility, methyl red, Voges–Proskauer (VP), and nitrate reduction and can utilize glucose as its sole carbon source. In addition to this, the strain is negative for hydrogen sulfide (H_2S), sucrose, lactose, and urease.

Table 1. Morphological and biochemical characteristics of strain BF2P4-5.

Characteristics	Results	Characteristics	Results
Colony and Cellular Morphology		Biochemical characteristics	
Form	Circular	Oxidase	Positive
Elevation	Raised	Catalase	Positive
Margin	Entire	Citrate Utilization	Positive
Size	Punctiform	Starch Hydrolysis	Positive
Pigmentation	Cream	Hydrogen Sulphide (H_2S)	Negative
Texture	Smooth	Motility	Positive
Surface	Glistening	Methyl red	Positive
Opacity	Translucent	Voges–Proskauer (VP.)	Positive
Gram reaction	Positive	Glucose	Positive
Cell shape	Staphylococci	Lactose	Negative
PGPR attributes		Sucrose	Negative
Nitrogen-fixing	+++	Urease	Negative
Phosphate solubilization	+++	Nitrate reduction	Positive
Potassium solubilization	KCL (−), K_2SO_4 (+), Mica (+), K_2PO_4 (−)	Indole Acetic Acid (IAA)	Positive

2.2. In Vitro Screening of Strain BF2P4-5 for Potential Plant Beneficial Traits

The results of the growth-promoting abilities of strain BF2P4-5 are shown in Table 1 and Figure 1. Bacterial strain BF2P4-5 can grow normally on a nitrogen-free medium, suggesting a potential capacity for nitrogen fixation. On Pikovskaya's (PVK) agar medium, BF2P4-5 formed a transparent zone around the inoculated area, indicating its ability to solubilize phosphate. Additionally, when an amendment of different potassium sources was made in Aleksandrov agar medium, strain BF2P4-5 formed a significant zone of potassium solubilization in media supplemented with KCl and mica powder but failed to solubilize the medium supplemented with K_2PO_4 and K_2SO_4. The formation of a cherry ring pink color when Sulfide Indole Motility (SIM) media were used indicates the bacteria's ability to produce IAA. However, no activity of hydrogen cyanide production was observed when the strain was inoculated in nutrient agar supplemented with glycine.

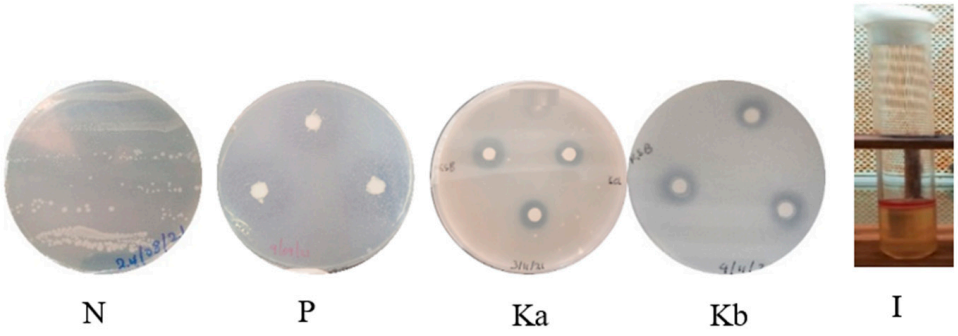

Figure 1. Screening of strain BF2P4-5 for potential plant beneficial traits. N—nitrogen fixation, P—phosphate solubilization, Ka—potassium solubilization (in KCL), Kb—phosphate solubilization (in Mica powder), and I—Indole Acetic Acid production.

2.3. Sequencing of 16S rRNA and Phylogenetic Analysis of Strain BF2P4-5

The 16S rRNA of strain BF2P4-5 was sequenced and deposited in the GenBank database to generate its accession number (OP135548.1), which shows 98.43% homology with the *Pseudarthrobacter chlorophenolicus* strain WK2 (KX214642.1), *Pseudarthrobac-*

ter chlorophenolicus strain IITA-TZ101 (OM909352.1), *Pseudarthrobacter equi* strain CH-33 (MH712960.1), and the *Pseudarthrobacter equi* strain NH21 (MF440330.1). The BF2P4-5 strain also showed 98.17% similarity to *Pseudarthrobacter phenanthrenivorans* strain HF-BPR41(MW092894.1) (Table 2). After comparative sequence analysis using BLAST tools in NCBI, analysis of the 16S rRNA sequence-based phylogenetic tree revealed that BF2P4-5 belonged to the family *Micrococcaceae*, genus *Pseudarthrobacter*, and grouped with *Pseudarthrobacter chlorophenolicus* and *Pseudarthrobacter equi* clade (Figure 2).

Table 2. Identification of strain BF2P4-5 using 16S rRNA gene sequence similarity in BLAST.

Bacterial Strain	Accession Number	% Coverage	% Identity
Pseudarthrobacter equi strain CH-33	MH712960.1	100	98.43
Pseudarthrobacter equi strain NH21	MF440330.1	100	98.43
Pseudarthrobacter chlorophenolicus strain WK2	KX214642.1	100	98.43
Pseudarthrobacter chlorophenolicus strain IITA-TZ101	OM909352.1	100	98.43
Pseudarthrobacter defluvii strain CNMUM20.6	MN004832.1	100	98.03
Pseudarthrobacter defluvii strain E182	MF681873.1	100	98.04
Pseudarthrobacter phenanthrenivorans strain HFBPR41	MW092894.1	100	98.17
Pseudarthrobacter phenanthrenivorans strain LMR746	MW559667.1	100	98.03
Pseudarthrobacter siccitolerans strain SMW-538	OM568656.1	100	98.03
Pseudarthrobacter siccitolerans strain TRM85691	OK299065.1	100	98.03
Paenarthrobacter ureafaciens strain AT63	MZ676045.1	100	98.03

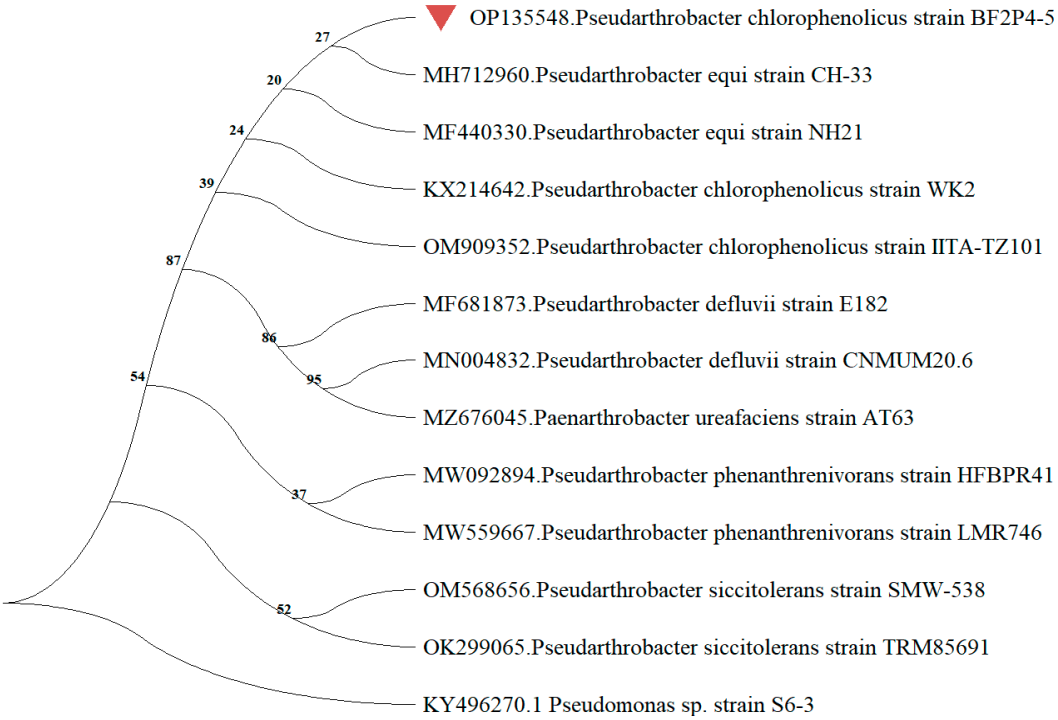

Figure 2. Phylogenetic tree based on 16S rRNA sequences showing the relationship between strain BF2P4-5 and the most closely related species of the genus *Pseudarthrobacter* using the neighbor-joining method. The sequence of *Pseudomonas* sp. Strain S6-3 was used as an outgroup. Bootstrap of 1000 replications was used, and the values are shown in the tree. Evolutionary analyses were conducted in MEGA11 [20].

2.4. Physiological Analysis of the Bacterial Strain BF2P4-5

The tested growth characteristics of pH, NaCl, and temperature ranges for strain BF2P4-5 are given in Table 3. Strain BF2P4-5 had a growth range of pH 4 to 9. Optimal growth was recorded at pH 7. As presented in Table 3, BF2P4-5 grew well at low to high

levels of NaCl (0% and 15% NaCl), while at 20% and 25% NaCl, weak growth was observed. The optimal sodium chloride concentration was 15% for the growth of strain BF2P4-5. When exposed to different temperature ranges, the optimal strain growth was at 35 °C.

Table 3. Growth characteristics of strain BF2P4-5.

Characteristics	Range of Growth	Optimum Growth
pH for growth	4 to 9	7
Growth at different NaCl concentrations (%)	0 to 20	15
Growth in different temperature ranges (°C)	20 to 45	35

2.5. Validation of BF2P4-5 Strain Inoculation in Tomato Plants under Greenhouse Conditions

The efficient phosphate, potassium-solubilizing, and nitrogen-fixing strain BF2P4-5 was evaluated for plant growth promotion activity for tomatoes under greenhouse conditions. The results are presented in Figure 1 and Table 4. The inoculation of the strain BF2P4-5 into the three tomato varieties significantly improved stem length, stem girth, number of leaves per plant, plant height, leaf length, leaf diameter, and chlorophyll content. The inoculated plants with the BF2P4-5 strain with the Anna F1, Eva F1, and Star9086 reached a height of 44.6 cm, 46.1 cm, and 43.1 cm, respectively. However, significant height differences were observed between the treated BF2P4-5 strain plants of the two varieties, Eva F1 and Star9086, and untreated plants compared to NPK-treated plants with a height of 44.3 cm, 32.4 cm, and 37.8 cm. The negative control recorded plant heights of 19.0 cm, 20.9 cm, and 23.0 cm, respectively (Table 3). Likewise, inoculation with BF2P4-5 significantly improved the number of leaves per plant, leaf length, and leaf diameter compared to the uninoculated (negative control) (Figure 3). The total chlorophyll content of single-truss tomato leaves was measured. Chlorophyll was measured from leaves at each level of the canopy (lower, middle, and top canopy). The content of chlorophyll was also incremented by microbial inoculation over uninoculated control. The stem girth was measured at two levels of each plant (5 cm above and 15 cm above). The positive effects of BF2P4-5 on growth among the three tomato varieties tested were observed in all parameters measured compared to uninoculated (negative control).

Table 4. Effect of *Arthrobacter chlorophenolicus* BF2P4-5 and NPK on growth attributes and phenology of tomato plants.

Treatments	Number of Leaves/Plants	Leave Length	Leave Diameter	Chlorophyll Content (SPAD Lower Canopy)	Chlorophyll Content (SPAD Mid Canopy)	Chlorophyll Content (SPAD Upper Canopy)	Plant Height at 55 DAS	Stem Length	Stem Girth (5 cm above)	Stem Girth (15 cm above)	LSD	F Value	CV%
NPK + Anna F1	$8.6 \pm 0.5^{abc}{}_{f}$	$22.1 \pm 1.3^{b}{}_{d}$	$14.8 \pm 0.8^{de}{}_{e}$	$31.5 \pm 1.1^{abcd}{}_{c}$	$36.9 \pm 1.4^{a}{}_{b}$	$37.2 \pm 1.9^{a}{}_{b}$	$44.3 \pm 2.8^{a}{}_{a}$	$24.4 \pm 1.9^{a}{}_{d}$	$0.5 \pm 0.03^{c}{}_{fg}$	$0.4 \pm 0.04^{c}{}_{g}$	4.11	118.6	14.6
NPK + Eva F1	$8.0 \pm 0.5^{c}{}_{d}$	$15.5 \pm 1.2^{c}{}_{c}$	$12.8 \pm 1.0^{e}{}_{c}$	$30.0 \pm 0.7^{bcd}{}_{b}$	$34.3 \pm 0.3^{ab}{}_{a}$	$34.7 \pm 0.9^{a}{}_{a}$	$32.4 \pm 2.3^{c}{}_{ab}$	$14.2 \pm 1.1^{c}{}_{c}$	$0.5 \pm 0.03^{c}{}_{e}$	$0.4 \pm 0.05^{c}{}_{e}$	2.95	173.7	12.60
NPK + Star9086	$8.2 \pm 0.4^{bc}{}_{e}$	$22.1 \pm 1.3^{b}{}_{c}$	$17.8 \pm 0.8^{bc}{}_{d}$	$33.3 \pm 2.1^{ab}{}_{b}$	$35.3 \pm 1.3^{ab}{}_{ab}$	$37.4 \pm 1.1^{a}{}_{a}$	$37.8 \pm 1.5^{bc}{}_{a}$	$19.6 \pm 0.7^{b}{}_{cd}$	$0.6 \pm 0.03^{b}{}_{f}$	$0.5 \pm 0.02^{bc}{}_{f}$	3.16	175.1	11.61
BF2P4-5 + AnnaF1	$8.6 \pm 0.4^{abc}{}_{f}$	$23.3 \pm 1.2^{b}{}_{d}$	$16.8 \pm 1.1^{cd}{}_{e}$	$32.1 \pm 0.9^{abc}{}_{c}$	$36.9 \pm 0.8^{a}{}_{b}$	$36.7 \pm 1.1^{a}{}_{b}$	$44.6 \pm 2.3^{a}{}_{a}$	$23.2 \pm 1.2^{a}{}_{d}$	$0.7 \pm 0.04^{a}{}_{g}$	$0.6 \pm 0.05^{ab}{}_{g}$	3.13	210.6	10.94
BF2P4-5 + Eva F1	$9.4 \pm 0.3^{ab}{}_{f}$	$27.2 \pm 0.8^{a}{}_{cd}$	$19.2 \pm 1.1^{ab}{}_{e}$	$29.8 \pm 0.9^{cd}{}_{c}$	$33.7 \pm 0.7^{b}{}_{b}$	$34.4 \pm 0.8^{ab}{}_{b}$	$46.1 \pm 2.3^{a}{}_{a}$	$24.6 \pm 1.2^{a}{}_{d}$	$0.6 \pm 0.03^{ab}{}_{g}$	$0.5 \pm 0.03^{ab}{}_{g}$	2.93	215.9	10.18
BF2P4-5 + Star9086	$9.8 \pm 0.4^{a}{}_{f}$	$27.8 \pm 0.7^{a}{}_{c}$	$20.3 \pm 0.7^{a}{}_{e}$	$33.8 \pm 1.2^{a}{}_{b}$	$36.2 \pm 0.9^{ab}{}_{b}$	$34.3 \pm 0.7^{ab}{}_{b}$	$43.4 \pm 1.8^{ab}{}_{a}$	$24.1 \pm 0.8^{a}{}_{d}$	$0.6 \pm 0.03^{ab}{}_{g}$	$0.6 \pm 0.03^{a}{}_{g}$	2.49	299.1	8.43
NC + Anna F1	$5.2 \pm 0.5^{d}{}_{d}$	$8.58 \pm 1.2^{d}{}_{c}$	$7.06 \pm 0.5^{f}{}_{cd}$	$25.9 \pm 1.8^{de}{}_{a}$	$28.6 \pm 1.2^{c}{}_{a}$	$28.5 \pm 1.8^{c}{}_{a}$	$19.0 \pm 1.9^{d}{}_{b}$	$9.20 \pm 0.7^{d}{}_{c}$	$0.3 \pm 0.03^{d}{}_{e}$	$0.2 \pm 0.03^{d}{}_{e}$	3.26	96.99	19.27
NC + Eva F1	$6.0 \pm 0.3^{d}{}_{e}$	$9.60 \pm 0.7^{d}{}_{d}$	$7.70 \pm 0.3^{f}{}_{de}$	$25.6 \pm 0.6^{e}{}_{b}$	$29.6 \pm 1.6^{c}{}_{a}$	$31.1 \pm 0.9^{bc}{}_{a}$	$20.9 \pm 1.2^{d}{}_{c}$	$8.6 \pm 0.4^{d}{}_{d}$	$0.3 \pm 0.02^{d}{}_{f}$	$0.2 \pm 0.02^{d}{}_{f}$	2.22	230.9	12.41
NC + Star9086	$5.0 \pm 0.6^{d}{}_{d}$	$10.6 \pm 1.1^{d}{}_{c}$	$8.60 \pm 0.3^{f}{}_{c}$	$28.1 \pm 0.8^{e}{}_{a}$	$28.6 \pm 1.2^{c}{}_{a}$	$29.1 \pm 1.1^{c}{}_{a}$	$23.0 \pm 2.4^{d}{}_{b}$	$8.4 \pm 0.6^{d}{}_{c}$	$0.3 \pm 0.03^{d}{}_{e}$	$0.2 \pm 0.03^{d}{}_{e}$	3.04	122.5	16.77
L.S.D $_{(0.05)}$	1.209	3.086	2.238	3.504	2.977	3.509	6.039	3.016	0.093	0.103			
F value	18.04	49.67	42.64	5.94	11.49	7.60	27.69	47.48	22.26	19.7			
p-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001			
CV%	12.333	12.979	12.559	9.099	6.964	8.113	13.596	13.541	14.114	20.36			

The mean values with the same superscript letter(s) within the same column are not significantly different by the Fisher test. In the Fisher test, the mean values with the same subscript letters within the same row are not significantly different. According to the Fisher test, different subscript and superscript letters indicate significantly different means within a column and the same row. NC = Negative control (without fertilization).

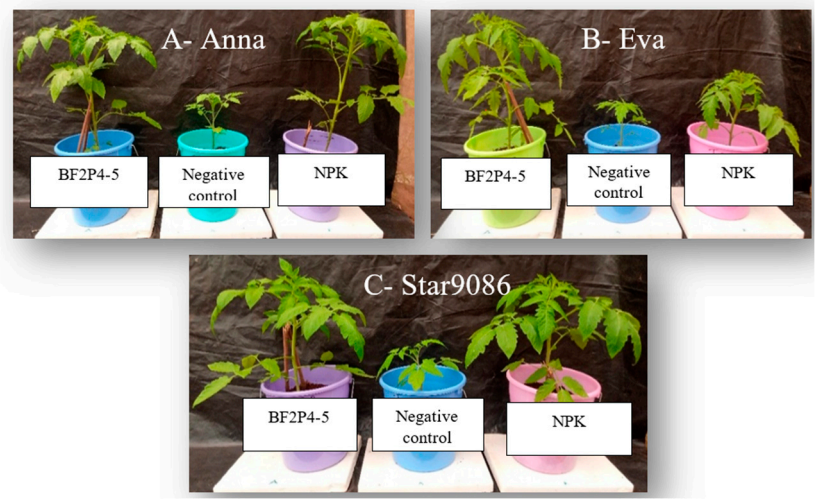

Figure 3. Plant tests on tomato growth promoted by bacterial strains BF2P4-5, NPK fertilizer, and a negative control after 55 days of growth for three varieties of tomato; Anna F1 Eva F1, and Star9086.

2.6. Analysis of Cocopeat Growing Medium

The results of pre-and post-treatment analysis on the cocopeat growing medium are presented in Table 5. The EC values differed between treatments, with the NPK treatment recording an elevated EC value (1.3 mS/cm^{-1}, 1.8 mS/cm^{-1} and 1.89 d mS/cm^{-1}) while the negative control treatment recorded the lowest EC values (0.21, 0.31 and 0.28 mS/cm^{-1}), respectively, compared to the initial value of 0.40 mS/cm^{-1}. The initial pH for cocopeat was higher (pH 7.19). The lowest recorded pH was that of Cocopeat + NPK+ Eva F1, but the values were eventually similar, ranging from (pH 5.96 to pH 6.31) at the end of the study. The pH of the treatments dropped slightly from the initial pH of 7.19 to pH 5.96 after 55 days of growth.

Table 5. Influence of NPK fertilizer and *Pseudarthrobacter chlorophenolicus* BF2P4-5 on pH, available nitrogen, available phosphorus (mg/kg), exchangeable potassium (K) (meq/100 g), and percentage moisture in cocopeat substrate before and after treatment.

Treatment	pH	Electrical Conductivity (EC) (mS/cm)	% Available Nitrogen	Available Phosphorus (mg/kg)	Exchangeable Potassium (meq/100 g)	% Moisture	L.S.D $_{(0.05)}$	F Value	*p*-Value	CV%
Pre-treatment										
Fresh Cocopeat media	7.19	0.40	0.12	3.00	0.77	27				
Post-treatment										
Cocopeat + NPK + Anna F1	$6.16 \pm 1.73^{a}{}_{cd}$	$1.33 \pm 0.08^{b}{}_{d}$	$1.21 \pm 0.28^{a}{}_{d}$	$3007.29 \pm 8.66^{c}{}_{a}$	$21.62 \pm 1.73^{a}{}_{b}$	$14.84 \pm 5.77^{d}{}_{cb}$	13.456	78,587.0	<0.0001	1.487
Cocopeat + NPK + Eva F1	$5.96 \pm 1.66^{a}{}_{c}$	$1.83 \pm 0.09^{a}{}_{c}$	$1.39 \pm 1.33^{a}{}_{c}$	$3148.80 \pm 6.65^{b}{}_{a}$	$21.98 \pm 1.66^{a}{}_{b}$	$20.09 \pm 5.78^{d}{}_{b}$	13.456	86,091.6	<0.0001	1.418
Cocopeat + NPK + Star9086	$6.28 \pm 1.73^{a}{}_{c}$	$1.89 \pm 0.07^{a}{}_{c}$	$1.38 \pm 0.82^{a}{}_{c}$	$3279.82 \pm 7.66^{a}{}_{a}$	$23.13 \pm 1.73^{a}{}_{b}$	$29.72 \pm 4.99^{dc}{}_{b}$	13.456	93,304.1	<0.0001	1.357
Cocopeat + BF2P4-5 + Anna F1	$6.21 \pm 1.73^{a}{}_{cd}$	$0.73 \pm 0.09^{c}{}_{d}$	$1.28 \pm 0.41^{ab}{}_{d}$	$1763.32 \pm 8.68^{d}{}_{a}$	$15.38 \pm 1.73^{b}{}_{c}$	$51.64 \pm 5.77^{ab}{}_{b}$	13.461	26,713.9	<0.0001	2.469
Cocopeat + BF2P4-5 + Eva F1	$6.07 \pm 1.75^{a}{}_{dc}$	$0.53 \pm 0.07^{dc}{}_{d}$	$1.29 \pm 0.40^{ab}{}_{dc}$	$1220.59 \pm 7.68^{f}{}_{a}$	$14.31 \pm 1.75^{b}{}_{c}$	$62.58 \pm 5.87^{a}{}_{b}$	13.461	12,682.1	<0.0001	3.479
Cocopeat + BF2P4-5 + Star9086	$6.30 \pm 1.72^{a}{}_{bc}$	$0.79 \pm 0.08^{c}{}_{c}$	$1.28 \pm 0.35^{ab}{}_{c}$	$1371.47 \pm 8.68^{e}{}_{a}$	$16.16 \pm 1.72^{b}{}_{b}$	$15.19 \pm 5.67^{d}{}_{b}$	13.461	16,240.9	<0.0001	3.217
Negative control + Anna F1	$6.5 \pm 1.74^{a}{}_{c}$	$0.21 \pm 0.09^{e}{}_{c}$	$0.02 \pm 0.01^{c}{}_{c}$	$711.21 \pm 6.98^{i}{}_{a}$	$0.22 \pm 1.74^{c}{}_{c}$	$20.24 \pm 4.77^{d}{}_{b}$	13.451	5490.97	<0.0001	6.094
Negative control + Eva F1	$6.31 \pm 1.67^{a}{}_{b}$	$0.31 \pm 0.06^{de}{}_{b}$	$0.07 \pm 0.00^{bc}{}_{b}$	$797.11 \pm 8.66^{h}{}_{a}$	$0.35 \pm 1.76^{c}{}_{b}$	$13.07 \pm 5.78^{d}{}_{b}$	13.451	5490.97	<0.0001	5.524
Negative control + Star9086	$6.06 \pm 1.80^{a}{}_{c}$	$0.28 \pm 0.07^{de}{}_{c}$	$0.02 \pm 0.35^{bc}{}_{c}$	$944.11 \pm 7.88^{g}{}_{a}$	$0.23 \pm 1.88^{c}{}_{c}$	$40.00 \pm 5.79^{bc}{}_{b}$	13.451	7637.63	<0.0001	4.557
L.S.D $_{(0.05)}$	5.146	0.262	0.8545	25.73	5.146	17.154				
F value	0.01	53.50	3.91	14851.7	18.40	9.59				
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001				
CV%	48.34	16.89	66.63	0.83	21.03	33.66				

The mean values with the same superscript letter(s) within the same column are not significantly different by the Fisher test. In the Fisher test, the mean values with the same subscript letters within the same row are not significantly different. According to the Fisher test, different subscript and superscript letters indicate significantly different means within a column and the same row.

3. Materials and Methods

3.1. Isolation of Bacterial Strain BF2P4-5 from Rhizosphere Soil

The bacterial strain BF2P4-5 was isolated from the rhizosphere soil of tomato plants in Juja, Kiambu county, Kenya. A standard serial dilution procedure was followed to isolate the bacterial strain BF2P4-5. About 1 g of the rhizospheric soil sample was weighed and then homogenized in a sterile test tube containing 9 mL of distilled water. The resulting rhizosphere soil suspension was then vigorously vortexed for 1 min. A four-fold serial dilution was carried out with the soil suspension from the rhizosphere and 0.1 mL aliquots of each dilution were spread and plated onto plates containing solid nutrient agar with a pH of 7.2. These plates were then incubated at 30 °C for 24 h and stored at 4 °C and −80 °C for further tests in a 20% glycerol suspension.

3.2. Morphological and Biochemical Observation of Bacterial Strain BF2P4-5

The morphological characteristics of the colony were observed as follows: form, elevation, margin, size, pigmentation, texture, surface, and opacity, following standard microbiological techniques [21]. Cellular morphology was observed by Gram staining and transmission electron microscope to determine the shape and Gram reaction (Gram positive or negative) of the bacterial cells using the standard classical Gram staining procedure described by [22]. Biochemical tests include oxidase, catalase, citrate utilization, starch hydrolysis, hydrogen sulfide production, Methyl Red-Voges-Proskauer Test (MR-VP), Triple sugar Iron, Urease Test, and nitrate reduction test after the staining process to identify the bacteria strain BF2P4-5, following a standard procedure described by [23].

3.3. In Vitro Screening of Strain BF2P4-5 for Potential Plant Beneficial Traits

Qualitative screening for biological nitrogen fixation of bacterial isolates was performed using nitrogen-free media (NFM) and incubated at 30 °C for three days. Nitrogen fixating potency was determined using the method described by Jiang et al. [9]. The phosphate solubilization ability of the bacterial strain was determined using Pikovskaya's agar medium. The size of the phosphate solubilizing area was determined using the method described by Chen and Liu [24]. An uninoculated medium served as a negative control. The potassium solubilization was analyzed as described by Bechtaoui et al. [25] with slight modifications. The qualitative analysis of potassium solubilization was carried out using Aleksandrov medium supplemented with four different types of minerals as substitutes for potassium sources, i.e., potassium chloride (KCl), potassium sulfate (K_2SO_4), potassium phosphate (K_3PO_4), and mica powder ($K_2O{\cdot}3AI_2O_3{\cdot}6SiO_2{\cdot}2H_2O$). A clear zone formed around the colonies due to the utilization of the potassium source being measured. Sulfur Indole Motility (SIM) media were used to screen the strain BF2P4-5 for IAA production [26]. The HCN production was carried out on modified nutrient agar supplemented with glycine (4.4 g/1 L) and then press on Whatman filter paper disc impregnated with 2.5% sodium carbonate in a 0.5% picric acid solution [27].

3.4. DNA Extraction, PCR Amplification, Sequencing, and Phylogenetic Analysis of 16S rRNA

Template DNA was prepared from a single colony of BF2P4-5 using a bacterial DNA extraction kit according to the manufacturer's instructions. Nucleic acid quantification was performed with a nanodrop spectrophotometer. Amplification of the 16S rRNA gene was carried out using universal primers (0.025 µM); 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT), with a size of 20 to 22 bp [28] in a final volume of 50 µL for the PCR reaction. The reaction mixture was composed of 2.0 µL of the purified bacterial DNA template in (901×10^{-6} µg), 25 µL quick master mix, 1.0 µL each of the universal forward and reverse primers, and 21 µL sterile double distilled water. The profile conditions used to program the PCR reaction were as follows: initial denaturation at (95 °C) for 5 min, final denaturation (95 °C) for 30 s, annealing (58 °C) for 30 s, extension (72 °C) for 1.5 min, and final extension at (72 °C) for 5 min for 35 cycles. PCR water and Genomic DNA from *E. coli* were used as negative and positive controls, respectively. Visualization

of the PCR products was performed under ultraviolet (UV) fluorescence on a 1% (*w*/*v*) agarose gel in 1X TAE buffer after staining with ethidium bromide [29]. A 1 kb base pair molecular ladder was used to determine the size of the amplified PCR product.

The 16S rRNA purified PCR product was subjected to sequencing by a commercial service provider (Macrogen Europe BV Inc., Amsterdam, The Netherlands). DNA sequences were generated in both directions. The obtained sequence was viewed and edited using chromaspro version 2.6.6 [30] and was then subjected to BLASTn [31] search for the determination of bacteria species. The percentages of sequence matching were analyzed and the sequence was submitted to NCBI (http://www.ncbi.nlm.nih.gov accessed on 30 August 2022) Gen Bank, and the accession numbers were generated and imported into and aligned by MUSCLE programming [32] based on the nearest neighbor joining. The 16S rRNA gene sequence with the highest percentage similarity from the BLAST results was selected and retrieved. Pairwise and multiple sequence alignment was performed and phylogenetic relatedness was constructed through the neighbor-joining method using MEGA11 [20]. Evolutionary distances were calculated using the maximum composite likelihood method. Gaps were treated by partial deletion and bootstrap analysis was performed for 1000 replications.

3.5. Physiological Analysis of the Bacterial Strain BF2P4-5

The growth of *Arthrobacter chlorophenolicus* BF2P4-5 was investigated in various pH ranges (4, 5, 6, 7, 8, 9, and 10), different salt concentrations (0%, 5%, 10%, 15%, 20%, and 25%) and different temperature ranges (20 °C, 25 °C, 30 °C, 35 °C, 40 °C, and 45 °C). Luria Bertani broth medium was used as the base medium, with uninoculated broth used as a control for each growth parameter. After incubation at 30 °C for 24 h, two hundred microtiters (200 µL) of the bacterial strain adjusted overnight broth culture medium was dispensed into a sterile 96-well flat-bottomed microtiter plate. The adjusted bacterial inoculum of the strain was then distributed into a well in the microtiter plate containing the growth medium of Luria Bertani (LB) Broth (200 µL/well). Optical Densities (ODs) were measured after 5 s auto-mixing and recorded at 600 nm for each well using a multi-detection microplate reader (VERSA max spectrophotometer, Molecular Devices, Sunnyvale, CA, USA) equipped with SoftMax Pro software version 7.1.

3.6. Analysis of the Cocopeat Growing Medium

Cocopeat was used for the greenhouse experiment. Thus, 100 g of cocopeat was sent to the Department of Horticulture and Food Security Soil Science lab to determine the initial pH, nitrogen, phosphorus, potassium, magnesium, manganese, zinc, moisture, and electrical conductivity. Soil pH and EC were analyzed based on a 1:2.5 cocopeat to water ratio using a Potentiometric and Electric Conductivity Meter. Available phosphorous by colorimetric or UV-vis spectrophotometer by ammonium molybdate/vanadate at 880 nm, available nitrogen by Kjeldahl method, basic cations (K, Ca, and Mg) by ammonium acetate pH seven method, organic carbon matter by Walkley's Black method, texture by pipette method, and moisture content by gravimetric method were all utilized.

3.7. In Plant Tests

3.7.1. Plant Material, Seed Sterilization, and Germination

The experiment was carried out in a greenhouse at Jomo Kenyatta University of Agriculture and Technology, Juja, Nairobi, Kenya (1.0912° S, 37.0117° E, 1.527 m). Three varieties of tomato seeds, Anna F1, Eva F1, and Star9086, were used for the experiment. The seeds were surface sterilized with 3% sodium hypochlorite (NaClO) for 10 min and rinsed six times with sterilized double-distilled water. A healthy, well-germinated plantlet from each tomato variety was selected after three days of pregermination and then transplanted into 66-cell seedling tray (11 × 21 × 0.87 inches) filled with sterilized cocopeat substrate. The experimental greenhouse experiences an average temperature of 20 °C to 25 °C, relative

humidity of 60–85%, and light intensity of 34,500.00 ± 6362.10 lux. Fifteen tomato plants were established for each treatment modality, for a total of 45 plants.

3.7.2. Inoculation of *Solanum lycopersicum* with BF2P4-5 Strain

The potency of the bacterial strain in promoting tomato growth was assessed by inoculation with the BF2P4-5 strain. Bacteria inoculation occurred after five days once the plants had pregerminated and transferred singly into the seedling tray containing the sterile cocopeat substrate. A pure culture of the *Arthrobacter chlorophenolicus* strain BF2P4-5 was revived on nutrient agar for 24 h. A single colony of the bacterial strain was transferred into a 250 mL flask containing 200 mL Luria Bertani broth and was incubated overnight on a rotary shaker (130 rpm) at 30 °C [33]. The plants were then inoculated with BF2P4-5 suspensions (10 mL of OD_{600} 2.0). Positive control plantlets were inoculated with NPK 17:17:17 (2 g in 1 L sterile distilled water) and uninoculated (negative control) plants used sterilized distilled water. The experiment was carried out with five replications for each treatment modality. After 25 days of growth and with five true leaves, the seedlings were transplanted into a 10 L bucket containing 4 kg of unsterile cocopeat substrate. The inoculation of tomato seedlings was continued by drenching the growing medium with 20 mL of BF2P4-5 suspension (OD_{600} = 2.0) in a 100 mL measuring cylinder. The bacterial strains BF2P4-5 and NPK were used to establish the treatments for the three different tomato varieties used for the experiment: T1-NPK + Anna F1, NPK + Eva F1, NPK + Star9086; T2-BF2P4-5 + Anna F1, BF2P4-5 + Eva F1, and BF2P4-5 + Star9086. The BF2P4-5 strain and NPK treatments were complemented with a negative control in which only tap water was applied to the tomato roots. T3-No fertilization + Anna F1, No fertilization + Eva F1, No fertilization + Star9086. The bacterial strain used in this study belongs to bacterial genera or species known to exhibit PGP traits according to the literature [9,11,12,33,34]. Data on plant growth and phenology were taken when the plants reached 55 days old.

3.7.3. Determination of Plant Phenology and Growth Attributes

The growth parameters, including plant height, number of leaves per plant, stem length, stem girth (at 5 cm above and 15 cm above the base of the plant), leaf length, and leaf diameter of plants, were measured after 55 days of treatment. The chlorophyll content was determined using a soil plant analysis development (SPAD) meter for each plant's lower, midportion, and top canopy to ascertain the level of chlorophyll content.

3.8. Statistical Analysis

Five replicates were used under greenhouse conditions. Data were imputed into Microsoft Excel and subjected to analysis of variance (ANOVA) to find the significance between the values. Means were compared using Fisher-LSD ($p < 0.001$) in R version 4.2. To examine significant differences, such as those with a P value less than (0.05); the probability level was determined to assess significant differences between various treatments. The standard error and LSD results were calculated.

4. Discussion

Soil microorganisms are as essential for plant growth as water and sunlight. Plant-growth-promoting bacteria (PGPB) are rhizospheric bacteria that help plants acquire nutrients by nitrogen fixation, potassium, phosphate solubilization, indole acetic acid production, siderophore production, etc., or by synthesizing plant hormones that stimulate growth and development [34]. Low availability of nitrogen, phosphorus, and potassium in natural or artificial (soil or soilless) media limits plant growth, yield, and quality. Scientists are interested in developing and deploying PGPB strains because of their potential to fix nitrogen, dissolve phosphorus, and mobilize potassium. The strain could produce IAA, solubilized phosphate, potassium, and fixed nitrogen. BF2P4-5 was studied for its ability to promote tomato growth. Tomato rhizosphere soil being the original isolation source of BF2P4-5, we hypothesized that it might be helpful as a bioinoculant to stimulate tomato growth and

development; hence, it was used as a model for this study. The bacterial strain BF2P4-5 was identified as *Pseudarthrobacter chlorophenolicus* on cultural, biochemical, physiological analysis, and 16S rRNA gene sequence. This study chronicles the plant-growth-promoting activity of *Pseudarthrobacter chlorophenolicus* and demonstrates that it could significantly promote tomato plants.

Nitrogen (N), a crucial element required in nucleic acid and protein synthesis, is fixed from the surrounding atmosphere with the assistance of the plant-growth-promoting bacteria. By producing nitrogenase, bacteria reduce the nitrogen gas (N_2) to ammonia (NH_3), an organic form available to plants. Phosphate solubility is a common trait through which rhizobacteria could substantially enhance plant growth. Phosphorus is an essential macronutrient that is difficult to utilize as it is typically in its insoluble form [35]. However, phosphorus-solubilizing microorganisms can excrete H^+ ions or organic acids that hydrolyze the insoluble forms of phosphorus, which plants can then assimilate. The result that BF2P4-5 could fix nitrogen and solubilize inorganic phosphate highlights that the strain can produce nitrogenase and secretes organic acids and phosphatases that reduce nitrogen gas from the atmosphere to ammonia and solubilize the insoluble phosphate, respectively. Consequently, the essential nutrient for plant growth, nitrogen, and phosphorus becomes available for roots to sustain plant growth. According to [16], *Arthrobacter chlorophenolicus* produces heteroauxin at a high rate and uses insoluble phosphate as a source of phosphor, improving fertilizer use, plant root development, fertilizer absorption, and increasing soil phosphorus content.

The *Arthrobacter chlorophenolicus* showed optimum growth at 30 °C temperature, pH 7.0, and 15% NaCl. These results agree with those of [36], who reported that *Arthrobacter chlorophenolicus* could tolerate elevated levels of NaCl. Tolerance to salinity levels confers a selective advantage for rhizobacterial species populating the same soil localities suffering from high levels of salts [15].

The results of *Pseudarthrobacter chlorophenolicus* after 55 days of treatment were very close to those obtained with the positive control (NPK fertilizer), indicating that the bacteria have a fertilization action equal to the chemical fertilizers. The role of the selected strain on the growth parameters of the three varieties of tomato plants was recorded after 55 days of growth. The growth parameters of BF2P4-5-treated plants were analyzed with those of NPK-treated and untreated plants. Remarkably, the results of the rhizobacteria-treated plants led to a significant increase in most of the analyzed plant growth parameters compared to the NPK-treated plants. Several studies have recently reported increased growth parameters inoculated with beneficial plant microbes over uninoculated control [1,9,34,37]. Plant height was significantly higher upon inoculation of *Pseudarthrobacter chlorophenolicus* compared to NPK over the negative uninoculated control treatment. Kumar et al. [33] reported that wheat plant height was significantly higher by application of *A. chlorophenolicus* singly and in combination with *Enterobacter* sp. and triple combination of strains *B. megaterium*, *A. chlorophenolicus*, and *Enterobacter* sp. by 60, 65, and 67, respectively, under pot experiment. The chlorophyll content is vital to indicate plant stresses and nutrient status. A comparison of means showed that the chlorophyll content in the lower, middle, and top canopy was not significantly different between the NPK and BF2P4-5 treatments. It seems that the involvement of *Pseudarthrobacter chlorophenolicus* accelerates iron uptake in plant cells, which helps in nucleic acid metabolism in the chloroplast, resulting in better chlorophyll content. These results corroborate the findings of [38] that at 45 DAS, the chlorophyll content in soybean leaves increased significantly after inoculation of two *Arthrobacter* isolates (AR2 and AR12). Furthermore, Alzate Zuluaga et al. [39] reported that in plants deficient in phosphate, the inoculation of the Enterobacter 15S phosphate-solubilizing bacteria increased the allocation of phosphate in roots (+31%) and shoots (+53%) contributed to better photosynthetic activity (chlorophyll content), similar to that in phosphate-sufficient plants ($p < 0.05$). In the inoculated series with NPK and BF2P4-5, a significant increase in stem length and girth was detected. However, the highest stem length and stem girth (5 cm and 15 cm above) were observed in the BF2P4-5 treatment, which was equally well followed

by the NPK treatment. The uninoculated negative control recorded the least values. Inoculation of *Arthrobacter* sp. GN70 showed a significantly positive effect on the shoot length and shoot girth of rice [34]. Adding nitrogen to the soilless media (cocopeat) encourages plant growth, particularly in the leaves, making the leaves appear greener. Therefore, the strain's nitrogen-fixing capabilities may have contributed to increased chlorophyl content, many leaves per plant, longleaf length, and wide leaf width, as recorded in this study.

Cocopeat has an acceptable pH, electrical conductivity (EC), other chemical attributes, and a high water-holding capacity. The initial pH and EC of the media directly influence the availability of nutrients. The final pH differences recorded were insignificant. Although plant species have different pH ranges for optimal growth, the optimal pH of soilless media (cocopeat) for good element availability is around 6.0 [40]. Despite a slight drop in pH, the plants grew normally and did not show symptoms of elemental deficiency or toxicity. EC values reflect the media extracting inorganic ion concentration. The low EC value indicates that the media do not contain enough nutrients to support healthy plant growth. NPK contains a high concentration of soluble salts, which may benefit plant growth. Good soilless cocopeat medium should have an initial EC of 0.4–1.5 mS/cm^{-1} [41]. This study's initial EC of 0.4 mS/cm^{-1} was acceptable for plant growth.

Cocopeat was low in nitrogen, phosphorus, and potassium in this experiment. However, the increased NPK bioavailability in the media could be attributed to the application of strain BF2P4-5 and NPK fertilizer. *Pseudarthrobacter chlorophenolicus* BF2P4-5 can improve cocopeat's macronutrient content by fixing nitrogen and solubilizing phosphorus and potassium. Reports from [16] revealed that *Arthrobacter chlorophenolicus L4* had excellent growth-promoting effects on peanuts and high-yield heteroauxin promotes peanut growth and development of peanuts and increases the content of available phosphorus in the soil, leading to a higher utilization rate of peanut phosphate fertilizer. Previous findings showed that inoculation with beneficial *Azotobacter* sp., *Trichoderma* sp., *Azotobacter chroococcum*, *Klebsiella variicola*, *Rhizobium larrymoorei*, and *Klebsiella pneumoniae* increased nitrogen, available phosphorus, and potassium in cocopeat and soil [42,43]. Various authors have linked microorganisms that solubilize inorganic insoluble phosphates to the production of organic acids, the chelation of oxoacids from sugars, and the exchange reactions in the growth environment [9,44–47].

In general, *Pseudarthrobacter* species have been shown to boost the growth of peanut [16], panax ginseng [9], wheat [33], and rice [34]. These findings that BF2P4-5 promoted tomato growth are consistent with previous studies of rhizobacteria that improved plant growth, suggesting that BF2P4-5 has a growth-promoting effect on tomato plants. BF2P4-5′s multiple associative properties, phosphate and potassium solubility, nitrogen fixation, and IAA synthesis promote tomato plant growth. The multifunctional plant-growth-promoting traits of rhizobacteria are widespread and they may exert their beneficial effects directly or indirectly.

5. Conclusions

This study identified and characterized the bacterial strain BF2P4-5 as a kind of rhizospheric *Pseudarthrobacter chlorophenolicus* and could promote the growth of tomatoes. Furthermore, the results showed that strain BF2P4-5 exhibited traits to promote plant growth and could be deployed as an eco-friendly microbial biofertilizer for tomatoes and probably other essential crops. This study contributed to the study of the interaction between BF2P4-5 and its host tomato. Although tomato is an essential traditional plant, further research is needed on the effect of *Pseudarthrobacter chlorophenolicus* on stress resistance and yield components of tomato fruits.

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