

Article

Rhizobacterial Isolates from the Native Plant *Ceanothus velutinus* Promote Growth in Two Genotypes of Tall Fescue

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Abstract: Global warming-induced climate change haunts the world, posing a critical threat to plant health and crop production. Overusing chemical fertilizers and pesticides poses a significant threat to soil health. *Ceanothus velutinus* (snowbrush) is a drought-tolerant, actinorhizal native plant found in the Intermountain West region of the US that harbors many plant growth-promoting rhizobacteria (PGPR). In this study, we evaluated the effects of PGPR CK-06, CK-22, CK-44, and CK-50 from *C. velutinus* on the growth and development of two tall fescue genotypes: (i) a lawn-type tall fescue blend and (ii) an endophyte-free forage-type tall fescue known as Armory. Tall fescue plants were grown in field soil and sand mix in pots and treated twice with 5 mL of bacterial inoculum. Two isolates, CK-06 and CK-22, significantly increased tiller numbers ($p < 0.05$) in the lawn-type tall fescue blend, and all isolates showed a significant increase in fresh and dry weight compared to the control. Isolate CK-22 significantly increased the tiller number and fresh and dry weight of the forage-type tall fescue Armory compared to the control. Isolates CK-44 and CK-50 tested positive for sulfur-oxidizing properties, and CK-44 was able to restore the sulfur content in sulfur-deficient soil compared to the control.

Keywords: *Ceanothus velutinus*; snowbrush; plant growth-promoting bacteria; tall fescue; forage; biofertilizer; *Pseudomonas*



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1. Introduction

The world is experiencing climate change, which threatens the adaptability and productivity of plants through alterations in climate and weather patterns [1–5]. The excessive use of chemicals for increased production and to protect crops against diseases and pests is degrading soil health [6] and contributing to greenhouse gas production and, ultimately, climate change. In addition, the world population is projected to reach the 9.8 billion mark in 2050 and 11.2 billion in 2100 (United Nations). To meet the food requirements of this increasing population while conserving the environment, we need to devise an adaptation strategy that will lessen the use of chemicals in agriculture and help mitigate the effects of climate change on plants. Utilizing the growth-promoting bacteria associated with plants in their natural ecosystems might be an alternative to the use of chemicals [7,8].

In natural agricultural ecosystems, plants are associated with various beneficial and pathogenic microbes, most of which are bacteria or fungi [9]. The beneficial microbes help plants in nutrient acquisition and hormone production and can be used as biocontrol agents against biotic and abiotic stresses [10–15]. Therefore, there is a growing trend of incorporating microbes into agricultural systems for sustainable crop production [16–18]. Native plants such as *Ceanothus velutinus* and *Shepherdia* sp. are excellent resources of plant growth-promoting bacteria [19,20].

A recent study reported that when turf grass lawns were replaced with native plants in North America, the native plants supported more microbial diversity and enhanced the relative abundance of potentially beneficial taxa in the soil [21]. Utah is rich in native

plants, and half of Utah is made up of wildlands rich in endemic species [22]. These native plants are promoted for low-water landscaping because of their drought- and disease-resistant capabilities [23]. Most of these are actinorhizal plants; they form nodules with the actinobacteria *Frankia* and can fix atmospheric nitrogen and help plant growth [24,25]. However, some native plants, such as *C. velutinus*, are difficult to propagate in landscape media, as their cuttings are susceptible to rotting [26,27]. In our previous study, we propagated *C. velutinus* cuttings by adding 50% native soil from the native locations of *C. velutinus* plants to the propagation media, which enhanced the cutting survival rate [28]. Several plant growth-promoting bacteria isolated from the rhizosphere of native soil-treated cuttings produced more than 10 µg/mL of indole acetic acid (IAA). They had several plant-promoting traits, such as the ability to fix nitrogen, solubilize phosphorus, and produce catalase, siderophore, ammonia, protease, and ACC (1-Aminocyclopropane-1-carboxylate) deaminase activity [28]. Eight isolates from the rhizosphere of these cuttings showed increased shoot biomass in *Arabidopsis thaliana* [28].

Given these isolates' promising plant growth-promoting activities in Arabidopsis, we anticipate that our research will significantly benefit for crop production. To explore this potential, we conducted a study to test the growth promotion of four isolates (CK-06, CK-22, CK-44, and CK-50) on two tall fescue varieties: a lawn-type tall fescue blend and an endophyte-free forage-type tall fescue—Armory. Tall fescue (*Festuca arundinacea* Schreb.), a cool-season perennial grass, is used globally as an essential forage and turf grass. Natural populations are distributed throughout temperate and cool climates of Europe, North-West Africa, North America, and West and Central Asia. However, tall fescue accessions have several genetic variations [29]. In the late 1970s, tall fescue was discovered as a natural host of fungal endophytes, which produce alkaloids in plants that are toxic to grazing animals [30]. In cattle, tall fescue toxicosis causes decreased milk production, birth weight, pregnancy rate, serum prolactin levels, and an increased respiration rate. Researchers have tried to create endophyte-free tall fescue varieties to avoid this toxicity [31]. However, in plants, endophytes are associated with several benefits, such as plant growth and development and mitigated environmental stress. Therefore, the survival rate of endophyte-infected tall fescue is better than that of endophyte-free tall fescues. Endophyte-infected tall fescue shows improved seedling survival, insect and nematode resistance, drought resistance, improved nitrogen assimilation, and higher seed set [32]. Using plant growth-promoting rhizobacteria in lawn-type tall fescue and endophyte-free forage-type tall fescue could alleviate the effect of endophyte absence and promote the growth and development of both varieties. Promoting PGPR to be used as biofertilizers promotes plant health and improves soil health.

2. Materials and Methods

2.1. Plant Material and Bacterial Isolates

Seeds for the tall fescue lawn-type blend were obtained from Dr. Paul Johnsons, Plants, Soils, and Climate, USU, Logan, Utah, and endophyte-free forage-type Armory was obtained from Dr. Shaun Bushman, USDA—Forage and Range Research, Logan, Utah. The four bacterial isolates, named CK-06, CK-22, CK-44, and CK-50, were isolated from the rhizosphere of *C. velutinus* (snowbrush) plants propagated by cuttings in native soil from a previous study and identified as *Pseudomonas* sp. by 16S rRNA sequencing. A 1.4 KB DNA fragment of 16S rRNA was amplified for each isolate and sequenced by Sanger sequencing. The obtained sequences were searched against the 16S rRNA database at NCBI and submitted to GenBank. The accession numbers are OR795732, OR795735, OR795740, and OR795742 for CK-06, CK-22, CK-44, and CK-50, respectively [28]. All four isolates tested positive for seven plant growth-promoting traits, such as the ability to fix nitrogen; phosphate solubilization; siderophore, ammonia, indole acetic acid, and catalase production; and the ability to use ACC as a nitrogen source, except CK-50, which showed protease activity too (Table 1). They all showed an increase in shoot biomass in Arabidopsis [28].

Table 1. Plant growth-promoting characteristics of bacterial isolates [28].

Isolates	IAA ($\mu\text{g/mL}$)	PSI	NH_3 ($\mu\text{g/mL}$)	NF		SP	ACC	PA	Cat
				Media	<i>nifH</i> ⁺				
CK-06	10.95 \pm 0.02	2.4 \pm 0.23	56.84 \pm 5.35	++	+	++	+++	-	++
CK-22	23.78 \pm 0.36	2.40 \pm 0.39	17.34 \pm 2.86	+++	-	++	++	-	+++
CK-44	28.79 \pm 0.54	2.01 \pm 0.13	50.32 \pm 5.83	+++	-	++	+	-	+++
CK-50	10.31 \pm 0.23	2.43 \pm 0.03	100.69 \pm 8.39	+++	-	++	+	++	+++

'-'—negative/absent, '+'—mild positive/present, '++'—moderately positive, '+++—strongly positive. SP—siderophore production, PSI—phosphate solubilization index, IAA—indole acetic acid production ($\mu\text{g/mL}$), ACC—ACC deaminase activity, PA—protease activity, Cat—catalase production, NF—nitrogen fixation, *nifH*⁺—Fe subunit of nitrogenase gene, NH_3 —ammonia production. \pm Standard error for triplicates.

2.2. Bacterial Treatment and Plant Growth Promotion Experiment

The bacteria were inoculated in liquid Luria–Bertani (LB) and kept in a shaker incubator at 28 °C overnight. The bacterial cells were pelleted by centrifugation and resuspended in 1/8th MS media, maintaining an OD₆₀₀ of 0.8–1.0 in the bacterial inoculum. The tall fescue seeds were surface-sterilized: they were soaked in 75% ethanol for 5 min and sodium hypochlorite (5% active chlorine) for 15 min, then washed with sterile water 4–5 times [33]. The seeds were then carefully transferred to 9 cm diameter Petri plates fitted with two sheets of Whatman filter paper wetted with 3 mL of double-distilled and autoclaved water to maintain moisture (Supplementary Figure S1A). The Petri plates were sealed with parafilm, wrapped in aluminum foil, and stored in a growth chamber (25° C) for five days. After five days, the pre-germinated seeds were transplanted into pots filled with a blend of field soil and sand carefully measured in a 1:1 ratio to provide optimal growing conditions for the seedlings (Supplementary Figure S1B). The soil was collected from USU fields, brought to the lab, and mixed with sand purchased from The Home Depot in a 1:1 ratio, filling the pots. The pots were arranged in a completely randomized design in the growth chamber, which was set at 24 °C Day/Night with 16H day/8H night cycles and a light intensity of 200 $\mu\text{M m}^{-2} \text{s}^{-1}$. Humidity was set to 50%. The lawn-type tall fescue blend experiment was conducted from January to May 2023. Two seedlings were planted per pot, and five pots per treatment were used. The forage-type tall fescue Armory experiment was completed from July to November 2023. Here, we used one plant/pot, as two plants per pot was very crowded, and the roots were coming out of the pots. Ten pots per treatment were used in this experiment to make ten biological replicates. The first bacterial inoculation was performed two weeks after the transfer of seedlings to the pots by pouring 5 mL of bacterial inoculum in 1/8MS onto the base of each plant. The second bacterial inoculation was undertaken two weeks after the first treatment. The plants were irrigated every third day with 1/8th MS.

2.3. Collection of Data for Plant Growth Parameters

The first harvest was performed 75 days after sowing, and data were recorded for the number of tillers, fresh weight, dry weight, and plant height. The plants were cut about 2 cm from the soil level and reinoculated by pouring the corresponding bacterial suspension onto the base of the plants. They were kept in the same growth chamber for another two months before being harvested again, with data recorded similarly. The data were analyzed using Analysis of Variance (ANOVA); Tukey's test (HSD) was used to test the significance of the differences among the sample means.

2.4. Soil Analysis

Bulk soil samples were collected from each pot of the treatment, pooled together, and mixed homogeneously after the second harvest. Two cups of soil samples from each treatment, including the control, were sent for soil mineral analysis at Utah State University analytical laboratories (USUAL). The following test methods were used by USUAL:

pH + EC (salinity) + SAR by saturated paste; phosphate and potassium by Olsen sodium bicarbonate extract [34]; NO₃-N by CaOH extract⁺ cadmium [35]; SO₄-S by CaHPO₄ ICP; organic matter (OM) by Walkley–Black method [36]; and Zn, Fe, Cu, Mn by DTPA + ICP [37].

2.5. Screening for Sulfur-Oxidizing Capability

A plate assay was conducted to evaluate the sulfur oxidation by the bacterial isolates. Agar Petri plates were prepared with a modified thiosulfate medium containing 5.0 g/L glucose, 5.0 g/L sodium thiosulfate, 0.1 g/L dipotassium hydrogen phosphate, 0.2 g/L sodium bicarbonate, 0.1 g/L ammonium chloride, 5.0 g/L yeast extract, and 20 g/L agar, adjusted to a pH of 8.0 [38]. The medium was supplemented with 0.5% thiosulfate and bromocresol purple as a pH indicator, following the method described in [39]. Aliquots of 20 µL from each bacterial isolate were placed onto the plates and incubated at 30 °C. The color of the medium changed from purple to yellow, indicating the oxidation of thiosulfate (a reduced form of sulfur) by the isolates, as shown by the translucent halo around the bacterial colonies. Three replicates per isolate were used for the assay, and the assay was repeated twice. The diameters of the yellow halo and the bacterial colonies were measured, and the solubilization potential was calculated using the solubilization index formula.

$$\text{Solubilization index} = (\text{Total diameter of colony} + \text{halo}) / (\text{Diameter of the colony})$$

The solubilization index was assessed at 24, 72, and 120 h of incubation.

3. Results

3.1. Effect of Bacterial Isolates on Tiller Number and Biomass of Lawn-Type Tall Fescue Blend

The results suggest that the mean tiller number in plants treated with CK-06 and CK-22 was significantly higher ($p \leq 0.05$) than in the control (untreated plants) (Table 2). Plants treated with CK-44 and CK-50 also showed an increase in tiller number, but this was not statistically significant ($p \geq 0.05$) compared to the control. The fresh weight and dry weight of the plants treated with CK-06 and CK-22 were significantly greater ($p \leq 0.05$) than the control in the first harvest, but in the second harvest, all treatments showed significantly greater weight ($p \leq 0.05$) than the control plants (Table 2). However, no significant differences were observed regarding plant height between the treatments and the control (Table 2, Figure 1A).

Table 2. Effect of bacterial isolates on lawn-type tall fescue blend.

Lawn-Type Tall Fescue Blend—First Harvest								
Treatment	Mean Tiller No	SE Tiller No	Mean Fresh WT	SE Fresh WT	Mean Dry WT	SE Dry WT	Mean Height	SE Height
Control	7.42 ^b	0.87	6.10 ^b	0.56	1.66 ^b	0.20	37.00 ^a	2.63
CK-06	18.67^a	2.83	11.38^a	1.12	3.37^a	0.31	35.93 ^a	1.68
CK-22	15.83^a	1.75	11.38^a	1.50	3.20^a	0.40	40.50 ^a	1.72
CK-44	14.25 ^{ab}	1.45	10.61 ^{ab}	1.49	2.84 ^{ab}	0.36	41.58 ^a	2.12
CK-50	15.67 ^{ab}	2.75	10.12 ^{ab}	1.44	2.69 ^{ab}	0.39	37.86 ^a	2.65
Lawn-Type Tall Fescue—Second Harvest								
	Mean Fresh WT	SE Fresh WT	Mean Dry WT	SE Dry WT				
Control	6.78 ^b	0.5	1.71 ^b	0.13				
CK-06	15.52^a	1.26	3.87^a	0.32				
CK-22	12.61^a	1.43	3.32^a	0.41				
CK-44	14.10^a	1.37	3.51^a	0.36				
CK-50	14.13^a	2.07	3.51^a	0.5				

The same letters within a column denote no significance among different samples according to Tukey's method for multiplicity at $\alpha = 0.05$. Numbers in bold are significant changes. SE—standard error.

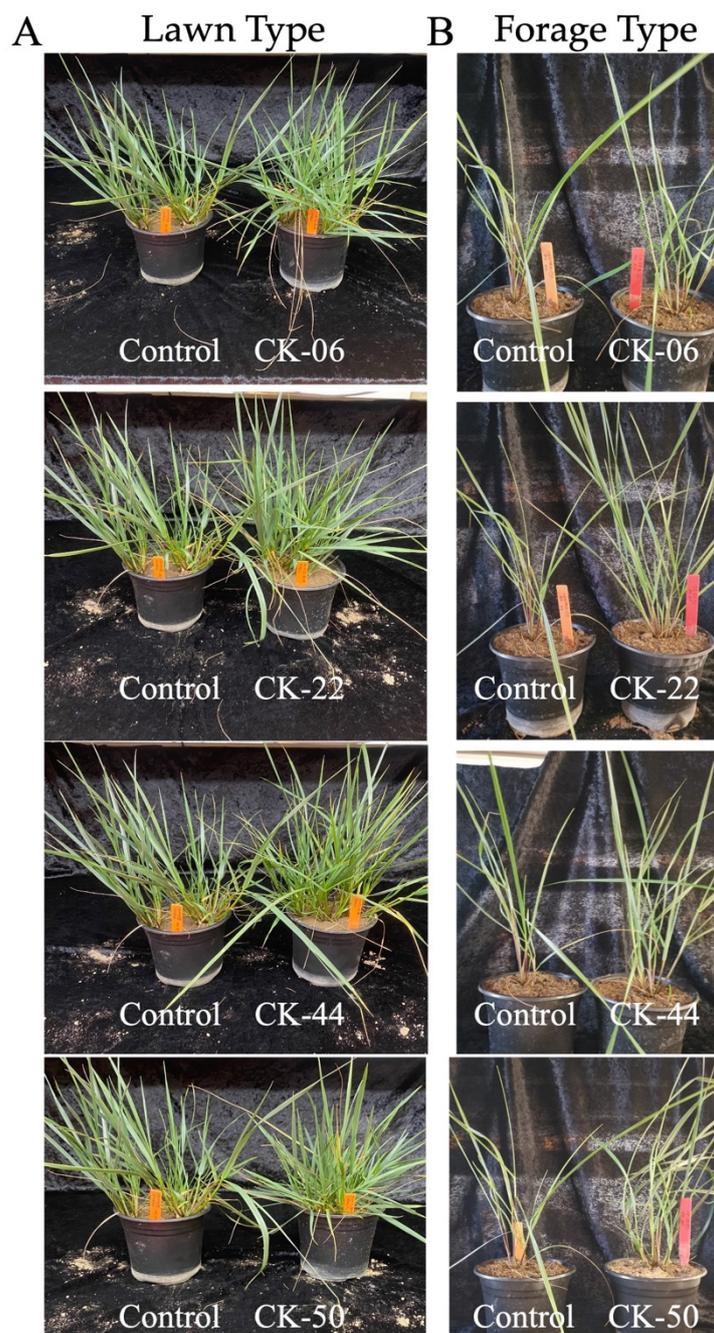


Figure 1. Effect of bacterial isolates on (A) lawn-type tall fescue blend (2 plants/pot (10 replicates/treatment)). (B) Forage-type tall fescue Army (1 plant/pot at second harvest pot (10 replicates/treatment)).

3.2. Effect of Bacterial Isolates on Tiller Number and Biomass of Forage-Type Tall Fescue Army

For the forage-type tall fescue variety Army, all bacterial isolates showed an increase in tiller number compared to the untreated plants; however, only the bacterial isolates CK-22 and CK-50 were significantly different ($p \leq 0.05$) from the control (Table 3). Similarly, all isolates showed an increase in fresh and dry weight compared to the control. Still, only CK-22 was significantly different from the control for fresh weight, and CK-06 and CK-22 were significantly different ($p \leq 0.05$) from the control for dry weight after the first harvest (Table 3). In the second harvest, all isolates showed an increase in tiller number, fresh weight, and dry weight, but only CK-22 was significantly different ($p \leq 0.05$) from the

control. The height of the tall fescue plants was not affected by the treatments compared to control (Table 3, Figure 1B).

Table 3. Effect of bacterial isolates on forage-type tall fescue Armory.

Forage-Type Tall Fescue Armory—First harvest								
Treatment	Mean Tiller No	SE Tiller No	Mean Fresh WT	SE Fresh WT	Mean Dry WT	SE Dry WT	Mean Height	SE Height
Control	4.9 ^b	0.4	5.1 ^b	0.6	1.2 ^b	0.1	52.0 ^a	2.5
CK-06	7.1 ^{ab}	0.7	7.5 ^{ab}	0.5	2.0^a	0.1	57.0 ^a	2.8
CK-22	8.1^a	0.7	8.2^a	0.7	2.1^a	0.2	53.8 ^a	2.3
CK-44	6.2 ^{ab}	0.5	5.8 ^{ab}	0.8	1.4 ^{ab}	0.2	48.3 ^a	3.1
CK-50	7.6^a	0.5	7.3 ^{ab}	0.8	1.9 ^a	0.2	50.4 ^a	2.7
Forage-Type Tall Fescue Armory—Second harvest								
Treatment	Mean Tiller No	SE Tiller No	Mean Fresh WT	SE Fresh WT	Mean Dry WT	SE Dry WT	Mean Height	SE Height
Control	12.5 ^b	1.3	6.5 ^b	0.7	1.7 ^b	0.2	43.2 ^a	1.9
CK-06	15.9 ^{ab}	2.1	9.8 ^{ab}	0.9	2.3 ^{ab}	0.2	49.0 ^a	2.3
CK-22	22.9^a	2.3	12.7^a	1.3	3.1^a	0.4	47.7 ^a	2.0
CK-44	16.6 ^{ab}	1.5	7.6 ^b	0.7	1.9 ^b	0.2	46.2 ^a	2.3
CK-50	19.3 ^{ab}	1.6	9.7 ^{ab}	0.7	2.5 ^{ab}	0.2	46.7 ^a	2.2

The same letters within a column denote no significance among different samples according to Tukey's method for multiplicity at $\alpha = 0.05$. Numbers in bold are significant changes. SE—standard error.

3.3. Soil Nutrient Analysis

Soil nutrient analysis was undertaken for both experiments. The soil texture was sandy clay loam for both experiments (Tables 4 and 5). In both experiments, the organic content was between 1 and 2%. According to the USUAL analysis report, the soil in the experiment with lawn-type tall fescue was deficient in sulfur in the control, CK-22, and CK-50 treatments; however, in the CK-06 and CK-44 treatments, the sulfur content was adequate. In the experiment with the forage-type tall fescue Armory, the soil samples were adequate for all nutrients except iron in all treatments. Our experiment, conducted under unique conditions, yielded some interesting results. CK-06 showed higher N content (153 mg/Kg) in the lawn-type tall fescue compared to the control (31 mg/Kg) and the other treatments, i.e., CK-22 (12.4 mg/Kg), CK-44 (23.2 mg/Kg), and CK-50 (32.6 mg/Kg) (Table 4). A higher Zn content (9.98 mg/Kg) was found in Armory compared to the control (0.9 mg/Kg) and the other treatments (CK-22 (0.91 mg/Kg), CK-44 (0.96 mg/Kg), and CK-50 (0.93 mg/Kg) (Table 5). We also observed higher K content in the control of the lawn-type blend compared to the control of Armory and higher S content in the control of Armory compared to the control of the lawn-type blend (Tables 4 and 5). The unique experimental conditions may have contributed to these differences, including different times of experimentation, varied collections of field soil, and different lots of sand.

Table 4. Soil analysis of lawn-type tall fescue after harvest.

Treatment	pH	Salinity- ECe (dS/m)	P (mg/Kg)	K (mg/Kg)	N (mg/Kg)	Zn (mg/Kg)	Fe (mg/Kg)	Cu (mg/Kg)	Mn (mg/Kg)	S (mg/Kg)	OM (%)
Control	8.1	1.72	8.08	566	31	1.3	5.53	0.71	4.37	5.6	1.8
CK-06	7.7	5.58	13	494	153	1.41	5.48	0.66	4.5	11.3	1.8
CK-22	8.1	1.05	9.98	556	12.4	1.52	5.65	0.79	5.64	5.3	1.7
CK-44	8.1	1.53	9.86	616	23.2	1.51	5.87	0.88	6.03	9.9	1.8
CK-50	8	1.82	7.33	529	32.6	1.33	5.39	0.74	5.17	6.9	1.4

P—phosphorus, K—potassium, N—nitrate nitrogen, Zn—zinc, Cu—copper, Mn—manganese, S—sulfate-sulfur, OM—organic matter. Numbers in bold are deficient.

Table 5. Soil analysis of Armory after harvest.

Treatment	pH	Salinity- ECe (dS/m)	P (mg/Kg)	K (mg/Kg)	N (mg/Kg)	Zn (mg/Kg)	Fe (mg/Kg)	Cu (mg/Kg)	Mn (mg/Kg)	S (mg/Kg)	OM (%)
Control	8	2.09	7.18	60.07	22	0.9	4.31	0.57	3.21	23.7	1.3
CK-06	8.1	1.29	5.57	61.2	7.86	9.98	4.65	0.60	3.25	12.2	1.5
CK-22	8.1	1.38	3.96	60.4	12.4	0.91	4.25	0.58	2.69	20.5	1.1
CK-44	8.0	2.16	4.83	63.6	30.9	0.96	4.22	0.59	2.89	24.7	1.2
CK-50	8.1	1.59	4.83	66.2	22.1	0.93	4.29	0.55	3.09	14.4	1.3

P—phosphorus, K—potassium, N—nitrate nitrogen, Zn—zinc, Cu—copper, Mn—manganese, S—sulfate sulfur, OM—organic matter. Numbers in bold are deficient.

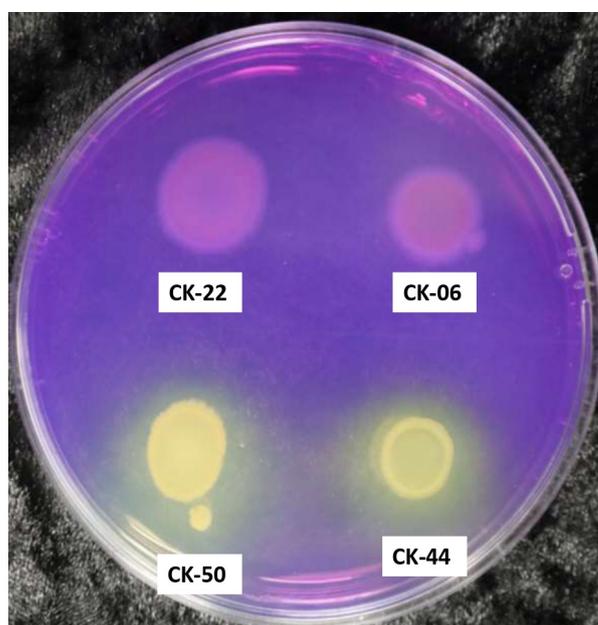
3.4. Sulfur-Oxidizing Capability

All four isolates were tested for sulfur-oxidizing potential. Only two of them, CK-44 and CK-50, showed a media color change and halo formation. CK-44 and CK-50 showed sulfur-oxidizing indices (SOIs) of 1.40 ± 0.05 and 1.63 ± 0.00 after 120 h of incubation (Table 6 and Figure 2).

Table 6. Sulfate solubilization index (SSI) by bacterial isolates after 24 h, 72 h, and 120 h of incubation.

Isolates	24 h	72 h	120 h
CK-06	-	-	-
CK-22	-	-	-
CK-44	1.39 ± 0.10	1.32 ± 0.05	1.40 ± 0.05
CK-50	1.32 ± 0.04	1.42 ± 0.08	1.63 ± 0.00

“-” negative; \pm standard error. Three replicates per isolate.

**Figure 2.** All four isolates on modified thiosulfate media.

4. Discussion

Plant growth-promoting rhizobacteria impact plant growth drastically by providing nutrients, producing phytohormones, and helping mitigate environmental stress [40]. Applying microbial fertilizers improves the soil environment, maintains soil microbial ecology, and promotes the absorption and utilization of nutrient elements, ultimately increasing crop yield [41]. This study showed that applying rhizobacteria isolated from native *C. velutinus* plants significantly increased the tiller number, fresh weight, and dry

weight of tall fescue plants (Tables 2 and 3). All four isolates were identified as *Pseudomonas*, sp., possessing more than 10 µg/mL of IAA and the ability to use ACC as a nitrogen source for ACC deaminase activity [28]. In this study, two isolates also exhibited sulfur-oxidizing capabilities (Figure 2 and Table 6). Several studies have reported the effect of plant growth-promoting *Pseudomonas* sp. on the growth and development of wheat and rice [42–44]. Several studies testing rhizobacterial isolates on tall fescue and bermudagrass forage systems in Alabama (USA) have reported their positive effects on growth and nutritional values [45]. A rhizobacteria *Pseudomonas* sp. SB isolated from tall fescue produced a biosurfactant, and its inoculation of tall fescue led to the bioremediation of oily sludge-contaminated soils [46]. Several PGPRs belonging to *Bacillus* spp., *Stenotrophomonas rhizophila*, and *Paenibacillus Ronchi* were tested to mitigate the effect of nematodes on turfgrass [47]. The stem length, shoot and root fresh weight, and dry weight of the rice plants significantly increased when treated with the plant growth-promoting rhizobacteria (ST-PGPR) *Pinoculum. atacamensis* compared to the control [48]. Similar positive changes in root and shoot dry weight, fresh weight, and root and shoot length were reported in Finger millet when the seeds were inoculated with the plant growth-promoting bacteria *Pseudomonas* spp. [49].

All isolates showed a significant increase in shoot biomass in lawn-type tall fescue. However, only CK-22 showed a significant increase in shoot biomass in the endophyte-free forage-type tall fescue Armory (Tables 2 and 3). Similarly, only CK-06 and CK-22 showed a significant increase in the tiller number of lawn-type tall fescue; however, in Armory, only CK-22 showed a significant increase (Tables 2 and 3). These results indicate that genetic variation plays a role in plant-microbe interactions. A genome-wide association study (GWAS) on several *Arabidopsis* accessions and PGPR *P. simiae* WCS417r revealed that plants possess genetic variation for plant-microbe interaction to benefit from PGPR [50]. Another GWAS on the interaction between PGPR *P. siliginis* and 162 *A. thaliana* accession reported strong genetic variation of plant response to the PGPR [50]. Another GWAS on the interaction between the PGPR *P. siliginis* and 162 *A. thaliana* accessions reported strong genetic variation of the plant's response to the PGPR [51].

In the lawn-type tall fescue experiments, CK-06 and CK-44 were reported to enhance the sulfate sulfur content in soil (Table 4). The CK-44 and CK-50 isolates showed sulfur-oxidizing capabilities (Figure 2 and Table 6). Though CK-50 did not increase the sulfate sulfur content in the soil to an adequate level, it increased the sulfate sulfur content (6.9 mg/Kg) compared to the control (5.6 mg/Kg) (Table 4). CK-06 showed a significant increase in sulfate sulfur content but did not have sulfur-oxidizing activity. CK-06 may reorganize the soil's microbial network and increase the colonization of sulfur-oxidizing bacteria. More microbial diversity and microbiome network studies are needed to study this concept.

The rhizosphere contains sulfur-oxidizing bacteria that mineralize elemental sulfur to sulfate, which plants can take up [52]. Sulfur-oxidizing bacteria (SOB) have emerged as promising agents in sustainable agriculture due to their dual role as plant growth regulators and biofertilizers. In one study, five potential sulfur-oxidizing bacteria—*Enterobacter cloacae* KDNC31 (AD31; OR083345.1), *Klebsiella oxytoca* KDNC1 (OR083341.1), *Raoultella planticola* KDNC3 (OR083342.1), *E. cloacae* KDNC9 (OR083344.1), and *K. pasteurii* KDNC8 (OR083343.1)—were isolated from the rhizosphere of chickpea in India [53]. The AD31 isolate demonstrated significant sulfur oxidation capabilities and several plant growth-promoting substances, including indole-3-acetic acid (IAA), solubilized tricalcium phosphate, and hydrogen cyanide (HCN). AD31 combined with elemental sulfur in a pot experiment significantly enhanced shoot and root length, leaf and branch count, and fresh and dry biomass compared to the control group [53]. Another study conducted a field experiment on calcareous soil to assess the impact of elemental sulfur and farmyard manure enriched with sulfur-oxidizing bacteria (termed microbial soil conditioner—MSC) on phosphorus and micronutrient availability, as well as wheat growth, concluded that using sulfur and farmyard manure enriched with SOB is a practical approach to improving

nutrient availability and promoting better plant growth in calcareous soils [54]. The sulfur-oxidizing bacteria (SOB) *Burkholderia cepacia*, *Enterobacter cloacae*, and *Klebsiella oxytoca* were isolated from mustard fields. They were found to significantly enhance nitrogen and sulfur uptake in wheat and mustard crops, demonstrating their effectiveness in oxidizing sulfur in both in vitro and in vivo conditions [55]. Utah soil is deficient in sulfur in several locations [56]. Isolating sulfur-oxidizing microbes from Utah's native plants can promote their development as biofertilizers for sulfur-deficient soil.

CK-06 also resulted in higher N content in the lawn-type tall fescue compared to the control and other treatments (Table 4). CK-06 exhibits N fixation ability and amplifies the *nifH*⁺ fragment of the Nitrogenase gene (Table 1). This could be the reason for the higher N content in CK-06-treated soil, as this microbe interacts with the lawn-type blend genotype. CK-06 also resulted in higher Zn content in Armory compared to the control and other treatments (Table 5). Zn deficiency is another problem in crops that not only affects the plant's development but also reduces the accumulation of Zn in edible parts of plants and affects human health. Microbial biofortification is desirable to mitigate plant Zn deficiency [57]. CK-06 is a potential candidate for further study in Zn solubilization activities.

A recent review discusses exploring the arid rhizosphere microbiome of date palms to develop biofertilizers that enhance the palms' resilience to climate change [58]. Another study on the rhizosphere microbiome of medicinal plants from arid climates reveals that microbial communities vary with location, host, and soil conditions [59]. Therefore, exploring PGPR from the rhizosphere of native plants in the arid region of Utah presents the potential to develop biofertilizers for sustainable agriculture under climate change.

5. Conclusions

Our results suggest that these rhizobacterial isolates from the native plant *C. velutinus* have promising plant growth-promoting traits, translating into Arabidopsis and tall fescue. All isolates resulted in a significant increase in fresh and dry weight in lawn-type tall fescue, underscoring the potential of these bacterial isolates. However, isolates CK-06 and CK-22 resulted in a significant increase in tiller number in lawn-type tall fescue. Isolate CK-22 resulted in a significant increase in tiller number and fresh and dry weight in both genotypes. Isolates CK-44 and CK-50 showed sulfur-oxidizing capability, and both increased the amount of sulfur in sulfur-deficient soil; however, CK-44 showed an adequate increase in sulfur. These findings highlight the potential of these beneficial microbes to be exploited and commercialized as biofertilizers, offering a promising alternative to chemical fertilizers. Natural beneficial microbes could be a sustainable resource in farming and help mitigate the effects of climate change on agriculture. However, more studies are needed to test these isolates on different genotypes and locations to develop these isolates as biofertilizers because plant genotype and soil composition greatly influence plant-microbe interactions. Further evaluation of these microbes on other crops is essential to promote them as biofertilizers.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microbiolres15040173/s1>, Figure S1: (A) Surface sterilized seeds were pregerminated in Petri plates for five days at 25 °C (B) and transferred to pots containing field soil and sand mix (1:1).

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References

1. Wang, L.; Wang, L.; Li, Y.; Wang, J. A Century-Long Analysis of Global Warming and Earth Temperature Using a Random Walk with Drift Approach. *Decis. Anal. J.* **2023**, *7*, 100237. [\[CrossRef\]](#)
2. de Gorter, H.; Drabik, D.; Just, D. Biofuel Policies and Food Grain Commodity Prices 2006–2012: All Boom and No Bust? *AgBioForum* **2013**, *16*, 1–13.
3. Kanojia, A.; Dijkwel, P. Abiotic Stress Responses Are Governed by Reactive Oxygen Species and Age. In *Annual Plant Reviews Online*; John Wiley and Sons Inc.: Hoboken, NJ, USA, 2018; pp. 1–32, ISBN 9781119312994.
4. Dhankher, O.P.; Foyer, C.H. Climate Resilient Crops for Improving Global Food Security and Safety. *Plant Cell Environ.* **2018**, *41*, 877–884. [\[CrossRef\]](#) [\[PubMed\]](#)
5. van Dijk, M.; Morley, T.; Rau, M.L.; Saghai, Y. A Meta-Analysis of Projected Global Food Demand and Population at Risk of Hunger for the Period 2010–2050. *Nat. Food* **2021**, *2*, 494–501. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Hossain, M.E.; Shahrukh, S.; Hossain, S.A. Chemical Fertilizers and Pesticides: Impacts on Soil Degradation, Groundwater, and Human Health in Bangladesh. In *Environmental Degradation: Challenges and Strategies for Mitigation*; Singh, V.P., Yadav, S., Yadav, K.K., Yadava, R.N., Eds.; Springer International Publishing: Cham, Switzerland, 2022; pp. 63–92, ISBN 978-3-030-95542-7.
7. Raza, A.; Razzaq, A.; Mehmood, S.S.; Zou, X.; Zhang, X.; Lv, Y.; Xu, J. Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. *Plants* **2019**, *8*, 34. [\[CrossRef\]](#)
8. Khan, A.; Panthari, D.; Sharma, R.S.; Punetha, A.; Singh, A.V.; Upadhayay, V.K. Chapter 6—Biofertilizers: A Microbial-Assisted Strategy to Improve Plant Growth and Soil Health. In *Advanced Microbial Techniques in Agriculture, Environment, and Health Management*; Chandra Pandey, S., Pande, V., Sati, D., Samant, M., Eds.; Academic Press: Cambridge, MA, USA, 2023; pp. 97–118, ISBN 978-0-323-91643-1.
9. Wille, L.; Messmer, M.M.; Studer, B.; Hohmann, P. Insights to Plant–Microbe Interactions Provide Opportunities to Improve Resistance Breeding against Root Diseases in Grain Legumes. *Plant Cell Environ.* **2019**, *42*, 20–40. [\[CrossRef\]](#)
10. Vocciant, M.; Grifoni, M.; Fusini, D.; Petruzzelli, G.; Franchi, E. The Role of Plant Growth-Promoting Rhizobacteria (PGPR) in Mitigating Plant’s Environmental Stresses. *Appl. Sci.* **2022**, *12*, 1231. [\[CrossRef\]](#)
11. Lugtenberg, B.; Kamilova, F. Plant-Growth-Promoting Rhizobacteria. *Annu. Rev. Microbiol.* **2009**, *63*, 541–556. [\[CrossRef\]](#)
12. Kuan, K.B.; Othman, R.; Abdul Rahim, K.; Shamsuddin, Z.H. Plant Growth-Promoting Rhizobacteria Inoculation to Enhance Vegetative Growth, Nitrogen Fixation and Nitrogen Remobilisation of Maize under Greenhouse Conditions. *PLoS ONE* **2016**, *11*, e0152478. [\[CrossRef\]](#)
13. Glick, B.R. Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica* **2012**, *2012*, 963401. [\[CrossRef\]](#)
14. Orozco-Mosqueda, M.d.C.; Santoyo, G.; Glick, B.R. Recent Advances in the Bacterial Phytohormone Modulation of Plant Growth. *Plants* **2023**, *12*, 606. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Bhattacharyya, C.; Banerjee, S.; Acharya, U.; Mitra, A.; Mallick, I.; Haldar, A.; Haldar, S.; Ghosh, A.; Ghosh, A. Evaluation of Plant Growth Promotion Properties and Induction of Antioxidative Defense Mechanism by Tea Rhizobacteria of Darjeeling, India. *Sci. Rep.* **2020**, *10*, 15536. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Compant, S.; Cassan, F.; Kostić, T.; Johnson, L.; Brader, G.; Trognitz, F.; Sessitsch, A. Harnessing the Plant Microbiome for Sustainable Crop Production. *Nat. Rev. Microbiol.* **2024**, *23*, 9–23. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Acharya, B.R.; Gill, S.P.; Kaundal, A.; Sandhu, D. Strategies for Combating Plant Salinity Stress: The Potential of Plant Growth-Promoting Microorganisms. *Front. Plant Sci.* **2024**, *15*, 1406913. [\[CrossRef\]](#)
18. Burlakoti, S.; Devkota, A.R.; Poudyal, S.; Kaundal, A. Beneficial Plant–Microbe Interactions and Stress Tolerance in Maize. *Appl. Microbiol.* **2024**, *4*, 1000–1015. [\[CrossRef\]](#)
19. Ganesh, J.; Singh, V.; Hewitt, K.; Kaundal, A. Exploration of the Rhizosphere Microbiome of Native Plant *Ceanothus velutinus*—an Excellent Resource of Plant Growth-Promoting Bacteria. *Front. Plant Sci.* **2022**, *13*, 979069. [\[CrossRef\]](#)
20. Devkota, A.R.; Wilson, T.; Kaundal, A. Soil and Root Microbiome Analysis and Isolation of Plant Growth-Promoting Bacteria from Hybrid Buffaloberry (*Shepherdia utahensis* ‘Torrey’) across Three Locations. *Front. Microbiol.* **2024**, *15*, 1396064. [\[CrossRef\]](#)
21. Baldi, D.S.; Humphrey, C.E.; Kyndt, J.A.; Moore, T.C. Native Plant Gardens Support More Microbial Diversity and Higher Relative Abundance of Potentially Beneficial Taxa Compared to Adjacent Turf Grass Lawns. *Urban. Ecosyst.* **2023**, *26*, 807–820. [\[CrossRef\]](#)
22. Stein, B.A. *States of the Union: Ranking America’s Biodiversity*; NatureServe: Arlington, VA, USA, 2002.

23. Rupp, L.; Wheaton, A. *Nurturing Native Plants: A Guide to Vegetative Propagation of Native Woody Plants in Utah*; Utah State University Extension: Logan, UT, USA, 2014; pp. 1–150.
24. Kratsch, H.A.; Graves, W.R. Nitrogen Fixation as a Stress-Avoidance Strategy among Actinorhizal (Non-Legume) Trees and Shrubs. *J. Crop Improv.* **2004**, *10*, 281–304. [[CrossRef](#)]
25. Chen, J.-J.; Kratsch, H.; Norton, J.; Sun, Y.; Rupp, L. Nodulation and Plant Growth of *Shepherdia* × *utahensis* ‘Torrey’ Topdressed with Controlled-Release Fertilizer. *HortScience* **2020**, *55*, 1956–1962. [[CrossRef](#)]
26. Hooper, V.H.; Endter-Wada, J.; Johnson, C.W. Theory and Practice Related to Native Plants: A Case Study of Utah Landscape Professionals. *Landsc. J.* **2008**, *27*, 127–141. [[CrossRef](#)]
27. Paudel, A.; Sun, Y.; Rupp, L.A.; Carman, J.G.; Love, S.L. Vegetative Propagation of *Ceanothus velutinus* Using Stem Cuttings. *Nativ. Plants J.* **2022**, *23*, 123–129. [[CrossRef](#)]
28. Ganesh, J.; Hewitt, K.; Devkota, A.R.; Wilson, T.; Kaundal, A. IAA-Producing Plant Growth Promoting Rhizobacteria from *Ceanothus velutinus* Enhance Cutting Propagation Efficiency and Arabidopsis Biomass. *Front. Plant Sci.* **2024**, *15*, 1374877. [[CrossRef](#)] [[PubMed](#)]
29. Lou, Y.; Chen, L.; Xu, Q.; Zhang, X. Genotypic Variation of Morphological Traits in Tall Fescue (*Festuca arundinacea* Schreb.) Accessions. *HortScience* **2015**, *50*, 512–516. [[CrossRef](#)]
30. Stuedemann, J.A.; Hoveland, C.S. Fescue Endophyte: History and Impact on Animal Agriculture. *J. Prod. Agric.* **1988**, *1*, 39–44. [[CrossRef](#)]
31. Waller, J.C. Endophyte Effects on Cattle. In *Tall Fescue for the Twenty-First Century*; American Society of Agronomy: Madison, WI, USA, 2009; Volume 53, pp. 289–310.
32. Pedersen Jeffrey, F.; Lacefield, G.D.; Ball, D.M. A Review of the Agronomic Characteristics of Endophyte-Free and Endophyte-Infected Tall Fescue. *Appl. Agric. Res.* **1990**, *5*, 188–194.
33. Zhang, Z.-F.; Rao, L.-Q.; Xiao, J. Research on the Sterilization Method for Endophytic Fungi of Tall Fescue Seeds. In *Multifunctional Grasslands in a Changing World II*; China Scientific Books: Hong Kong, China, 2008; p. 629.
34. Olsen, S.R.; Cole, C.V.; Watandbe, F.; Dean, L. Estimation of Available Phosphorus in Soil by Extraction with Sodium Bicarbonate. *J. Chem. Inf. Model.* **1954**, *53*, 1689–1699.
35. Haby, V.A. Soil NO₃-N Analysis in CA(OH)₂ Extracts by the Chromotropic Acid Method. *Soil Sci. Soc. Am. J.* **1989**, *53*, 308–310. [[CrossRef](#)]
36. Poudel, S. Organic Matter Determination (Walkley-Black Method). 2020. Available online: https://www.researchgate.net/publication/339941885_Organic_Matter_determination_Walkley_-Black_method?channel=doi&linkId=5e6e50fc92851c6ba7063044&showFulltext=true (accessed on 1 September 2024).
37. Lindsay, W.L.; Norvell, W.A. Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper. *Soil Sci. Soc. Am. J.* **1978**, *42*, 421–428. [[CrossRef](#)]
38. Vidyalakshmi, R.; Sridar, R. Isolation and Characterization of Sulphur Oxidizing Bacteria. *J. Cult. Collect.* **2007**, *5*, 73–77.
39. Chaudhary, S.; Dhanker, R.; Tanvi; Goyal, S.L. Characterization and Optimization of Culture Conditions for Sulphur Oxidizing Bacteria After Isolation from Rhizospheric Mustard Soil, Decomposing Sites and Pit House. *World Acad. Sci. Eng. Technol. Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng.* **2017**, *11*, 427–431.
40. Backer, R.; Rokem, J.S.; Ilangumaran, G.; Lamont, J.; Praslickova, D.; Ricci, E.; Subramanian, S.; Smith, D.L. Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Front. Plant Sci.* **2018**, *9*, 1473. [[CrossRef](#)] [[PubMed](#)]
41. Wei, X.; Xie, B.; Wan, C.; Song, R.; Zhong, W.; Xin, S.; Song, K. Enhancing Soil Health and Plant Growth through Microbial Fertilizers: Mechanisms, Benefits, and Sustainable Agricultural Practices. *Agronomy* **2024**, *14*, 609. [[CrossRef](#)]
42. Shaharoon, B.; Jamro, G.M.; Zahir, Z.A.; Arshad, M.; Memon, K.S. Effectiveness of Various *Pseudomonas* Spp. and Burkholderia Caryophylli Containing ACC-Deaminase for Improving Growth and Yield of Wheat (*Triticum aestivum* L.). *J. Microbiol. Biotechnol.* **2007**, *17*, 1300.
43. Shruti, K.; Arun, K.; Yuvneet, R. Potential Plant Growth-Promoting Activity of Rhizobacteria *Pseudomonas* Sp. in *Oryza Sativa*. *J. Nat. Prod. Plant Resour.* **2013**, *3*, 38–50.
44. Mirza, M.S.; Mehnaz, S.; Normand, P.; Prigent-Combaret, C.; Moëgne-Loccoz, Y.; Bally, R.; Malik, K.A. Molecular Characterization and PCR Detection of a Nitrogen-Fixing *Pseudomonas* Strain Promoting Rice Growth. *Biol. Fertil. Soils* **2006**, *43*, 163–170. [[CrossRef](#)]
45. Cole, M.L. Use of Plant Growth-Promoting Rhizobacteria in Tall Fescue and Bermudagrass Forage Systems. Master’s Thesis, Auburn University, Auburn, AL, USA, 2021.
46. Liu, W.; Sun, J.; Ding, L.; Luo, Y.; Chen, M.; Tang, C. Rhizobacteria (*Pseudomonas* sp. SB) Assist Phytoremediation of Oily-Sludge-Contaminated Soil by Tall Fescue (*Testuca arundinacea* L.). *Plant Soil* **2013**, *371*, 533–542. [[CrossRef](#)]
47. Groover, W.; Held, D.; Lawrence, K.; Carson, K. Plant Growth-Promoting Rhizobacteria: A Novel Management Strategy for *Meloidogyne incognita* on Turfgrass. *Pest Manag. Sci.* **2020**, *76*, 3127–3138. [[CrossRef](#)]
48. Arora, N.K.; Mishra, J.; Singh, P.; Fatima, T. Salt-Tolerant Plant Growth-Promoting *Pseudomonas atacamensis* KSS-6 in Combination with Organic Manure Enhances Rice Yield, Improves Nutrient Content and Soil Properties under Salinity Stress. *J. Basic Microbiol.* **2024**, *64*, 2300767. [[CrossRef](#)]

49. Chandra, D.; Srivastava, R.; Glick, B.R.; Sharma, A.K. Drought-Tolerant *Pseudomonas* spp. Improve the Growth Performance of Finger Millet (*Eleusine coracana* (L.) Gaertn.) Under Non-Stressed and Drought-Stressed Conditions. *Pedosphere* **2018**, *28*, 227–240. [[CrossRef](#)]
50. Wintermans, P.C.A.; Bakker, P.A.H.M.; Pieterse, C.M.J. Natural Genetic Variation in *Arabidopsis* for Responsiveness to Plant Growth-Promoting Rhizobacteria. *Plant Mol. Biol.* **2016**, *90*, 623–634. [[CrossRef](#)] [[PubMed](#)]
51. Ramírez-Sánchez, D.; Gibelin-Viala, C.; Roux, F.; Vailleau, F. Genetic Architecture of the Response of *Arabidopsis thaliana* to a Native Plant-Growth-Promoting Bacterial Strain. *Front. Plant Sci.* **2023**, *14*, 1266032. [[CrossRef](#)] [[PubMed](#)]
52. Vidyalakshmi, R.; Paranthaman, R.; Bhakayaraj, R. Sulphur Oxidizing Bacteria and Pulse Nutrition—A Review. *World J. Agric. Sci.* **2009**, *5*, 270–278.
53. Patel, K.; Kapadia, C.; Patel, N.; Patel, D.; Parmar, P.; Datta, R.; Alharbi, S.; Ansari, M. Effect of Supplementing Sulphur-Oxidizing Bacteria with Different Sulphur Sources on the Growth and Development of Chickpea (*Cicer arietinum*). *Plant Stress* **2024**, *12*, 100433. [[CrossRef](#)]
54. Nadeem, S.M.; Hanif, A.; Khan, M.Y.; Waqas, M.; Ahmad, Z.; Ashraf, M.R.; Naveed, M. Elemental Sulphur with Sulphur Oxidizing Bacteria Enhances Phosphorus Availability and Improves Growth and Yield of Wheat in Calcareous Soil. *Arch. Agron. Soil Sci.* **2022**, *69*, 1494–1502. [[CrossRef](#)]
55. Chaudhary, S.; Dhanker, R.; Singh, K.; Brar, B.; Goyal, S. Characterization of Sulfur-oxidizing Bacteria Isolated from Mustard (*Brassica juncea* L.) Rhizosphere Having the Capability of Improving Sulfur and Nitrogen Uptake. *J. Appl. Microbiol.* **2022**, *133*, 2814–2825. [[CrossRef](#)]
56. Koenig, R.; Hurst, C.; Barnhill, J.; Kitchen, B.; Winger, M.; Johnson, M. *Fertilizer Management for Alfalfa*; Utah State University Extension: Logan, UT, USA, 1999.
57. Upadhayay, V.K.; Singh, A.V.; Khan, A.; Sharma, A. Contemplating the Role of Zinc-Solubilizing Bacteria in Crop Biofortification: An Approach for Sustainable Bioeconomy. *Front. Agron.* **2022**, *4*, 903321. [[CrossRef](#)]
58. Ben Zineb, A.; Lamine, M.; Khallel, A.; Hamdi, H.; Ahmed, T.; Al-Jabri, H.; Alsafran, M.; Mliki, A.; Sayadi, S.; Gargouri, M. Harnessing Rhizospheric Core Microbiomes from Arid Regions for Enhancing Date Palm Resilience to Climate Change Effects. *Front. Microbiol.* **2024**, *15*, 1362722. [[CrossRef](#)]
59. Khan, A.L.; Asaf, S.; Abed, R.M.M.; Ning Chai, Y.; Al-Rawahi, A.N.; Mohanta, T.K.; Al-Rawahi, A.; Schachtman, D.P.; Al-Harrasi, A. Rhizosphere Microbiome of Arid Land Medicinal Plants and Extra Cellular Enzymes Contribute to Their Abundance. *Microorganisms* **2020**, *8*, 213. [[CrossRef](#)]

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