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Proceeding Paper

Assessment of Rhizospheric *Streptomyces* Strains as Potential Biopesticides for Further Applications on Wheat Crops †

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Abstract: Actinobacteria species, especially *Streptomyces*, are well known and widely studied as promising biocontrol and phytostimulation agents. They constitute an eco-friendly substitute for chemical fungicides. *Streptomyces*-like strains were isolated from wheat fields to suppress the growth of *Fusarium*, the leading wheat root-rot-associated fungi, and to stimulate plant growth. The isolates were characterized morphologically and biochemically and subjected to a comprehensive in vitro screening for various plant-growth-promoting (PGP) traits. The potential beneficial effects of these strains on wheat plants were evaluated upon their inoculation (germination rate, shoot and root lengths). Among 32 isolates, the strain Act 02 was positive in inhibiting *Fusarium* growth and showing vigorous antifungal activity. In vitro assays demonstrated the ability of Act 02 to produce ammonia and indole-acetic acid (IAA). The strain showed extracellular enzyme production, such as Chitinases, Cellulases, and solubilized phosphate (Ca_3PO_4). The strain Act 02 tolerated high concentrations of NaCl with a considerable interval of [1–8]% (*w*/*v*), with optimum ranges between [1–3]%. 16s RNA gene barcoding and phylogenetic analysis showed that the strain Act 02 belongs to *S. lividans* with a 99.04% similarity. Seed germination and pot experiments were conducted by inoculating *Triticum durum* seeds with a selected isolate extract. Act 02 was able to significantly increase plant lengths.

Keywords: Actinobacteria; agroactive compounds; biocontrol; *Fusarium culmorum*; plant growth promotion

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1. Introduction

Population growth is increasing global food demand, forcing agriculture to improve productivity. However, limited soil macronutrients necessitate agrochemicals, posing environmental and health risks. Innovative, cost-effective, and environmentally friendly strategies are needed to achieve similar agricultural goals [1,2]. Actinobacteria species, especially *Streptomyces*, constitute a group of abundant beneficial microorganisms, have significant biotechnological potential due to their ability to enhance soil fertility through mineral solubilization, nitrogen fixation, organic matter decomposition, and nutrient storage. This study explores Actinobacteria's role as promising biocontrol and phytostimulation agents and their growth-promoting traits, providing insights into their future potential as an eco-friendly substitute for chemical fungicides.

2. Materials and Methods

2.1. Isolation and Identification of Streptomyces-like Strains

Soil samples were collected from the wheat rhizosphere in the Taoura region of Souk-Ahras, Algeria (36°9′48.41″ N, 8°2′37.65″ E). Actinobacteria were isolated on International *Streptomyces* project (ISP2) and selected according to their morphology [2].

2.2. Antagonism Assay by Diffusible Compounds

The antifungal activity of the *Streptomyces*-like isolates was assessed against phytopathogenic *Fusarium culmorum* obtained from the LaMyBAM culture collection using the dual culture method and calculating the inhibition percentage (I%) of fungal growth [3].

2.3. In Vitro Assessment of Plant Growth Promotion (PGP) Traits

- Ammonia production (NH_3) and Hydrogen Cyanide (HCN) production were evaluated according to Farda et al. [4] and Alloun et al. [2], respectively.
- Indole 3-acetic acid (IAA) production was assessed on yeast–tryptone broth supplemented with 0.2% (*w*/*v*) L-tryptophan after eight days of incubation. The IAA quantification process involved centrifuging the culture supernatants for 30 min at 8000 rpm and using the Salkowski reagent with a ratio of (1:2) (*v*/*v*). The pink-red colour indicates indole compounds. A standard curve was generated using synthetic IAA, and optical density was measured at 530 nm using a UV–vis spectrophotometer [5,6].
- Phosphate solubilisation was investigated on Pikovskaya medium (PVK) amended with Ca_3PO_4 [7].

2.4. Enzymatic Profile of Selected Strains

- Protease production, cellulolytic and amylolytic activity, and chitinolytic activity were evaluated according to [1], [8,9], and [2], respectively.
- The ability of the isolate Act 02 to tolerate NaCl concentrations from 0 to 10% (*w*/*v*) (at intervals of 1.0 NaCl unit) was assessed on GYMA after 8 days of incubation at 28 °C.

2.5. Molecular Identification of Act 02

The molecular identification of Act 02 using 16S rRNA gene barcoding and taxonomic affiliation was determined based on sequence similarity with referenced strains from GenBank database. A phylogenetic tree was constructed using the Maximum Likelihood method on MEGA 11 software [10].

2.6. Seed Bio-Priming Assays

Wheat seeds (*Triticum durum*) were disinfected with sodium hypochlorite (2% NaClO for 5 min) and 90% ethanol (for 5 min), rinsed three times with sterile distilled water and air-dried under a laminar flow hood. Actinobacteria spore suspensions at various concentrations were prepared, consisting of the following treatments: A: 6×10^5, B: 2.5×10^6, C: 6.4×10^6, D: 7.2×10^6, E: 9×10^6, F: 3.4×10^7 (spores mL^{-1}), and sterile distilled water as control. Seeds were germinated in the dark and then transferred to pots and incubated at 25 °C/16 h of light per day for two weeks. The germination rates and seedling heights of the co-inoculated group were calculated and statistically compared with the control group.

2.7. Statistical Analysis

All experimental data consisted of the mean of three replicates ± standard deviation (*SD*). One-way analysis of variance (ANOVA), followed by a Tukey post hoc test, comparing mean values at a 5% significance level ($p < 0.05$), was used to determine the statistical significance of differences between groups.

3. Results

3.1. In Vitro Antagonism of Actinobacteria Isolates

Figure 1 displays the in vitro antagonism of Actinobacteria against *F. culmorum*.

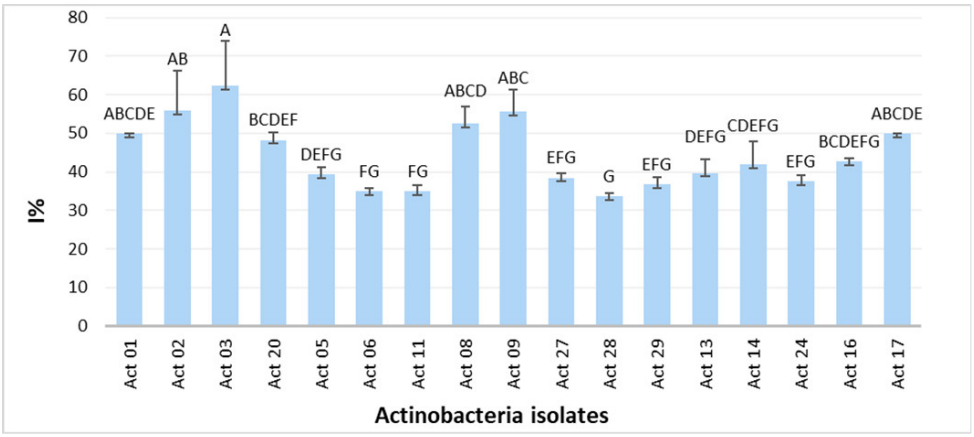

Figure 1. Growth inhibitory rate I% of *Fusarium culmorum*. One-way ANOVA ($p < 0.05$) was used to compare means. Each assay was performed three times with three biological replicates, and values represent their means ± standard deviation (*SD*). Values sharing a letter are not statistically different.

3.2. Plant-Growth-Promoting Activities

The IAA production from Actinobacteria isolates is represented in Figure 2.

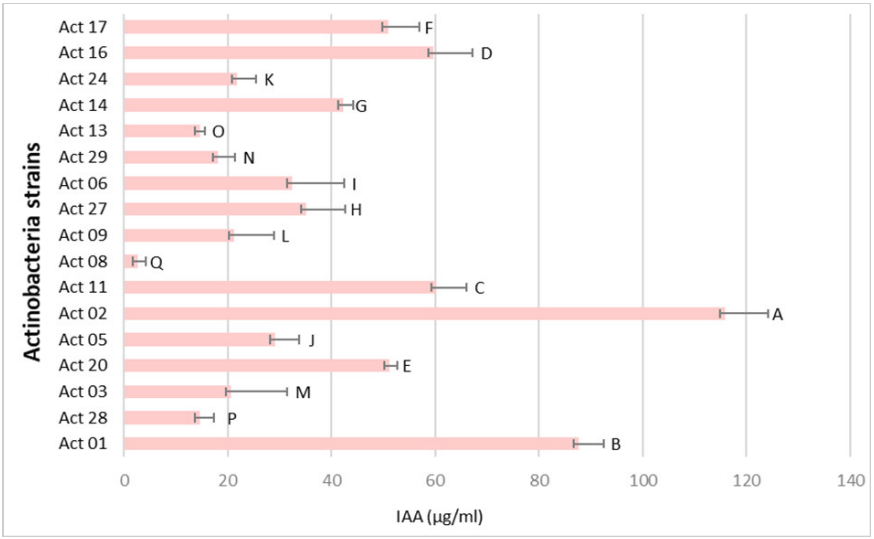

Figure 2. IAA production from Actinobacteria strains (µg mL^{-1}). One-way ANOVA ($p < 0.05$) was used to compare means. Each assay was performed three times with three biological replicates, and values represent their means ± standard deviation (*SD*). Values sharing a letter are not statistically different.

The PGP traits of the collection of rhizospheric Actinobacteria, including HCN, NH_3, phosphate solubilisation and enzyme production, are displayed in Table 1.

Table 1. Enzymatic profile, HCN and ammonia production and P solubilisation of a collection of Actinobacteria isolates.

Isolates	Act 01	Act 28	Act 03	Act 20	Act 05	Act 02	Act 11	Act 08	Act 09	Act 27	Act 06	Act 29	Act 13	Act 14	Act 24	Act 16	Act 17
Caseinases	ND	+	+	+	+	+	−	−	+	+	ND	+	−	−	−	+	−
Amylases	+	+	+	+	+	+	+	+	−	+	−	+	−	+	+	+	+
CMCases	+	−	+	−	+	−	+	+	+	−	−	−	−	−	+	−	+
Chitinases	−	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−
HCN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NH_3	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	+
Ca_3PO_4	−	−	+	−	−	+	−	−	−	+	−	−	+	+	−	−	−

Note: (+): positive; (−): negative.

3.3. Molecular Identification of Act 02

The phylogenetic and taxonomic analysis of the isolate ACT 02 is exhibited in Figure 3.

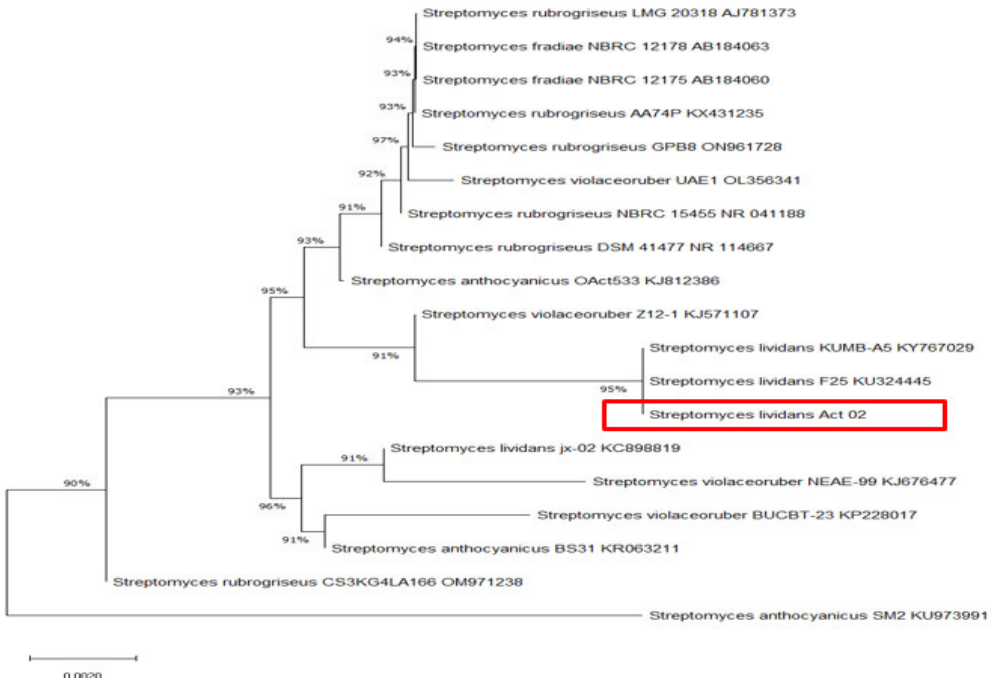

Figure 3. Phylogenetic tree based on NJ method of 16S rRNA gene sequences of *Streptomyces lividans* Act 02 (framed in red) and related strains.

Figure 4 depicts the germination percentages of *Triticum durum* under various Act 02 spore concentrations (A, B, C, D, E, F) and a control group.

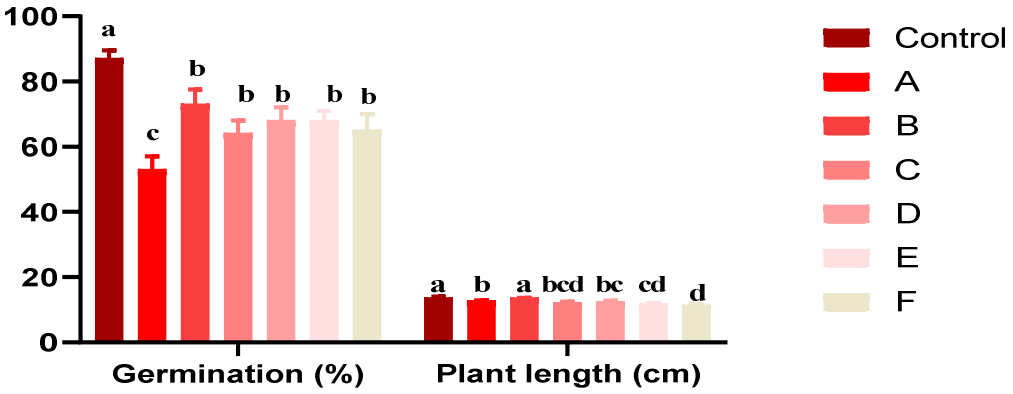

Figure 4. Effect of various concentrations of Act 02 on germination rates and *Triticum durum* plant lengths after 15 dpi. Results are reported as mean ± *SD* (n = 3). Data with different letters were significantly different (Tukey test, $p < 0.05$).

4. Discussion

The role of Actinobacteria biological agents in protecting plants from a variety of soil-borne diseases and to engage in hostile environments has been reported [11]. In this study, 17 out of 32 isolates presented *Streptomyces*-like morphological and cultural characteristics and considerable inhibition rates (I%) of *Fusarium culmorum* growth (Figure 1) with statistically significant differences between the isolates. Actinobacteria's biocontrol activity is correlated with host defence induction, lysis mechanisms and antibiosis [12]. Interest in integrating Actinobacteria strains in plant protection from phytopathogens is growing and

promising candidates for discovering novel secondary metabolites for biocontrol application on crops have been found [2]. Moreover, Actinobacteria can promote plant growth via nutrient solubilization, nitrogen fixation and phytohormone synthesis [13]. Significant levels of IAA have been generated by rhizospheric *Streptomyces* strains [2,14]. *Streptomyces griseorubens* BC10 produced up to 128.44 µg mL^{-1}, according to Boubekri et al. [15]. Our recent study reported the production of IAA from 28 Actinobacteria isolated from wheat rhizosphere in the region of Tiffeche, Algeria, with *Streptomyces rubrogriseus* AW22 exhibiting the highest IAA yields (24 µg mL^{-1}) [2]. According to Figure 2, Act 02 exhibited the highest IAA yield compared with those obtained by Alloun et al. [2]. Ammonia and HCN were discovered to be produced by these isolates. Actinobacteria's antagonistic potential may be linked to HCN generation and diffusible antifungal metabolites, as there was no direct contact between the target fungal pathogen and Actinobacteria [2].

Actinobacteria isolates had distinct enzymatic profiles from one isolate to another. These enzymes actively contribute to the organic matter degradation and the cycling of nutrients in soil, which are both necessary for the survival of strains under different growth conditions [16]. The isolate Act 02 was found to solubilize phosphate and tolerated high concentrations of NaCl 1–8% (optimal at 1–3%). The capacity of Actinobacteria to solubilize phosphate was reportedly less studied than the other PGPR features [17].

The isolate Act 02 presents 99.04% similarity with *S. lividans* using 16S rRNA gene barcoding, and the National Center for Biotechnology Information (NCBI) received its sequence submission for GenBank. Its phylogenetic tree is displayed in Figure 3.

This study investigated the effects of Actinobacteria strain Act 02 spore concentrations on *Triticum durum* germination and growth (Figure 4). The germination rate in the control group was the greatest at 87%, whereas in treatments with decreased spore concentrations (A and F), the germination rate was lower, potentially inhibiting germination. The effects of treatments (B, C, D, and E) with different spore concentrations on germination were variables; however, they were not statistically significant.

The length of the plants in these treatments was just marginally less than the control. Plant growth was unaffected significantly by Treatment B. Further statistical analysis is necessary in light of these findings, highlighting the complex link between Actinobacteria strain Act 02 spore concentrations and durum wheat germination and growth.

5. Conclusions

This study suggests Actinobacteria strains as a potential biocontrol agent against *Fusarium* spp. and plant-growth-promoting tools, offering an alternative to conventional pesticides and seed treatments for wheat seed emergence. However, further research is needed for sustainable cropping systems based on improved soil biodiversity. This report suggests enhancing soil and plant microorganisms for biocontrol, particularly *Fusarium* spp., by developing cost-effective fungicides and fertilizers for Algeria's durum wheat cropping systems.

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