Formulation of biofungicides based on Streptomyces lincolnensis strain SZ03 spores and efficacy against R. solani damping-off of tomato seedlings

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Abstract

This work aimed to explore an antagonistic actinobacterial strain isolated from roots of *Ziziphus lotus* in bioformulation processes and the biocontrol of *Rizoctonia solani* damping-off of tomato seedlings. The strain *Streptomyces lincolnensis* SZ03 was investigated for the principal *in vitro* biocontrol mechanisms and then formulated in three different biofungicides Wettable Talcum Powder (WTP), Sodium-Alginate Propagules (SAP) and Clay Sodium-Alginate Propagules (CAP). Compared to a marketed control products (Serenade® and Acil 060FS®), the formulated biofungicides were investigated against the *R. solani* damping-off of tomato *cv*. Aïcha seedlings. The strain *S. lincolnensis* SZ03 produced chitinases, cellulases, β-1,3-glucanases, cyanhydric acid and siderophores and it showed strong antagonistic effect on the mycelial growth of *R. solani*. Bioautographic and HPLC analysis revealed the production of a single antifungal compound. The biofungicide WTP showed an attractive biocontrol effect by reducing significantly the disease severity index (DSI) compared to untreated seeds. No significant differences were obtained compared to the chemical treatment with Acil 060FS®. The viability of spores and biocontrol efficacy of the WTP were confirmed after one year storage. *S. lincolnensis* strain SZ03 has never been reported in the biocontrol of phytopathogenic fungal diseases and it open up very attractive prospects in the fields of biocontrol and crop improvement.

Introduction

Rhizoctonia solani Kühn is one from the most important phytopathogenic fungi (Cao et al. 2004). This ubiquitous fungus is known to attack various spontaneous or cropped plants, and causes the damping-off, root-rot and crown rot. It can resist in the soil for many years as the form of viable sclerotia (Cao et al. 2004; Agrios 2005). This pathogen is attracted to the germinating seeds and young roots by chemical stimuli and causes important crop losses. The affection by *R. solani* can also causes to the death of non-germinated seeds of several plant species (Sneh et al. 1996).

Damping-off has been recognized as one of the most destructive diseases of tomato seedlings. It can be caused by various soil-borne pathogenic fungi. Nevertheless, *R. solani* has been reported to be the most important causative agent affecting tomato seeds and seedlings (Papadopoulos 1991).

Different cultural practices, such as solarization and burial are applied to manage the contamination of soils by *R. solani*. Nevertheless, all these methods failed to eradicate completely this pathogen (Keinath 1995). Chemical compounds seem to be the most effective treatment on the *R. solani* damping-off. However, the repeated and abusive uses of chemical pesticides against phytopathogens cause negative impact on the stability of ecosystems (Spence et al. 2014). Furthermore, the soil contamination by stable chemical compounds, effect on the human health, elimination of useful entomofaune and the appearance of resistant strains remain the most disadvantages of the intensive uses of chemical fungicides (Adam 2008).

In view of these drawbacks, it is important to reduce the use of chemical pesticides by looking for alternative solutions to manage the most problematic phytopathogens. The biological control methods are increasingly considered in agricultural production as an alternative to chemical fungicides for the biocontrol of phytopathogenic fungi in the soil (Elliott et al. 2009). Several researchers have reported the potential role of actinobacteria in the biological control of fungal diseases and in promoting plant-growth (Winding et al. 2004; Compant et al. 2005; El-Tarabily and Sivasithamparam 2006). From the genus *Streptomyces*, several species have been reported as potential biocontrol agents against phytopathogenic fungi such as *Phytophthora fragariae* (Valois et al. 1996), *Sclerotinia homeocarpa* (Trejo-Estrada et al. 1998), *Gaeumannomyces graminis* (Chamberlain and Crawford 1999), *Pythium ultimum* (Castillo et al. 2002), *Rhizoctonia solani* (Patil et al. 2010), *Fusarium* spp. (Bubici, 2018) and *Botrytis cinerea*, *Alternaria kikuchiana* and *Rhizoctonia solani* (Olanrewaju and Babalola 2019).

The convenience of using a bacteria-based biocontrol agent is mainly related by its bioformulation, which involves the mixing of viable spores or propagules with various carriers for the production of the most effective form (Santhosh 2015).

In this context, our work aimed to highlight the potential of the strong antagonistic actinobacterial strain *Streptomyces lincolnensis* SZ03 in the *in vivo* biocontrol of *R. solani* causing damping-off of tomato seedlings. Firstly, the strain SZ03 will be studied for the main biocontrol traits before the bioformulation process of spores as WTP, SAP and CAP biofungicides. Their biocontrol efficacy will be investigated *in vivo* against *R. solani* and compared to marketed biological and chemical control agents.

Materials And Methods

Actinobacterial strain

Streptomyces lincolnensis strain SZ03 belonged to the bacterial collection of our research laboratory (Laboratoire de Biologie des Systèmes Microbiens – LBSM – ENS de Kouba – Algiers – Algeria). It was isolated and identified by Goudjal et al. (2013) from root of the Saharan native plant *Zizyphus lotus*, which was well adapted in the arid climatic conditions and poor sandy soil of the Algerian Sahara, where symbiotic bacteria may play a major effect in such adaptation. The strain SZ03 was selected through a preliminary study showing strong antagonistic activities against several soil-borne phytopathogenic fungi.

Phytopathogenic fungus

The pathogen *R. solani* was obtained from the fungal collection of our research Laboratory (LBSM, ENS-Kouba, Algeria). This strain was isolated and identified by Zamoum et al. (2017) from typical symptoms of the disease observed on the crowns and roots of tomato plants *cv.* Aïcha harvested in an infected tomato field in Algiers (Algeria).

Biocontrol traits

Production of siderophores

Production of siderophores by strain SZ03 was carried out on chrome azurol (CAS) plates using a method of Sadeghi et al. (2012). Investigated actinobacterial strain was spot inoculated on CAS plates and incubated at 30°C for 7 days. The appearance of clear halo surrounding colonies was considered as positive results for siderophores production. Non-inoculated CAS plates were considered as control (Tamreihao et al. 2016).

Production of hydrogen cyanide (HCN)

Thestrain SZ03 was grown on the King's B medium supplemented with glycine (4.4g l^{-1}). A sterile filter paper (Whatman n°1) flooded with a 0.5% picric acid solution (prepared in 2% sodium carbonate for one minute) was stuck beneath the plate lid. The Petri dishes were then sealed with parafilm and incubated at 30°C until sporulation. Apparition of orange color on the filter paper indicated positive results for HCN production (Mahesh et al. 2015).

Extracellular enzymatic activities

Chitinolytic activity

The chitinolytic activity was investigated using colloidal chitin medium (Ramos-Solano et al. 2010). The strain SZ03 was spot inoculated onto the medium and incubated at 30°C for 5 days. Chitinolytic activity was evaluated by measuring the size of the hydrolytic halo surrounding the bacterial colony.

Cellulolytic activity

The cellulases production by strainSZ03 was determined using a modified methods of Sharma and Bajaj (2005) and Nirmala and Sindhu (2011). Actinobacterial strain was inoculated on ISP9 plates containing carboxymethyl cellulose (CMC) as a sole carbon source and then incubated at 30°C for 7 days. The activity of extracellular cellulase was revealed by flooding the colonies with 0.1 ml of red Congo for 15 min before washing three times with NaCl solution (0.1M). The appearance of clear halos surrounding colonies as well as their diameters was noted.

Protease activity

S. lincolnensis strain SZ03 was streaked on a skimmed milk agar plate and incubated at 37°C for 72h. The clear halo around the bacterial colonies was considered as positive result for protease production (Geetha et al. 2002).

β-1,3-Glucanase activity

The production of β-1,3-glucanase strain SZ03 was investigated according to the method of Singh andal. (1999). A 250 ml-conical flasks containing 50 ml of Tryptic Soya Broth (TSB) medium supplemented with 1% colloidal chitin are inoculated with 1 ml of the spore suspension ($\approx 10^6$ufc ml^{-1}) and incubated at

30°C under permanent agitation (200rpm) for 5 days. The cultures were then centrifuged for 10 min at ×10,000*g* and 1 ml of the supernatant was mixed with 0.1 ml of a laminarine solution and deposited at 40°C for 1 hour. Three ml of 3,5-dinitrosalicylic acid was added to stop the reaction before boiling for 10 min. The color of was measured at 530 nm. The level of reducing sugars was then calculated from a calibration curve drawn by a glucose concentration gradient.

Antifungal compounds

Production, extraction and antifungal activity

The growth of strain SZ03 and production of antifungal compounds was carried out in a 500 ml-conical flasks containing 100 ml of ISP2 broth and inoculated with 2 ml spore suspension ($\approx 10^{-6}$cfu ml^{-1}) before incubation at 30°C with constant shaking (200rpm). The kinetic of growth was carried out by daily sampling of 2 ml of the culture in a 2 ml-Eppendorf tubes. After centrifugation for 10 min at ×4000*g* and washing with distilled water, the tubes were dried at 105°C for 4 h and the dry cell weight was measured. The antifungal activity was studied by the well method (50µl filtrate per well 6 mm in diameter) against *R. solani*. The zones of inhibition were measured daily (incubation at 25°C for 5 days) and the incubation period corresponding to the maximum antifungal activity was noted.

In order to determine the best extraction solvent, the antifungal compounds were extracted separately with n-hexane, dichloromethane, n-butanol and ethyl-acetate before antibiography test against *R. solani*. Thus, 60 ml of filtrate were extracted with 60 ml of the solvent having allowed the best extraction rate. The organic phase was recovered and dehydrated by passage through a filter containing anhydrous sodium sulphate to remove hydrophilic contaminants and dried under vacuum conditions.

Antibiography, bioautography and HPLC purification

The inhibitory activity of the organic phase was tested by antibiography. Eighty µl of the organic extract were deposited on 6 mm in diameter paper discs, dried at 37°C for 30 min then UV sterilized for 30 min. The discs were aseptically placed on the surface of the ISP2 plates (10g of agar l^{-1}) previously inoculated with 6 mm diameter explants from an active culture of *R. solani* LRS1. Plates were then placed at 4°C for 2h and zones of inhibition were noted in millimeters after incubation at 25°C for 72h.

Thin layer chromatography (TLC) on silica gel (GF254, thickness 0.25 mm, Merck, Germany) was carried out by spot deposition of 60 µl of the organic extract, using the mobile phase B.A.E. (acetic acid/n-butanol/water at 1/3/1, v/v/v) until saturation of the tank. The plates were dried then observed under UV light at 254nm (absorption) to locate and delimit the visible spots. The method of Betina (1973) was used to localize the active spots from the organic extracts on the TLC plates and to determine their number and their frontal ratio (FR). The TLC plates were deposited for 12h at 37°C to completely evaporate the solvent and then placed on a glass support in a polyethylene box (22×24 cm). All the device was then UV sterilized for 30 min.

Fifty ml of supercooled ISP2 medium (10g of agar/l) was distributed as a uniform thin film on the plate. After solidification, 6 mm diameter explants of *R. solani* culture were placed on the plate margins. The cultures were placed at 4°C for 2h and then incubated at 30°C. After 72h, the zones of inhibition are noted and the FR's were calculated for the active spots according to the formula:

$$FR = \frac{\text{Active spot migration distance (mm)}}{\text{Solvent migration distance (mm)}}$$

The HPLC purification was carried out in reverse phase: JASCO apparatus, 600 controller, 600 pump, Dual λ Absorption 2487 detector, column C18 (250×7.8 mm UP ODS), mobile phase (methanol-H_2O gradient with linear growth from 50 to 100% in 39 min); flow rate of 0.8 ml min^{-1}, UV detection at 220nm. The final purification was obtained after a second re-injection and the activity of the eluates corresponding to each peak was checked by antibiography against *R. solani*.

Formulation of biofungicides and *in vivo* biocontrol essay

Microbial suspensions

Spore suspension of strain SZ03 was prepared as used by Goudjal et al. (2014). The strain was inoculated on ISP2 plates and incubated at 30°C for 10 days. Actinobacterial pores were recovered in a Tween20 solution (0.05%) and the spores concentration of was adjusted to ≈10^6 spores ml^{-1}.

The method of Dhanasekaran et al. (2005) was used for the preparation of *R. solani* suspension. Potato Dextrose Agar (PDA) plates were inoculates with the pathogen and incubated at 25°C for 6 days. Mycelial fragments were recovered by scraping the surface of the PDA medium and recovered in sterile distilled water. The fungal suspension was adjusted to ≈10^5cfu ml^{-1} using the same method cited above.

Formulation of biofongicides

Spores of strain SZ03 were formulated as WTP, SAP and CAP. All of the formulation processes were done under sterile laboratory conditions.

Formulation of wettable-talcum powder

A modified method of Sabaratnam and Traquair (2002) was used for the preparation of WTP biofungicide. Twenty-five ml spore suspension (≈10^6 spores ml^{-1}) was mixed with 100g of autoclaved talc powder (Fisher Scientific), 10g of CMC and 1.5g of calcium carbonate. The mixture was dried under a laminar flow hood for 12h and finely ground. The WTP biofungicide was weighted and stored in the dark at room temperature.

Formulation of propagules

SAP was prepared using a modified method of Zacky and Ting (2015). The sodium alginate (Sigma-Aldrich) solution (2%) was prepared with sterile distilled water and dissolved under agitation at 45°C. A 25 ml spore suspension (≈10^{-6}cfu ml^{-1}) was mixed with 200 ml sodium alginate solution and pumped using a peristaltic pump through two hypodermics syringes (45 mm length×0.6 mm diameter) and dropped into sterile $CaCl_2$ solution (2.5%) to form and solidify the propagules (0.6-0.8 mm in diameter). Propagules were then dried for 12h under a flow hood, weighted and stored at room temperature.

CAP was prepared using the same process as described above. A 200 ml alginate solution was mixed with 50g autoclaved green clay (0.2 mm in particle diameter). The mixture was dropped through hypodermic syringes (45 mm length×1.2 mm diameter) into the same $CaCl_2$ solution to form propagules (0.8-1.0 mm in diameter), which were dried as described above.

Viability of spores and purity of biofungicides

The viability of spores and purity of the formulated biofungicides were checked before the biocontrol essay and after one year storage at room temperature for the high effective biofungicide. Decimal suspension-dilutions were prepared from each biofungicide and inoculated on ISP2 plates. After incubation at 30°C for 7 days, the SZ03 colonies were authenticated and the possible growth of contaminant microbes was checked.

In vivo biocontrol trials

The tomato (*Solanum lycopersicum* L.) *cv*. Aïcha was selected to investigate the potential of formulated biofungicides in the biocontrol of *R. solani* damping-off. This variety is very wide cultured in fields and presents a great sensitivity to soil-borne phytopathogenic fungi.

In vivo trials were conducted thrice to ensure reproducibility. Five treatments were retained for the *in vivo* biocontrol essay both in autoclaved and non-autoclaved sandy soils. Negative control (NC): surface-disinfected tomato seeds were sown in sandy soils without any treatment to check the germination rate of the seeds. Positive control (PC): surface-disinfected seeds were sown in sandy soils infected with *R. solani*. This treatment confirms the fungus virulence. Biocontrol treatment (BT): the formulated biofungicides were used to control the damping-off of seedling in infected soils. Treatments with marketed chemical agent (AT): Efficacy of the formulated biofungicides was compared with commercially available fungicides: Acil 060FS® (with 60g l^{-1} of Tebuconazole belonging to the Triazoles family). Treatment with the marketed biocontrol agent (ST): Serenade® WP(based on spores of *Bacillus subtilis* strain QST713). These two marketed fungicides are commonly used against several soil-borne fungi such as *Pythium*, *Fusarium*, and *Rhizoctonia*.

The *in vivo* biocontrol assay was performed in a five repetitions of complete randomized block design. The experimental unit is represented by a pot containing 6 tomato seeds.

The sandy soil was treated with the formulated biofungicides. SAP was added at a rate of 1g Kg^{-1} of soil then homogenized using a soil propeller. The CAP was added at the rate of 5g Kg^{-1} of soil. The WTP was liquefied in sterile distilled water (50g l^{-1}).

The *R. solani* symptoms were noted using a 5-class scale (Dhanasekaran et al. 2005): no symptom (0); 0–25% of root browning (1); 26–50% of root browning (2); 51 – 75% of root browning (3); 76–100% of root browning (4) and plant death (5). Annotations were converted to DSI using the following equation:

$$DSI = \frac{[\sum(R \times N)] \times 100}{H \times T}$$

Where: R is the disease rating, N is the number of plants with this rating, H is the highest rating category and T is the total number of plants counted.

In the aim to evaluate the effect of the three formulated products on the plant growth, the root and shoot lengths, and the dry weight were measured for healthy seedlings. To answer the stability and efficacy, the high effective biofungicide was stored at room for one year and *in vivo* trials were then reproduced as described above.

Statistical analysis

Data were subjected to variance analysis (ANOVA). Significant differences between means were compared using Fisher's protected LSD test at P=0.05. Differences were considered significant when P<0.05.

Results

Hydrogen cyanide and siderophores production

The development of orange color on the Whatman filter paper revealed a positive result for HCN production by SZ03 strain (Fig.1A). However, the clear halo surrounding the *strain* SZ03 colonies indicated the production of siderophores (Fig.1B).

Production of extracellular enzymes

Results given in Table 1 showed that SZ03 strain was able to produce all the extracellular chitinases, cellulases, proteases and β-1,3-glucanases. Good growth and sporulation of the strain SZ03 were observed on all used media.

Production and activity of antifungal compounds

Kinetics of growth and antifungal activity

The kinetics of growth and production of antifungal compounds by strain SZ03 on ISP2 medium are given in Fig. 2. Growth of the strain SZ03 begins from the first day of incubation and evolves during the first 4 days (exponential phase), then slows down between the 4^{th} and 6^{th} days (slowing down phase), before stabilizing between the 6^{th} and 10^{th} day (stationary phase with a slight decline).

Antifungal activity against the mycelial growth of *R. solani* begins after 2 days of incubation (exponential phase) and gradually increases to its maximum after 6 days of incubation (beginning of the stationary phase) (Fig. 2, S1).

Antibiography results

Strong activity against *R. solani* was recorded by the ethyl acetate extract and less activity with the n-butanol extract. Nevertheless, dichloromethane and n-hexane did not show any antifungal activity (Fig. S2, Table S1). The ethyl acetate was retained for the extraction of antifungal compounds produced in a larger volume of broth.

Bioautography results

After migration on the B.A.E solvent system and observation of the spots at 254 nm, the TLC plates were biologically autographed by the targeted fungus to localize the active fractions. A clean profile containing only two visible (at 254 nm) fractions A (FR=0.8) and B (FR=0.5) was shown. Only the fraction A was found to be active against *R. solani* (Fig. S3).

HPLC analysis and antifungal compound activity

The chromatographic profile is shown in Fig. S4. After the dead time of the column has elapsed (8 min), we notice (at 220nm) the appearance of a clear profile showing three fractions (F1, F2 and F3) with the appearance of a major peak (28 min time of retention). Only the major fraction F2 has been found active against *R. solani*. This fraction was re-injected into the HPLC system, purified and the antifungal activity on the targeted fungus was confirmed (Fig. 3).

Formulation of biofongicides

Viability of spores and purity of biofungicides

Purity of the formulated biofungicides and viability of SZ03 spores were proved before proceeding to the *in vivo* trials and after one year storage at room temperature. After inoculation of the formulated biofungicides on ISP2 plates at 30°C for 7 days, colonies of actinobacteria with identical morphology to SZ03 colonies were successfully identified from the three formulated biofungicides. Furthermore, no contaminant microbes were observed (Fig. S5, S6). The densities of viable spores were evaluated at $\approx 2.0\times10^{6}$cfu g^{-1} and $\approx 4.5\times10^{5}$cfu g^{-1} for SAP and CAP biofungicides respectively. The high effective biofungicide WAP yielded $\approx 2.1\times10^{5}$cfu g^{-1} and 2.0×10^{5}cfu g^{-1} before and after one year storage at room temperature respectively.

Efficacy of formulated biofungicides

The results of the incidence of root and crown rot in autoclaved and non-autoclaved soils are given in Fig. 4. High DSI was observed for the PC, which prove the virulence of the targeted fungus (Fig. S7). The biocontrol treatments reduced significantly (P<0.05) the DSI and no significant differences (P<0.05) were obtained in autoclaved and in non-autoclaved soils.

The most effective biocontrol treatment was reached with the WTP biofungicide. Furthermore, no significant differences (P<0.05) were shown compared to the chemical treatment with Acil 060FS®. Nevertheless, significant differences (P<0.05) were observed compared to the marketed biocontrol agent Serenade® (Fig. 4).

In vivo biocontrol using WTP, SAP and CAP permeated to investigate their effect on the growth of healthy seedlings. Growth parameters were evaluated in the aim to select the high interesting biofungicide which can also promote the growth of tomato *cv.* Aïcha seedlings.

The WTP biofungicide improved all the plant growth parameters compared to healthy seedlings from the negative control, Serenade® and Acil 060FS® (Fig. 5, S8). However, the treatments with CAP biofungicide showed a weaker effect on the growth promotion of seedlings (Fig. 5).

Results from the *in vivo* trials carried out after one year storage of the WTP biofungicide at room temperature showed high protective effect against the *R. solani* root rot as well as for their PGP effect on tomato seedlings. This proves the viability of ZS03 spores and their biocontrol efficacy as WTP biofungicide (Fig. 4, 5).

Discussion

Actinobacteria are well known to colonize the rhizosphere on the basis of their competitive and antagonistic properties, especially against soil-borne fungi (Sarmiento-Vizcaíno et al. 2021). The small molecular weight iron scavengers named siderophores are mainly produced by microbes, and plants. They have attracted recently increasing attention because of their potential role in environmental bioremediation, PGPB and biocontrol (Roskova et al. 2022). *S. lincolnensis* strain SZ03 produced siderophores and showed high antifungal activity against *R. solani*. Getha et al. (2005) supported these findings by reporting the efficacy of *Streptomyces griseorubiginosus* producing siderophores in the biocontrol of *Fusarium oxysporum* f. sp. *cubense*.

The strain SZ03 produced the HCN recognized as volatile antifungal compound, which inhibits the cytochrome oxidase of the pathogenic fungus (Szilagyi-Zecchin et al. 2016). Several researchers reported the role of this compound in the suppression of soil-borne phytopathogenic fungi belonging to the genus *Fusarium* (Gopalakrishnan et al. 2011). Moreover, Blom et al. (2011) and Kai et al. (2010) reported that mutants unable to produce HCN failed to inhibit the mycelial growth of phytopathogenic fungi. In

addition, this compound has been reported for improving the root system growth (Luz 1996; Passari et al. 2015).

The production of lytic enzymes is another mechanism very widely involved in the parasitism of telluric fungi (Harman et al. 1993; Madi et al. 1997; Behera et al. 2019; Behera et al. 2020). The strain SZ03 produces the lytic enzymes chitinases, cellulases, proteases and glucanases, which can be involved in the degradation of the fungal wall (chitin and β-1,3-glucans), in the destruction of pathogenic molecules through proteases or in the degradation of fungal spores by cellulases (Singh et al. 1999; Behera et al. 2020).

Bioautography was performed determine the number of antifungal compounds active against *R. solani*. At this stage, it has been revealed only one inhibition zone, which could be explained by single or several antifungal compounds migrating for a same FR. Several works have reported the production of lincomycin by *S. lincolnensis* isolated from soil, with antioxidant and antitumor activities against the human breast cancer cell line (Kim et al. 2008). Mohamed et al. (2021) isolated equally lincolnenin from *S. lincolnensis* witch exhibited a great activity against Gram-positive pathogens. Nevertheless, the novelty of our work was to report for the first time the efficacy of *S. lincolnensis* strain SZ03 against *R. solani* damping-off of tomato seedlings.

The formulation of biofungicides WTP, SAP and CAP based on SZ03 spores gave homogeneous biofungicides. The viability of spores and purity were proved before the biocontrol trials and after one year storage at room temperature. Our results are confirmed by those of Sabaratnam and Traquair (2001) and Bencheikh and Setti (2010) which reported high purity and viability of *Streptomyces* spores formulated in talcum powder and sodium alginate beads.

The *in vivo* biocontrol trials showed a lowest DSI reached by the WTP biofungicide and no significant difference was obtained compared to chemical control agent Acil 060FS®. In addition, WTP biofungicide allowed more interesting biocontrol effect compared to the marketed Serenade®. According to Lumsden et al. (1995) and Green et al. (1998), the talcum powder requires simple technology and is more cost effective than alginate beads formulation. The high efficacy of the WTP can be explained, according to Bencheikh and Setti (2010), by the rapid propagation of *Streptomyces* spores in the rhizosphere. In addition, the size of talcum powder particles is very suitable for mixture and use as a soil amendment. On another hand, WTP biofungicide based on SZ03 spores showed also the best plant growth promoting effect on tomato seedlings by enhancing the root length, seedling length, fresh and dry weights. These results are in agreement to those of Bencheikh and Setti (2010) reporting the efficacy of formulated talcum powder spores of *Streptomyces* sp. both in the biocontrol of *Mycosphaerella pinodes* causing the foot rot in pea and in the improvement of pea seeds germination. The biocontrol and plant growth efficacy of the WTP biofungicide have been retained even after one year storage at room temperature. This can be related, as reported by Allali et al. (2019) by the high level of viable spores formulated in talcum powder carrier.

Conclusion

The strain *S. lincolnensis* SZ03 produces HCN, chitinases, proteases, cellulases, β-1,3-glucanases and siderophores, which can act directly and effectively in the biocontrol of *R. solani*. It produced a single antifungal compound detectable by bioautography.

Treatment with WTP biofungicide based on SZ03 spores is the most effective treatments. These results are closely related to those of Acil 060FS®. The WTP biofungicide promoted the growth of tomato *cv*. Aïcha seedlings and remains the most successful treatment. Furthermore, Efficacy of the WTP biofungicide was confirmed after one year storage at room temperature.

As an alternative of control chemical treatments, the biofungicide WTP based on *S. lincolnensis* strain SZ03 spores open very promising perspectives in the biocontrol of *R. solani* damping-off of tomato seedlings.

Declarations

Acknowledgements Dedication: This paper is dedicated to the memory of the defunct Pr. Nasserdine Sabaou (1956-2019), the excellent researcher and the great professor of microbiology (Ecole Normale Supérieure de Kouba, Alger-Algeria) and the Director of our research laboratory, the LBSM (Laboratoire de Biologie des Systemes Microbiens). Professor Nasserdine Sabaou has been a constant source of knowledge and inspiration. The authors acknowledge the support of the Ministry of Higher Education and Scientific Research of Algeria.

Authors' contributions We confirm that this research was conducted in our research laboratory. All authors contributed critically in performing and analyzing the results. YG, AZ and MZ conceived and designed the research. KA, MZ and AB conducted the experiments and analyzed the data. KA YG wrote the manuscript. MZ supervised the research, corresponding author. All authors read and approved the manuscript.

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Data availability All data and materials are available.

Conflict of interest Authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Human and animals rights This article does not contain any studies with human participants or animals.

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Tables

Table 1
Production of extracellular enzymes by *Streptomyces lincolnensis* strain SZ03

Strain	Chitinase		Protease		Cellulase		β-1,3-glucanase
	Growth	Halo diameter (mm)[a]	Growth	Halo diameter (mm)[a]	Growth	Halo diameter (mm)[a]	reducing sugars Activity (mg ml^{-1})[a]
SZ03	+	22 ± 1.3	+	12 ± 1.4	+	8 ± 1.6	14 ± 0.2 ++
a: Mean of three replicates ± standard error							

Figures

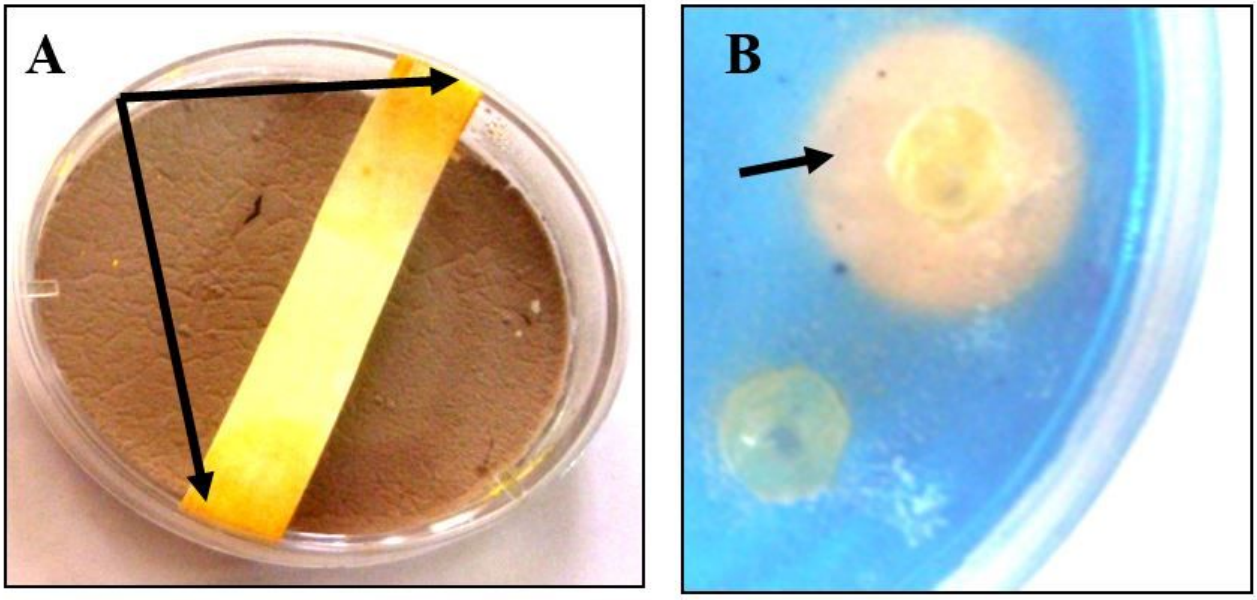

Figure 1

Production of hydrogen cyanide (A) and siderophores (B) by *Streptomyces lincolnensis* strain SZ03 (A). Arrows indicates the orange color on the filter paper and the clear halo on CAS agar. Photography were taken after 7 days culture at 30°C

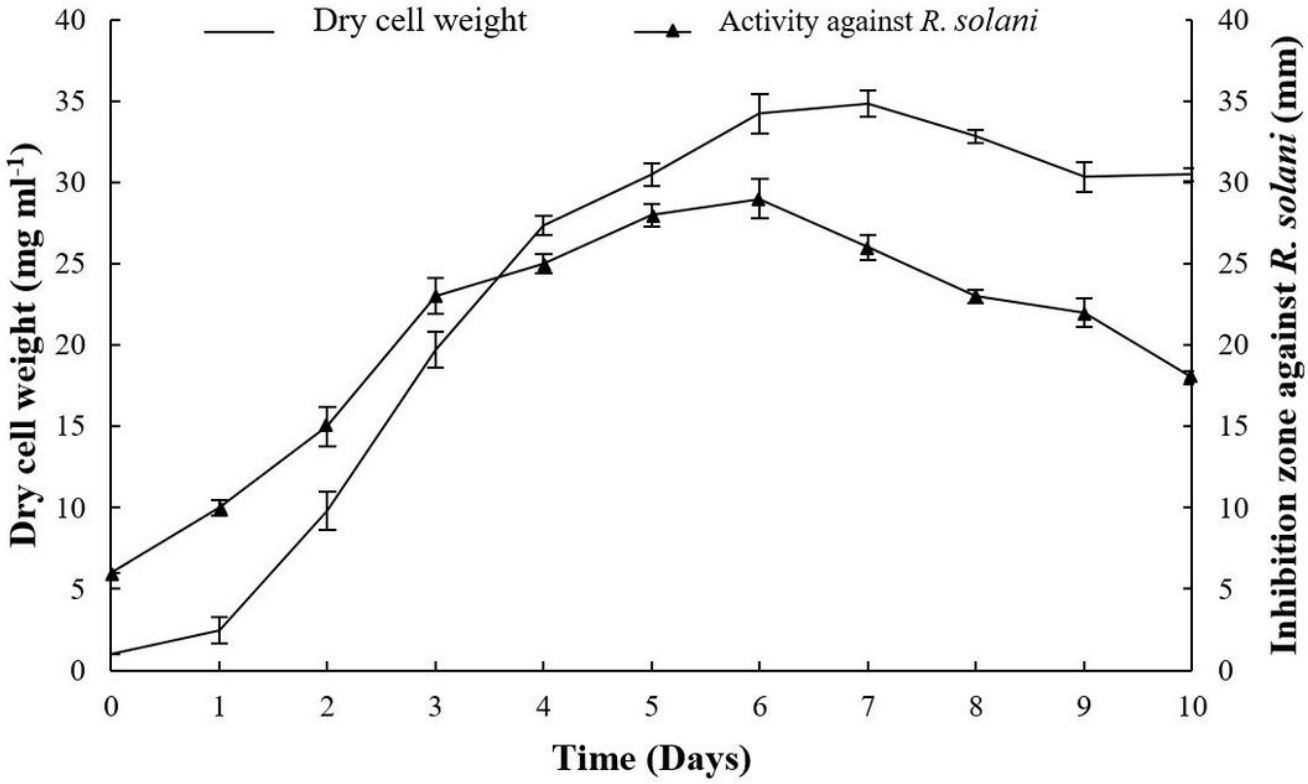

Figure 2

Kinetics of the dry cell weight of *Streptomyces lincolnensis* and the antifungal activity of culture against *Rhizoctonia solani*. Error bares represents the standard deviation from three replicates

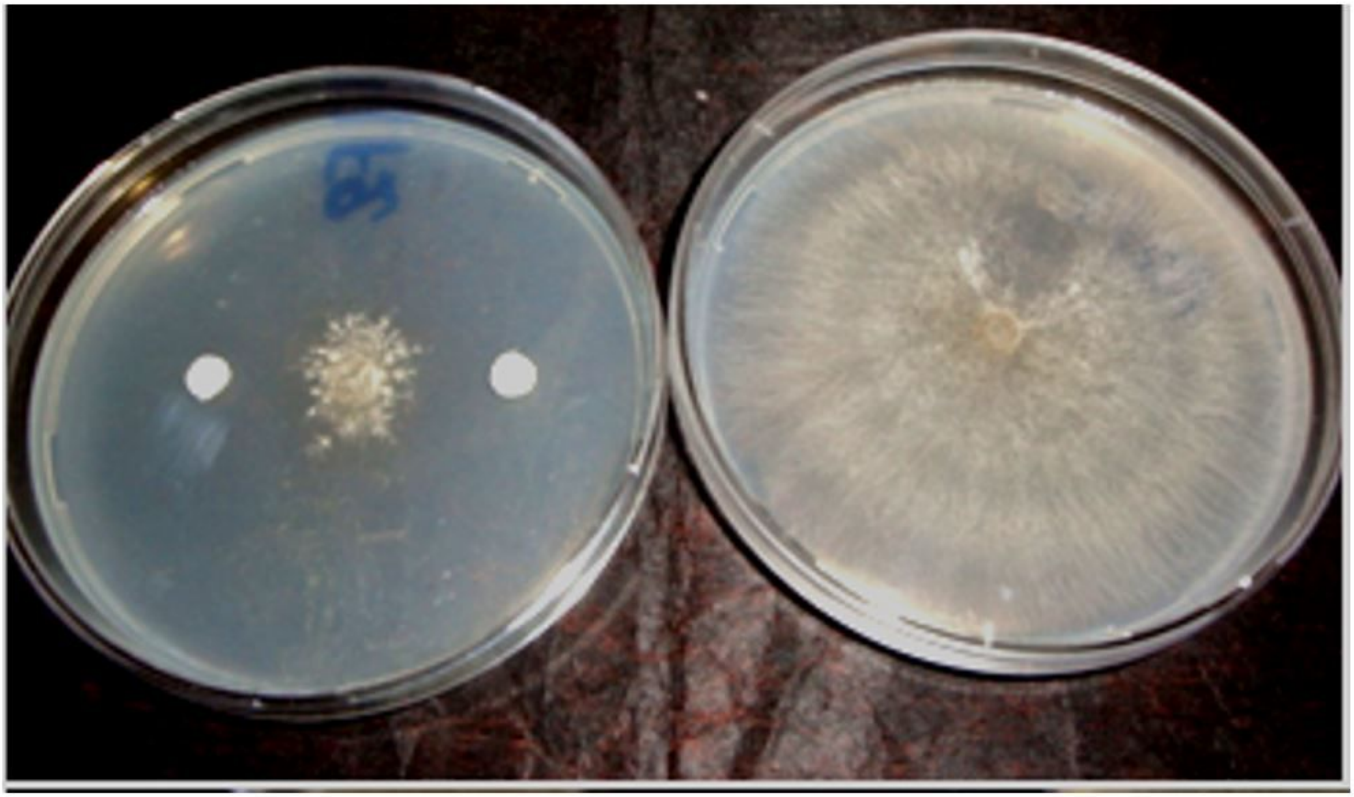

Figure 3

Antibiography showing the antifungal activity (left) of purified antifungal compound "F2" on the mycelial growth of *Rhizoctonia solani* and the control culture (right). Photography was taken after 3 days culture on ISP2 medium at 25°C.

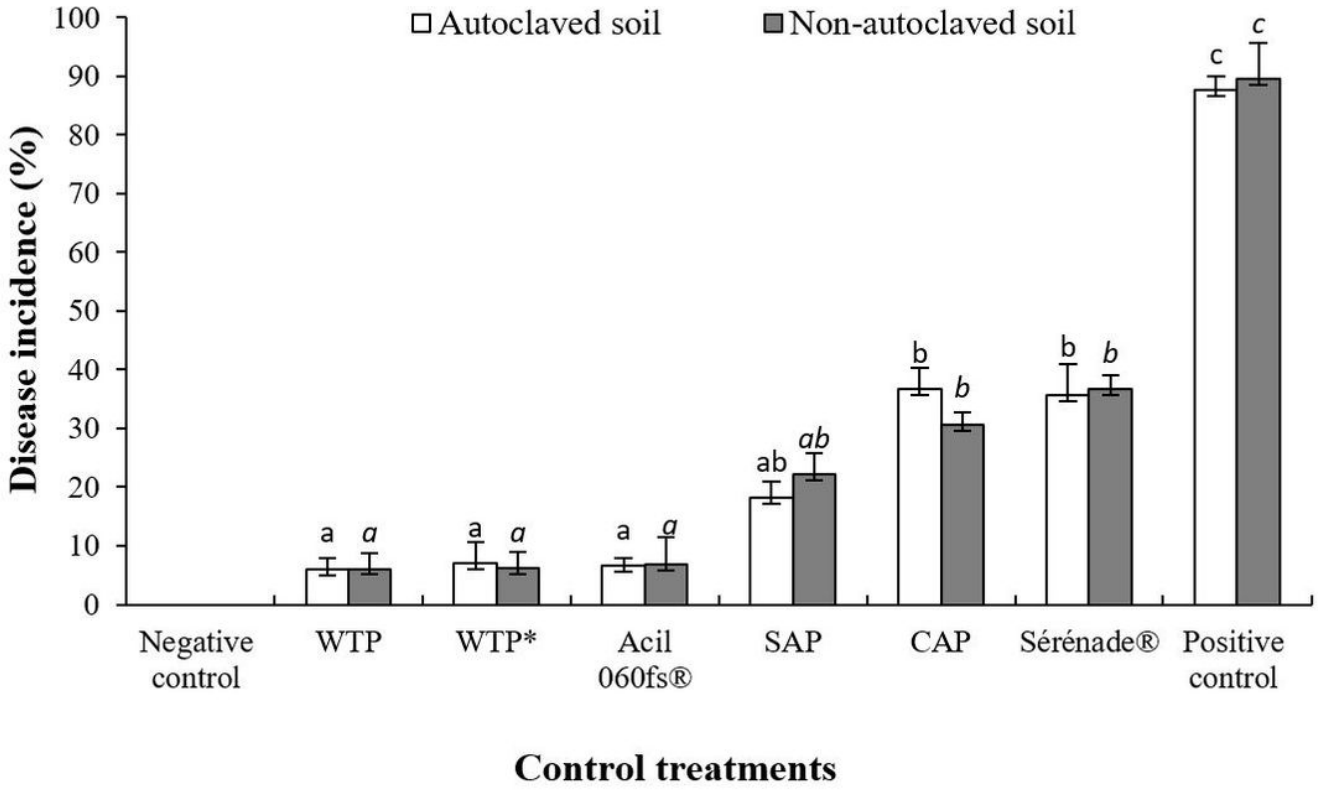

Figure 4

Effect of treatment with formulated biofungicides, Acil 060FS® and Serenade® on the disease incidence caused by *Rhizoctonia solani* in autoclaved (white) and non-autoclaved (grey) soils. The control treatments correspond to untreated seeds sown in non-infested soil (negative control) or in infested soil (positive control). Evaluation was made 30 days after planting. Control treatment with (*) was carried out after one year storage at room temperature. Bars labeled with the same letters are not significantly different according to Fisher's protected LSD test at $P = 0.05$. Error bars represent the standard deviation from 15 replicates

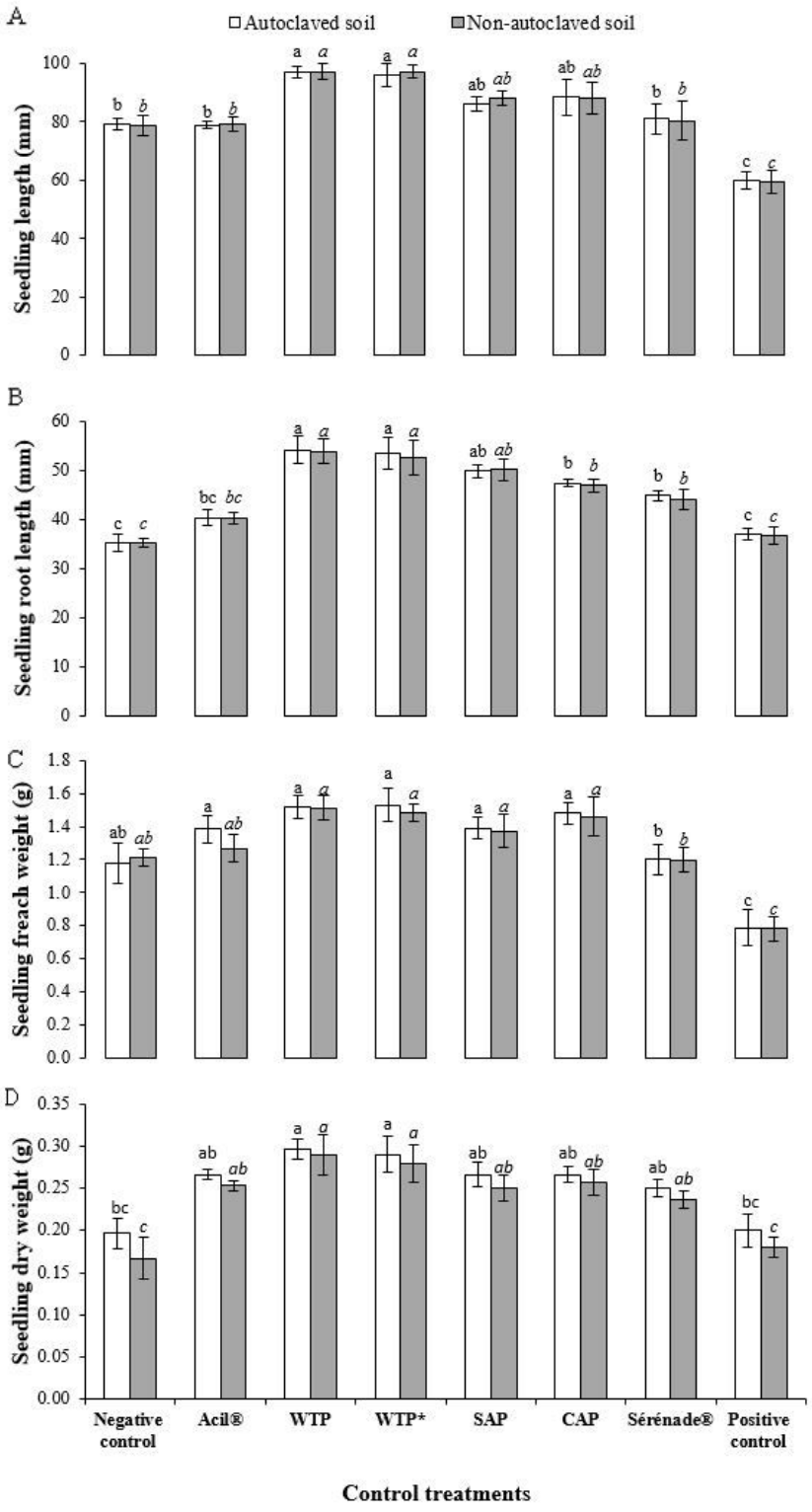

Figure 5

Effect of treatment with formulated biofungicides, Acil 060FS® and Serenade® on the seedling length (A), seedling root length (B), seedling fresh weight (C) and seedling dry weight (D) in autoclaved (whit) and non-autoclaved (grey) soils. The control treatments correspond to untreated seeds sown in non-infested soils (negative control) or in infested soils (positive control). Evaluation was made 30 days after planting. Control treatment with (*) was carried out after one year storage at room temperature. Bars labeled with

the same letters are not significantly different according to Fisher's protected LSD test at $P = 0.05$. Error bars represent the standard deviation from 15 replicates

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