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Research Article

Evaluation of the antagonistic potential of bacterial strains isolated from Algerian soils for the biological control of phytopathogenic fungi

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Abstract
Antagonistic bacteria contribute to the management of plant diseases by stimulating the natural defenses in the host and/or by ensuring direct biocontrol of the aggressors. The objective of this work was to isolate, identify and evaluate (*in vitro*) various *Bacillus* spp. for their potential to control phyopathogenic fungi. Selection of the 40 strains of *Bacillus* previously isolated from the soil in various areas of western Algeria was carried out by direct confrontation on the mycelial growth of four phytopathogens (*Fusariumoxysporumf.splycopersici, Alternaria tenuis , Phytophthorainfestans, Ascochytapisi*). This strategy involved using the antagonistic potential of microorganisms found in the plant environment in Algeria. The second part of this work consisted of the characterization and identification of tested strainsThe identification of the selected strains was carried out by biochemical tests. The results obtained showed that at the end of the fifth day, the most promising isolates showed antifungal activity and reached an inhibition rate of the mycelial growth of phytopathogenic fungi, respectively, *F. oxysporumf. splycopersici* 75%, *A. tenuis* 80%, *P. infestans* 83.30%, *Ascochytapisi* 67%. The potential antagonist of Bacillus tested in vitro by direct confrontation against 04 phytopathogenic fungi showed that all strains of Bacillus decreased fungal mycelial growth. Two strains of Bacillus B30 and B41 were found to have the most efficacy against *Fusarium oxysporum f.sp. lycopersici, Alternaria tenius, Phytophtora infestans et Ascochyta pisi*, with an inhibition rate of 65.25 and 72.25% respectively These results demonstrate that *Bacillus* sp. presenteds a potential for biological control. However, it is important to understand the mechanisms implemented by these bacteria to develop effective protection strategies.

Keywords: Antagonism, *Bacillus* , Biocontrol, Pest, Phytopathogens fungi

INTRODUCTION

For more than a century, many researchers have been interested in understanding the interactions between plants and microorganisms in the rhizosphere. Some soil-derived bacteria are beneficial for plants, both in terms of pathogen control and growth promotion. Phytopathogenic fungi are mainly responsible for plant crop losses and have generally been controlled by chemical pesticides. However, the emergence of treatment-resistant pathogens, public health concern and environmental issues are driving the search for viable alternatives to these products (Karkachi *et al*., 2010; Saeed *et al*., 2021; Zhang *et al*., 2021). Integrated Pest Management (IPM) aims to encourage the use of natural mechanisms to restrict the use of chemicals. Biological control of plant pathogens through the introduction of beneficial microorganisms into the rhizosphere has been proposed as an alternative to the use of chemicals. Antagonistic bacteria can secrete bioactive molecules that have antifungal abilities, to improve plant growth and induce plant resistance (Zhang *et al.,* 2017) .Several species of bacterial biological agents have been reported to be effective against soil-borne pathogens such as *Rhizoctonia solani*(Karimi *et al.,* 2016), *Phytophthora* spp. (Thampi *et al.,* 2017), and *Pythium* spp. (Gerbore *et al.,* 2014). Bacillus subtilis is one of the most commonly used bacteria to control plant diseases (Bettiol and Morandi. 2009). These bacteria are gram positive, aerobic (optionally anaerobic), spore forming in which sporulation provides a mechanism of resistance to changes in the environment (Tejera *et al.,* 2012). Biofungicides, derived from either microbes or plants, have emerged as promising alternative strategies. Among the toxinogenic fungi that contaminate many plants in the field, the most harmful belong to the genus of Fusarium, Alternaria, Phytophthora and Ascochyta(Kharayat&Singh, 2018; Perincherry et al., 2019). Therefore, this work aimed to isolate, identify and evaluate (in vitro) various Bacillus spp isolated from soil in different regions of western Algeria for their potential to control phytopathogenic fungi (Fusarium, Alternaria, Phytophthora and Ascochyta) and also

MATERIALS AND METHODS

Origin of bacteria isolates

Seven soil samples were collected from two regions of western Algeria: six samples from Oran and one sample from Ain Temouchent. Samples were collected at a depth of 10 cm from the rhizosphere of olive and apricot trees, and vegetable crops (carrot, tomato, bean, potato). All samples were placed in sterile paper bags and transferred to the laboratory (Fan *et al.,* 2016).

Isolation of antagonistic bacteria from soil

Isolation of the 40 potentially antagonistic bacteria was achieved by the dilution method 10 g of soil was mixed with 9 ml of sterile physiological water. After vortex agitation, dilution series up to $1/10^4$ were carried out. 100 µl of each dilution was inoculated into two sterile Petri dishes containing Potato Dextrose Agar (PDA) and Nutritive agar (NA) media. The plates were incubated at 37°C for 24 hours. The colonies obtained after incubation are then purified and kept at 4°C. *Bacillus* colonies were identified based on morphology, shape, color, Gram type, cellular form, and spore presence. The subsequent identification test includes the amylase test.

Origin of the phytopathogenic fungi

The Fusarium wilt, ascochyta blight, alternaria black spot and downy mildewsymptoms present on stems or leaves, were cut into 1 cm segments, surface-disinfested in 6% sodium hypochlorite for 3 min and rinsed in sterile distilled water for 3 min. Stem pieces were placed on culture medium PDA: 200 g potato, 20 g Dextrose (Sigma), 20 g Agar (Sigma), 1 L distilled water. Single conidia were germinated and grown on PDA medium at 21°C with a 12 h alternating light-photoperiod. After 6 days of incubation, the presence of phytopathogenic fungi was confirmed by microscopic observation of conidia (Gharbi *et al*., 2013 ;Gharbi *et al*., 2015)

Evaluation of *Bacillus* for the antagonist potential against plant pathogens

Isolates with antifungal activity were screened on PDA medium. For this purpose, the strains of *Bacillus* spp. tested for their ability to inhibit mycelial growth were spotted on the edge of the Petri dishes. Then a 7-day-old disc of the plant pathogen fungus (5 mm in diameter) was deposited in the center of the plate. Discs of the fungus were placed on the control plates in the absence of the antagonist bacterium. The incubation of the plates was done at 28°C for 5 days. The growth diameters of the fungi are then measured and compared to the control (Djellout *et al*., 2019).

The experiment was repeated four times for each bacterial isolate. The antagonistic capacity of bacterial isolates was evaluated after 5 days of incubation at 28°C by calculating the percentage of inhibition of mycelial growth compared to the control using the following formula (Kerroum *et al*., 2015).

$$\text{Mycelial inhibition} = \frac{D1-D2}{D1} \times 100 \qquad \text{....Eq. 1}$$

D1: Diametric average growth of the pathogen in the control plates

D2: Diametric average growth of the pathogen in the confrontation plates.

Amylase test

The test that indicated amylase activity was performed on starch agar media. After incubation at 37°C for 24 to 48 hours, iodine solution was dispersed over the entire surface of the medium. After a few minutes of contact, excess iodine solution was removed and the plates were washed with distilled water. The positive result was revealed by the presence of a clear zone around the colony.

RESULTS AND DISCUSSION

The criteria considered wassufficient according to (Bouali *et al*., 2016) to affirm the belonging to the genus *Bacillus*were the macroscopic appearance of the colonies on the agar, the positive reaction to the Gram staining, the mobility, and the presence of spores inside the bacterial cells (Fig.1).

Screening of antagonistic bacteria

The appearance of a clear zone around the bacterial colony after incubation demonstrated inhibition of the fungal strains by the antagonists. The results obtained showed that *Bacillus* inhibited mycelial growth (Fig.2). There wasaninhibition rateof 75% with *Fusariumoxysporumf.splycopersici*and 80% with *Alternaria tenuis*, 83.30% with *Phytophthora infestans*, and 67% with *Ascochytapisi*. Of the 40 bacterial strains tested, 28 (70%) had a growth inhibitory action greater than 20%and ; on *Fusariumoxysporumf.splycopersici* (28.57%), *Alternaria tenuis* (32.14%), *Phytophthorainfestans* (50%), *Ascochyta*pisi(46.42%), compared to controls.

Antifungal activity of *Bacillus* spp. on phytopathogenic fungi

Of the 28 bacteria having an action on phytopathogenic fungi, eight strains of *Bacillus* spp (28.57%) (B1, B12, B15, B12, B2, B3, B30, B41) have a remarkable inhibition on the mycelial growth of *Fusarium oxysporum* f. *splycopersici* on NA and PDA medium (Fig.3). It is also noted that nine strains of *Bacillus* (32.14%) have an effect on *Alternaria tenuis*, five of which (B30, B13,B1, B41, B11) have an inhibitory action on the mycelial growth of the fungus in both media (Fig.4). The inhibitory action of bacterial strains on *Phytophthora.infestans* showed that 14 strains, B42, B7, B8 B5, B32, B30, B1, B44, B41, B6, B14, B10, B4 inhibit the growth of the fungus more than 50 % (Fig.5). The percentage of inhibition on the mycelial growth of *Ascochyta.pisi* is obtained with the 14 bacteria (46.42%) B44 B30, B41,B8, B14, B9, B3, B4, B11, B1, B34 B38, B13, B42, which present a more antagonistic effect on the PDA medium than on the NA medium (Fig.6).

DISCUSSION

The evaluation of the antagonistic activity of three bacterial and a fungus with direct confrontation method and the filtrates culture against the growth of Fusarium oxysporum f. sp. *lycopersici* showed the inhibition of the mycelia growth of *Fusarium oxysporum f. sp. lycopersici* with *Bacillus cereus* energized the low activity and it was more significant with Serratia marcescens and *Trichoderma harzianum* for the 2nd day but with *Pseudomonas fluorescens*, it was for the 5th day. The filtrates of culture of these antagonists showed that only *Serratia marcescens* and *Trichoderma sp*. have a rate of inhibition which varies between (40-95%) and of (20-30%) with *Pseudomonas fluorescens*(Karkachi *et al*., 2010). The antifungal activity of *Bacillus* spp. is well known and has been reported and discussed in several studies. In this study, we focused on their action against the most common and problematic plant pathogens in Algeria. Isolation has occurred in the rhizosphere of plants to be protected, as this is not only the area where the microorganisms are most abundant (Adhya *et al*., 2017) but also as suggested by Weller (988), biological control agents are more effective

Fig. 1. A. *Microscopic and B. Macroscopic observation of Bacillus strain B2*

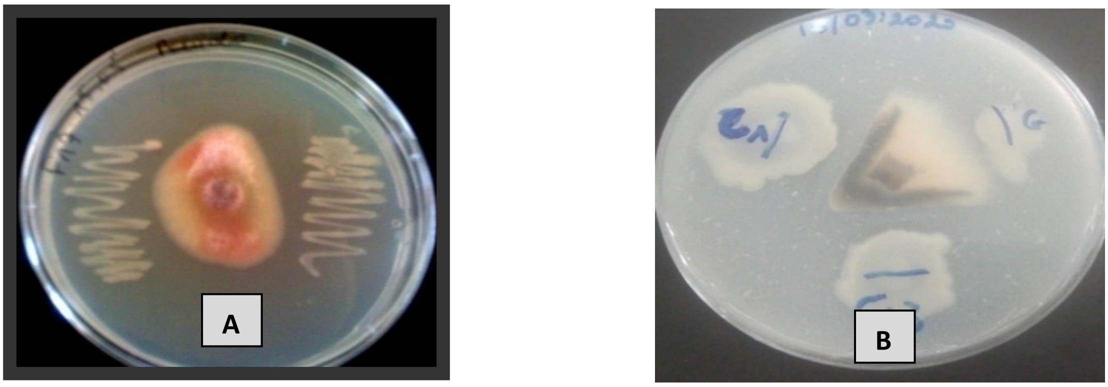

Fig. 2. *Direct confrontation of Bacillus between A. Fusarium and B. Ascochyta*

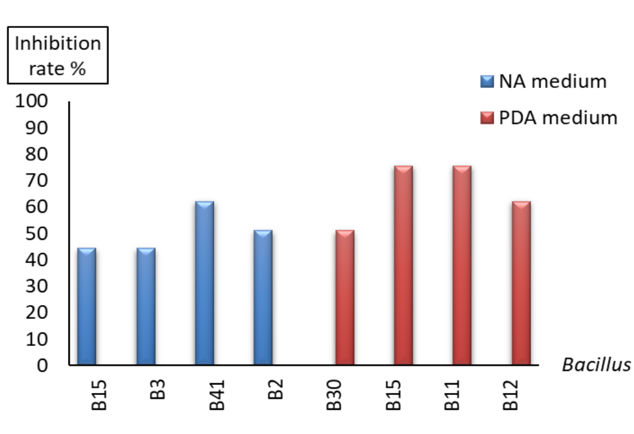

Fig. 3. *Inhibition rate of Bacillus against Fusarium oxysporum F. sp lycopersici*

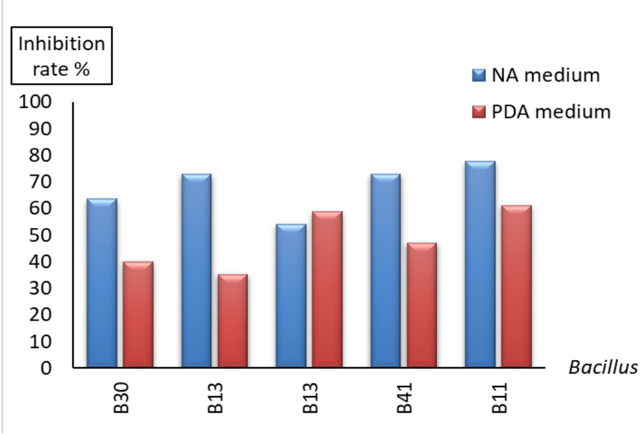

Fig. 4. *Inhibition rate of Bacillus against Alternaria tenuis*

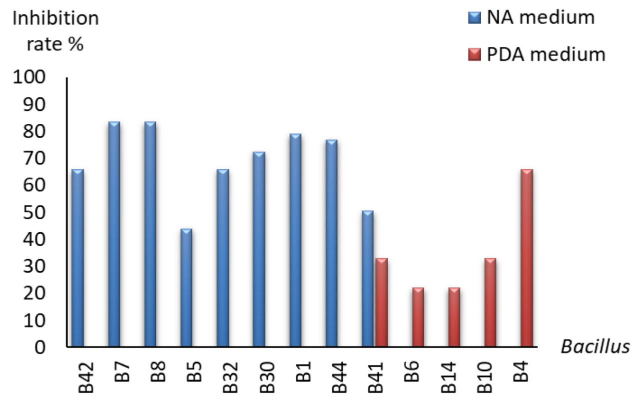

Fig. 5. *Inhibition rate of Bacillus against Phytophtora infestans*

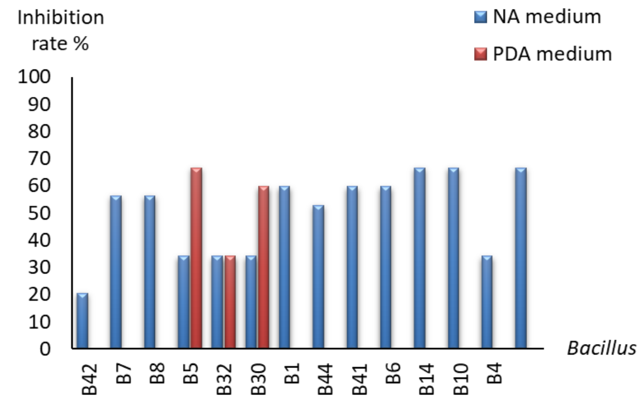

Fig. 6. *Inhibition rate of Bacillus against Ascochyta pisi*

when applied in the same environment as their isolation site. In addition, during in vitro confrontation tests, their activity is influenced by the type of medium used (Peighamy-Ashnaei *et al*., 2008). Therefore, in our study, the PDA and the NA media were used in the confrontation method. The results showed that in some strains, the antifungal activity differs depending on the medium used. However, some strains such as KH17 and KH12, tested against *Phytophthora* and *Alternaria* respectively, showed almost the same results regardless of the culture medium (Saleh *et al*., 2021) also carried out a similar study on the evaluation of the antifungal activity of *Bacillus* sp. depending on the medium type, in which three different media were used: NA, LB agar (Luria Bertani) and TSA (Tryptone soy agar). The antifungal activity of the strain of *Bacillus* tested varied between media. Moreover, the ability of a biological control agent to inhibit several plant pathogens can be a major asset since farmers often face several plant pathogens attacking their crop at the same time. In order to control these pathogens, a single biological agent application would be more cost-effective (Spadaro *et al*., 2005). Therefore, in this study, strains of *Bacillus* were evaluated against several phytopathogens. Strains B30, KH4 and B41 showed inhibition rates greater than 50% against all phytopathogens tested. (Diabankana *et al*.2021) reported the same results in which biological agents inhibited plant pathogens such as *Ascochyta pisi* and *Fusarium oxysporum*. This ability can be attributed to the production of antifungal secondary metabolites (Madriz-Ordeñana *et al*., 2022).

Conclusion

The use of biological control agents in the management of soil-borne fungal diseases is a promising alternative to chemical pesticides, both environmentally and economically. These biological agents can be applied to plants for the biological control of pathogens. In this study,The results showed that the majority of bacterial isolates of *Bacillus*spp isolated from the soil of western Algerian regions produce antifungal secondary metabolites capable of significantly inhibiting the in vitro development of phytopathogenic fungi (*Fusarium oxysporum*f.sp*lycopersici, Alternaria tenuis, Phytophthora infestans, Ascochyta pisi*). Finally, it can be said that *Bacillus* is one of the most well-known and widely used microorganisms in the field of biological control of several plant pathogens. Most species of the genus *Bacillus* are already in use successfully in agriculture.

Conflict of interest

The authors declare that they have no conflict of interest.

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