SCREENING OF ANTAGONISTIC EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA OF *Bacillus* GENERA

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Abstract. Phytobeneficial rhizobacteria can be involved in biocontrol through a process known as antagonistic phenomena, which entails the trophic competition and production of inhibitory compounds. The aim of this study is to assess the antagonistic effect of five Plant Growth Promoting Rhizobacteria of the genus *Bacillus* (PGPR-*Bacillus*) isolated from previous study (coded RP) on four reference strains (*Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27813). Direct confrontation, extraction of bioactive substances and competition techniques were used. The direct confrontation method showed an inhibitory effect of PGPR-*Bacillus* strains characterized by the growth slowing of tested ATCC strains. In addition, PGPR inhibitory constituents production whether constitutive, inducible or volatile were noted on three reference strains. *Pseudomonas aeruginosa* ATCC 27813 was the most sensitive to all RP extracts in contrary to *Staphylococcus aureus* ATCC 25923. Furthermore, PR5: *Bacillus cereus* ethyl-acetat extract was the most effective one with the largest inhibitory zone (15.00 ± 4.33 mm). This survey was able to show a very strong antibacterial and antifungal activity and described some important antagonistics mechanisms that PGPR-*Bacillus* could manifest for biological control purposes.

Key words: PGPR; *Bacillus*; antagonism; biocontrol; ATCC strains.

INTRODUCTION

The rhizosphere is an important ecological niche of microbial biodiversity, which interact with each other'sand with host plants roots [4]. Therefore, rhizospheric microorganisms exert various effects on plants by influencing their development [19]. This part of the soil is home to an incredible biodiversity of bacterial species capable of promoting and stimulating the growth of the plant (Plant Growth Promoting Rhizobacteria: PGPR) and fight against plant pathogens. The latter mechanism is called "biocontrol" or "biological control", which defines the ability of a biological agent to prevent or suppress disease. In general, the term biological control has also been applied to the use of natural products extracted or fermented from various sources [25]. At the same time, numerous studies were conducted to establish the role of these rhizobacteria in defending plants against plant pathogens such as bacteria, fungi, viruses and nematodes [5]. In addition, beneficial rhizosphere bacteria can secrete substances that inhibit the growth of phytopathogenic microorganisms, called antagonists. Antagonism is thus the ability of one bacterium to inhibit the growth of another in the same microbial community [8].

On the other hand, antagonist rhizosphere bacteria exert biocontrol effects by inhibiting the growth of a large number of phytopathogens [14]. Indeed, various biological control mechanisms involve the secretion of extracellular metabolites such as hydrogen cyanide, siderophores, antibiotics, hydrolases and/or competition for nutrients [14, 24]. Antibiotic production is one of the mechanisms PGPR used to prevent attack by plant pathogens and suppress biological diseases [6]. Some antagonistic bacteria are already marketed as biological agents for the treatment of certain plant diseases such MbI600 *Bacillus subtilis* damping off (horticulture) [16]. To this end, it is important to search of antagonistic microorganisms to be used as biological alternative to contraint plant pathogens instead of chemicals.

Furthermore, PGPR of *Bacillus* species are well known, for example the species of the *Bacillus subtilis* group, in particular *B. subtilis, B. licheniformis, B. amyloliquefaciens* and *B. pumilus* which are intensively studied for their importance in the biotechnology industry and agriculture, as a plant pathogen antagonist or promoter of plant growth [26]. In fact, *Bacillus* spp. strains can compete with other microbes that could harm crops; they can induce a host-plant defense system against potential pathogenic attacks or inhibit them, stimulate plant growth and improve nutrient uptake [10].

It is in this perspective that our work focuses on the conduct of *in vitro* tests to evaluate the antagonistic potential of rhizobacteria promoting plant growth (PGPR) of *Bacillus* genera isolated from previous study [7] against microbial reference strains as a preliminary step in the study of the biocontrol potential of these PGPR strains.

MATERIALS AND METHODS

The objective of this study was to assess the antagonistic potential of five successful strains of PGPR of the genus *Bacillus* (Table 1) isolated from the rhizosphere of the date palm and characterized in a previous study [7]. To this end, we proceeded to test the so-called strains against four reference strains (*Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27813).

Table 1. Performant PGPR strains of the genus *Bacillus* isolated from the rhizosphere of the date palm (Benaissa *et al.* 2024) [7]

Previous code (Benaissa et al. 2024) [7]	**New code**	**Species**
RP_{07}	RP_{01}	*Bacillus coagulans*
RP_{08}	RP_{02}	*Bacillus circulans*
RP_{10}	RP_{03}	*Paenibacillus polymyxa*
RP_{12}	RP_{04}	*Bacillus circulans*
RP_{13}	RP_{05}	*Bacillus cereus*

Antagonism assays were used to determine whether five (05) PGPR-*Bacillus* strains could have any inhibitory effects on the four (4) microbial reference strains. The methodology outlined by Bezert *et al.* (1996) [9] was applied to these assays on Müller-Hinton Agar (MHA).

A petri dish was split into two equal sections for the direct confrontation test of PGPR-*Bacillus* against reference strains using a smear made from a PGPR-*Bacillus* strain. Using a sterile Pasteur pipette, remove two 6 mm discs from the flat end and place them on either side of the stripe, 1 cm from the box's edge, for pectinolytic phytopathogen cultures on MHA agar. PGPR-*Bacillus* was not seeded into control boxes [9].

Previous research indicates that PGPR bacteria secrete various forms of active substances (volatile, inducible, and constitutive) that can affect the growth of other microorganisms. Thus, in accordance with Bezert *et al.* (1996) [9], we looked into the production of constituent and inducible substances on the growth of microbial reference strains. Certain bacteria consistently excrete constituent components, which are metabolites, regardless of the medium in which they are present.

The test involves inoculating PGPR-*Bacillus*, a straight strip in another box, with two gel slices containing pectin-decomposing plant pathogens on either side of another agar slice. Certain bacteria produce substances known as inducible components, which can only be produced in the presence of an inducer. In this instance, the inducer might be a material produced by the plant pathogen that breaks down pectin. We tested the inducing substance using the same methodology as the direct exposure test. We sandwiched an agar slice containing the microbial reference strains between two gel discs containing the pectinolytic phytopathogen and PGPR-*Bacillus*, which was located in the zone of inhibition between the two.

According to Hibar *et al.* (2005) [15], the search for a PGPR volatile substance production was carried out in the presence of reference strains. The purpose of this experiment is to assess diffused PGPR volatile compounds' antimicrobial activity. In a Petri dish with a thin layer of MHA medium previously applied, two agar discs—one from PGPR-*Bacillus* and the other from cultures that were previously seeded with reference strains and pectinolytic phytopathogen—are placed on the bottom and lid, respectively. To prevent the loss of volatile materials, we wrapped the Petri dish in Parafilm [11].

According to Belter (1979) [3], bioactive compounds were extracted in order to investigate the antimicrobial activity of PGPR-*Bacillus* strains. Following that, PGPR cultures were made and centrifuged for 20 minutes at 6000 turns. To create two phases (aqueous and organic), the obtained supernatant was divided into two test tubes and combined with an equivalent volume of organic solvents (chloroform and ethyl acetate). After recovering the organic phase, reference strains are used to evaluate the organic phase's antibacterial activity.

According to Miyadoh (1993) [24], the direct diffusion technique involves chopping wells (diameter 6 mm) in MHA agar that have previously been seeded by the test germs. Next, a few drops of agar are added to the bottom of each well to stop the 200 µL deposited extracts from leaking, and the wells are refrigerated for ten minutes before being incubated for twenty-four hours at 37°C. As a control method, the antimicrobial activity of pure solvents used in the bioactive substance extraction process is also assessed.

In order to investigate potential trophic competition between PGPR-*Bacillus* and reference strains, we employed Petri dishes that were seeded with both strains, reflecting a phenomenon of antagonism between them. In order to determine whether PGPR-*Bacillus* is trophic inhibiting reference strains, half of the central streak of the agar is then removed [9].

RESULTS

The various tests for screening antagonistic effects are carried out by the use of five (05) strains of PGPR-*Bacillus* that have demonstrated biofertilizing potential in cowpea according to Benaissa *et al.* (2024) [7]. These bacteria were tested against four (04) reference strains, giving varied results from one test to another.

The colony diameter in (mm) in relation to the control was used to estimate the sensitivity of reference strains in the direct confrontation test. Consequently, the results (Table 2) show that, in contrast to the other reference strains that exhibit resistance to various RP, the colony diameter of reference strain *C. albicans* ATCC 10231 was smaller than the diameter of their controls (Figure 1), indicating that it had a very sensitivity profile to all RP strains. Furthermore, we could clearly see that the RP05 strain outperforms all other reference strains.

The results of research into a constituent substance of RP strains and their effects on the development of reference strains were assessed by measuring the colony diameter in (mm) relative to control (Table 3). As a result, we distinguish that *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27813 represent sensitivity to certain RP (Figure 2). While *C. albicans* ATCC 10231 remained sensitive to all RP strains with a colony

diameter larger than diameter of their controls. In addition, *S. aureus* ATCC 25923 presented total resistance against RP strains.

The results of an inducible substance production (Table 4) demonstrated that RP strains have a high effect on the growth of *C. albicans* ATCC 102310, reflected by their sensitivity to all RP (Figure 3). Followed by *P. aeruginosa* ATCC 27813 which represents absolute resistance against RP strains. On other hand, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 indicated some sensitivity to different RP.

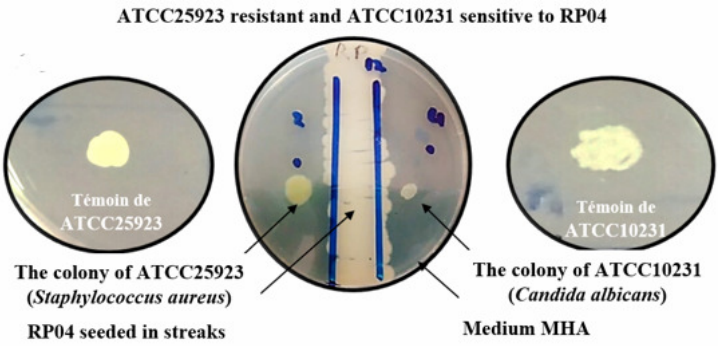

Figure 1. Photograph depicts the sensitivity of *C. albicans* ATCC 10231 strain and the resistance of *S. aureus* ATCC 25923 strain to RP04 in the comparison test of reference strains to RP

Table 2. Results of the direct confrontation test of PGPR-*Bacillus* strains (RP) against reference strains, represented by colony diameters in (mm).

Reference strains	Control (mm)	RP01	RP02	RP03	RP04	RP05
E. coli ATCC 25922	8	9 (R)	9 (R)	10 (R)	10 (R)	6 (S)
S. aureus ATCC 25923	7	9 (R)	10 (R)	12 (R)	12 (R)	6 (S)
P. aeruginosa ATCC 27813	8	9 (R)	9 (R)	9 (R)	9 (R)	6 (S)
C. albicans ATCC 10231	6	4 (S)	4 (S)	4 (S)	4 (S)	4 (S)

where: S - sensitive, R - resistant

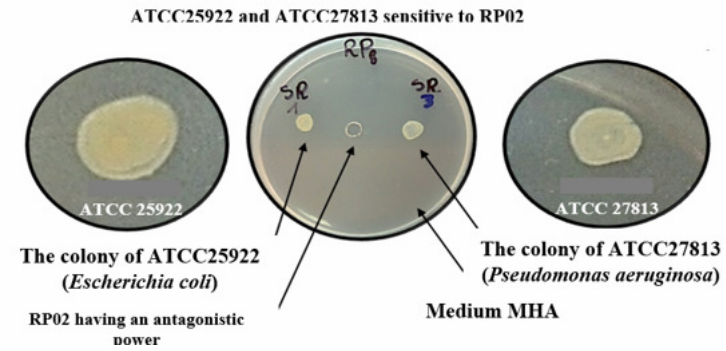

Figure 2. Photograph represents the sensitivity of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27813 strains to RP02 in the test for a constituent of RP strains on the development of reference strains.

Table 3. The results of constituent substance produced from PGPR-*Bacillus* strains (RP) inhibiting the development of reference strains, represented by diameters of colonies in (mm).

SR	Control (mm)	RP01	RP02	RP03	RP04	RP05
E. coli ATCC 25922	8	6 (S)	6 (S)	9 (R)	7 (S)	9 (R)
S. aureus ATCC 25923	7	10 (R)	11 (R)	11 (R)	9 (R)	10 (R)
P. aeruginosa ATCC 27813	8	9 (R)	7 (S)	9 (R)	6 (S)	9 (R)
C. albicans ATCC 10231	6	5 (S)	5 (S)	5 (S)	5 (S)	5 (S)

where: S - sensitive, R - resistant

Comparing the colony diameter of reference strains in the presence of RP strains with the diameter of their controls yielded the results presented in Table 5, which indicates that all reference strains sensitive to volatile substances secreted by certain RP strains (Figure 4), at the same time were resistant to the few RP.

Results of *in vitro* antimicrobial activity test of PGPR strains (Table 6) demonstrated that *P. aeruginosa* ATCC 27813 is sensitive to ethyl acetate extract from different RP (Figure 5) and *C. albicans* ATCC 10231 is sensitive to Chl and EA extract of RP02 et RP04, with the presence of inhibition zones varied between 6 and 13 mm. *S. aureus* ATCC 25923 showed resistance to both (02) extracts of RP02, RP03 and RP04, with a resistance of these strains to pure solvents as a control (Figure 5).

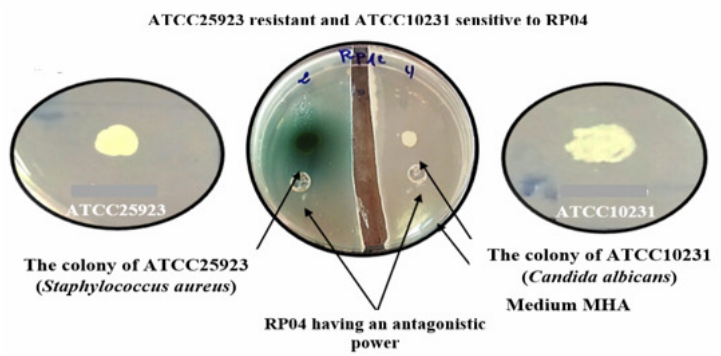

Figure 3. Photograph represents the sensitivity of *C. albicans* ATCC 102310 and *S. aureus* ATCC 25923 to RP04 in the inducible test for the development of reference strains.

Table 4. Results of inducible substance produced from PGPR-*Bacillus* strains (RP) against reference strains, represented by colony diameters in (mm)

SR	Control (mm)	RP01	RP02	RP03	RP04	RP05
E. coli ATCC 25922	8	6 (S)	7 (S)	6 (S)	9 (R)	9 (R)
S. aureus ATCC 25923	7	9 (R)	8 (R)	5 (S)	8 (R)	8 (R)
P. aeruginosa ATCC 27813	8	9 (R)	10 (R)	9 (R)	8 (R)	10 (R)
C. albicans ATCC 10231	6	5 (S)	5 (S)	5 (S)	5 (S)	5 (S)

where: S - sensible, R - resistant

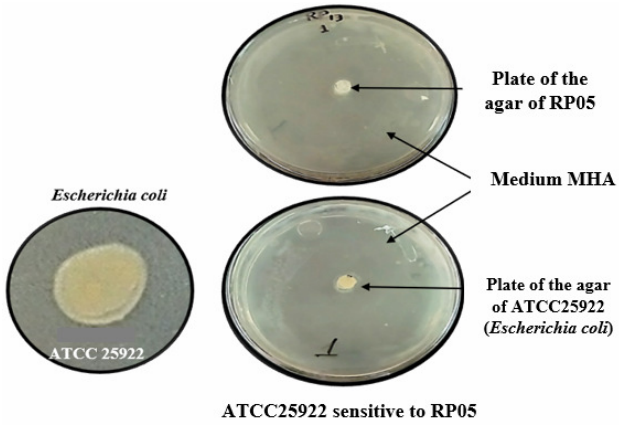

Figure 4. Photograph represents the susceptibility of strain *E. coli* ATCC 25922 to RP05 in the volatile test

Table 5. Results of direct confrontation test between PGPR-*Bacillus* strains (RP) and reference strains, represented by colony diameters in (mm).

SR	Control (mm)	RP01	RP02	RP03	RP04	RP05
E. coli ATCC 25922	8	10 (R)	11 (R)	7 (S)	6 (S)	5 (S)
S. aureus ATCC 25923	7	6 (S)	5 (S)	9 (R)	11 (R)	13 (R)
P. aeruginosa ATCC 27813	8	9 (R)	7 (S)	9 (R)	6 (S)	7 (S)
C. albicans ATCC 10231	6	5 (S)	7 (R)	8 (R)	5 (S)	4 (S)

where: S - sensitive, R - resistant

The results of trophic competition experiment between PGPR (RP) and test strain (SR) were interpreted in one of three ways: The reference strain continued to grow normally and uniformly in the presence or absence of PGPR streaks (Form 1), so there was no nutritional inhibition and thus this strain was resistant. Pectinolytic phytopathogens continue to grow on the side where PGPR had been eliminated

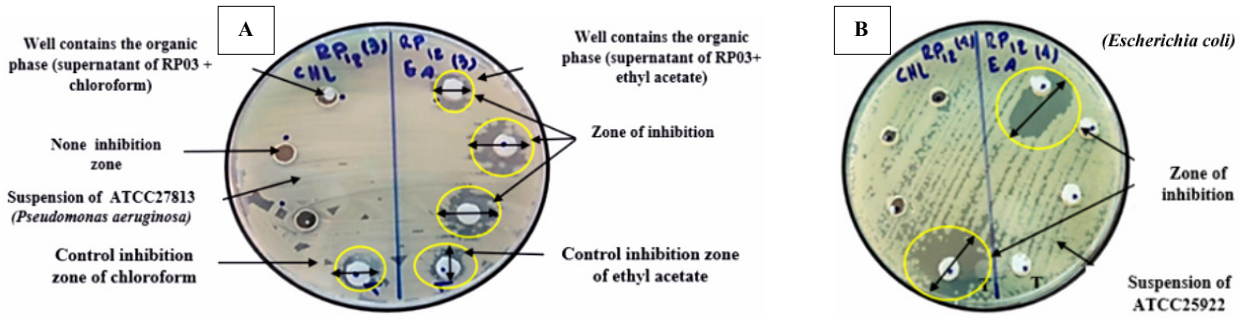

Figure 5. Photograph represents the antimicrobial activity of RP3 (A) and RP1 (B) extracts respectively against *P. aeruginosa* ATCC 27813 and *E. coli* ATCC 25922.

Table 6. Antimicrobial activity of PGPR-*Bacillus* organic extracts performed by direct diffusion technique of reference strains, represented by the observed inhibition zones subtracted from the inbition zones of the pure solvents (control). All values are the mean ± ES (standard error) of three replicates of a single sample for each experiment.

RP	Slv	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*P. aeruginosa* ATCC 27813	*C. albicans* ATCC 10231
RP01	**Chl**	00	12.33±1.26	00	00
	EA	00	00	5.67±2.57	00
RP02	**Chl**	00	00	00	6.00±2.65
	EA	2±1.73	00	9.00	12.00
RP03	**Chl**	00	00	00	00
	EA	3.00±2.60	00	13.33±1.04	00
RP04	**Chl**	00	00	7.00	3.33±2.89
	EA	15.00±4.33	00	10.00±2.18	13.00±0.58
RP05	**Chl**	00	3.33±2.89	7.33±0.29	00
	EA	2.00±1.73	00	12.33±1.26	00

where: Slv - solvent; Chl - chloroform; EA - ethyl-acetate

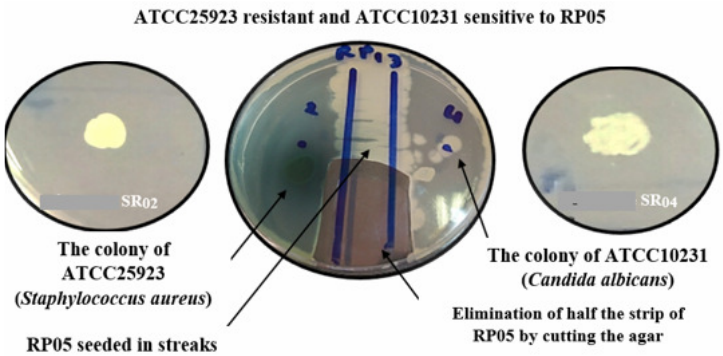

Figure 6. Photograph shows the resistance of *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231 strains to RP05 in the trophic competition research test between PGPR-*Bacillus* and reference strains.

Table 7. Test results for *in-vitro* trophic competition between PGPR-*Bacillus* strains (RP) and reference strains

SR	**Cs**	**RP_{01}**	**RP_{02}**	**RP_{03}**	**RP_{04}**	**RP_{05}**
E. coli ATCC 25922	GF	3	1	1	1	3
	TC	+	-	-	-	+
	R/S	S	R	R	R	S
S. aureus ATCC 25923	GF	1	1	1	1	1
	TC	-	-	-	-	-
	R/S	R	R	R	R	R
P. aeruginosa ATCC 27813	GF	1	**1**	**1**	**1**	**1**
	TC	-	-	-	-	-
	R/S	R	R	R	R	R
C. albicans ATCC 10231	GF	3	3	3	**3**	3
	TC	+	+	+	+	+
	R/S	S	S	S	S	S

where: R - resistant, S - sensitive, -: no trophic competition, +: possible trophic competition, GF - growth form, TC - trophic competition, R/S - resistance/sensitivity, Cs - characteristics, 1/ 2/ 3 - growth form of reference strains.

(form 2a), so there was no trophic competition, but did not continue to grow on the side where PGPR was present (form 2b) or do not stop growing again on each side (Form 3), so trophic competition is still possible and therefore this strain is susceptible.

The results presented in Table 7 showed that the reference strains *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27813 presented total resistance to all RP strains (Figure 6). On the other hand, *C. albicans* ATCC 10231 showed a high sensitivity to the latter (Figure 6), while *E. coli* ATCC 25922 showed a variable sensitivity to PR.

DISCUSSION

The PGPR-*Bacillus* had developed an inhibitory activity with respect to specific reference strains, according to the results. Additionally, the variation in the growth zone diameters of the reference strains raises the possibility that the PGPR-*Bacillus* strains produced different kinds and amounts of inhibitory substances in the medium. In support of this theory were Williams and Asher (1996) [27]. Numerous species, as previously mentioned, were capable of producing powerful antibacterial and antifungal molecules like subtilin, bacitracin, bacillin, and bacillomycin, which are related to the iturin family [1] and polymixin [21]. Additionally, they could produce hydrolytic enzymes like β-1,3-glucanase, which could break down the cell walls of plant pathogenic fungi [22].

We also discovered that *Bacillus* strains may still used this mechanism to compete for nutrients and space, as evidenced by the results we obtained, which showed that the diameter of the growth zone of some reference strains increased as the distance between them and the PGPR-*Bacillus* increased. But in contrast to reference strains, our PGPR-*Bacillus* strains had shown antimicrobial activity that is in line with findings from Eltem and Ucar (1998) [12], Aslim *et al.* (2002) [2], and Lounaci and Athmani-Guemouri (2014) [23].

A constituent substance produced by PGPR-*Bacillus* that inhibits or slows down the growth of specific reference strains was found. Moreover, we discovered that the compounds generated by the PGPR-*Bacillus* strains exhibit antifungal and antibacterial properties against *C. albicans* ATCC 10231 and *P. aeruginosa* ATCC 27813 and *E. coli* ATCC 25922. Furthermore, *Paenibacillus polymyxa* had the capacity to produce antibiotics that inhibit *P. aeruginosa* growth [22]. Furthermore, no inhibitory effects were observed against *S. aureus* ATCC 25923. According to Khelil *et al.* (2013) [18]. There was a chance that the amount of compounds produced by our *Bacillus* strains was insufficient to cross the inhibition threshold.

Searching for inducible substances appears to produce better outcomes than the initial two tests. As a result, we presume that the characteristics of molecules carried by agar discs differ from those of constituent materials. In comparison to direct challenge assays, their numbers and concentrations were therefore adequate to stop the growth of phytopathogenic strains, guaranteeing continuous inhibitor secertion and permitting the inhibition threshold to be crossed. Our findings differed from those of Bezert *et al.* (1996) [19], but they were comparable to those of Lounaci and Athmani-Guemouri (2014) [23].

The various results obtained in our study lead us to believe that the gelded discs used, support inducible substances inhibiting the growth of *E. coli* ATCC 25922 and *C. albicans* ATCC 10231. Unlike the study by Klouche Khelil *et al.* (2013) [20], which reported no effects against *E. coli* ATCC 25922 and yeast *C. albicans* ATCC 10231 by our *Bacillus* strains, although the method used to study antagonistic activity is not the same. Also, no inhibitory effects were reported for *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27813. This negative result may likely be due to resistance of the latter to substances produced by the *Bacillus* strains, or to the absence of receptors specific to the bioactive substances produced by this strain [28].

According to the same perspective, when PGPR-*Bacillus* directly confronted the test strains, they demonstrated an antagonistic effect that resulted in the production of volatile compounds that prevented the reference strains' growth. As per Faraga *et al.* (2013) [13] and Sicuia *et al.* (2015) [26], a multitude of *Bacillus* species synthesized volatile substances like ammonia and hydrogen cyanide (HCN), which were crucial for biological control. However, the outcomes of our *in vitro* tests on the antimicrobial activity (direct diffusion) of PGPR-*Bacillus* strains demonstrated that the activity of the supernatant positively impacted the development of some reference strains' extracellular metabolism. This indicated that *Bacillus*'s extracellular metabolites were equally potent to the bacterium itself. This can be explained by the supernatant containing enough antimicrobial activity to prevent the growth of the strains that were tested. Our findings aligned with the findings published by Benaissa *et al.* (2019) [8]. The results indicated that the supernatant's antimicrobial activity was more important than the raw culture when it comes to the reference strains. Notably, a number of pilot studies have evaluated the antibacterial activity of various bacteria and actinomycetes that produce bioactive molecules using the strains of *E. Coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 as a reference model [17, 26].

As a result, we can speculate that the activity might be connected to the extract's bioactive molecule content. Moreover, we discovered that the ethyl acetate extract outperformed the chloroformic acid extract in terms of antibacterial antagonistic activity against the reference strain. The final result aligned with the research conducted by Benaissa *et al.* (2019) [8]. The efficiency of bioactive substances was actually influenced by a number of factors, including the type

of solvent used, the method employed, and the bacterial species and susceptibility [8].

It was demonstrated that the growth of the tested strains was inhibited, indicating a potential for trophic competition between PGPR-*Bacillus* and reference strains. This inhibition is brought on by either nutritional competition or the inhibitory compounds that PGPR-*Bacillus* secreted into the medium both before and after half-agar was eliminated. This also applies to specific PGPR-*Bacillus* strains of *C. albicans* ATCC 10231 and *E. coli* ATCC 25922. Furthermore, with regard to the strains that continue to grow regularly and uniformly both in the presence and absence of the PGPR-*Bacillus* streak, this indicated that either there was no trophic competition or the inhibitory substance was insufficient both qualitatively and quantitatively. This was the case with *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27813 and *E. coli* ATCC 25922 with some PGPR-*Bacillus* strains. These results were also found in similar studies such as that of Bezert *et al.* (1996) [9], which could not prove trophic competition between antagonistic agents and plant pathogens. Indeed, competition for sources of nutrients and energy is an important mechanism characteristic of PGRPs allowing them to absorb root exudates and colonize roots plant [6].

In vitro antagonism tests showed that our PGPR-*Bacillus* strains have an inhibitory power on the development of microbial reference strains, which is varied depending on the test performed and the strain tested. In fact, direct confrontation revealed an inhibition of the reference strains growth demonstrated through a reduction in the diameter of the reference strains colonies compared to control. This indicates that PGPR-*Bacillus* can exhibit antagonistic effect through competition phenomon, production of inhibitory constitutive and inducible substances, and extracellular bioactive molecules. In perspective, we intend to test the PGPR strains against *in vitro* and *in vivo* plant pathogens to validate their applications in the agricultural field as biofertilizers and biopesticides.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Alippi, A., Monaco, C., (1994): Antagonismo *in vitro* de especies de *Bacillus* contra *Sclerotium rolfsii y Fusarium solani.* Revista Facultad Nacional de Agronomía Medellín, 70: 91-95.

[2] Aslim, B., Saglam, N., Beyatli, Y., (2002): Determination of some properties of *Bacillus* isolated from soil. Turkish Journal of Biology, 26: 41-48.

[3] Belter, P.A., (1979): General procedures for isolation of fermentation products. In Peppler H.J., Perlman D., (eds.): Microbial Technology. Academic press, Wisconsin, pp. 403-432.

[4] Benaissa, A., (2019): Plant growth promoting rhizobacteria. A Review. Algerian Journal of Environmental Science and Technology, 5(1): 873-880.

[5] Benaissa, A., (2023a): Plant beneficial bacteria from rhizosphere as biocontrol agents of phytopathogens. Vegetos, pp. 1-4.

[6] Benaissa, A., (2023b): Rhizosphere: role of bacteria to manage plant diseases for sustainable agriculture. Journal of Basic Microbiology., 65: 2300361.

[7] Benaissa, A., Basseddik, A., Chegga, A., Djebbar, R., (2024): Halotolerant *Bacillus* species as plant growth promoting rhizobacteria from hyper – arid area of Algeria. Journal of Agricultural Sciences, 30(2): 400-412.

[8] Benaissa, A., Djebbar, R., Abderrahmani, A., (2019): Antagonistic effect of plant growth promoting rhizobacteria associated with *Rhus tripartitus* on Gram positive and negative bacteria. Analele Universitatii din Oradea, Fascicula Biologie, 2(26): 67-72.

[9] Bezert, G., Chappe, P., Mourey, A., Loubinoux, B., (1996): Action de *Bacillus* et d'actinomycètes sur les champignons du bleuissement du bois. Bulletin de l'Academie et de la Societe Lorraines des Sciences, 35(3): 177-190.

[10] Constantinescu, F., Sicuia, O.A., (2013): Combaterea biologică a bolilor plantelor cultivate. ISBN: 978-973-0-14196-2. București, pp. 99.

[11] Daami-Remadi, M., Dkhili, I., Jabnoun-Khiareddine, H., El Mahjoub, M., (2012): Biological control of potato leak with antagonistic fungi isolated from compost teas and solarized and non-solarized soils. Pest Technology, 6: 32-40.

[12] Eltem, R., Ucar, F., (1998): The determination of antimicrobial activity spectrum of 23 *Bacillus* strains isolated from Denizli-Acigol (Bitter Lake) which is soda lake (Na_2SO_4). Journal of Kukem, 21: 57-64.

[13] Farag, M.A., Zhang, H., Ryu, C.M., (2013): Dynamic chemical communication between plants and bacteria through airborne signals: induced resistance by bacterial volatiles. A review. Journal of Chemical Ecology, 39: 1007-1018.

[14] Fernando, W.D., Nakkeeran, S., Zhang, Y., (2005): Biosynthesis of antibiotics by PGPR and its relation inbiocontrol of plant diseases. In PGPR: Biocontrol and Biofertilization, Springer, Netherlands, pp. 67-109.

[15] Hibar, K., Daami-Remadi, M., Khiareddine, H., El Mahjoub, M., (2005): Effet inhibiteur *in vitro* et *in vivo* du *Trichoderma harzianum* sur *Fusarium oxysporum* f. sp. *radicis-lycopersici.* Biotechnologie, Agronomie, Société et Environnement, 9(3): 163-171.

[16] Jijakly, M.H., (2003): La lutte biologique en phytopathologie pp. 289-317, In: Lepoivre, P. (Ed): Phytopathology, De Boeck, Bruxelles.

[17] Kamat, T.K., Kiran, S., Kerkar, S., (2011): Antimicrobial potential of *Bacillus marismortui*, a salt pan isolate of *Cavellosim,* Goa, India. International Journal of Advanced Biotechnology and Research, 3: 321-328.

[18] Khelil, O., Choubane, S., Cheba, B.A., (2016): Polyphenols content of spent coffee grounds subjected to physico-chemical pretreatments influences lignocellulolytic enzymes production by *Bacillus sp.* R2. Bioresource Technology, 211: 769-773.

[19] Kloepper, J.W., Beauchamp, C.J., (1992): A review ofissues related to measuring colonization of plant rootsby bacteria. Canadian Journal of Microbiology, 38: 1219-1232.

[20] Klouche Khelil, N., Aissaoui, N., Nas, F., Luc Cayol, J., Ghellai, L., (2013): A novel halotolerant bacterium isolated from el Goléa lake in Algeria and antimicrobial

potential of this strain. Journal of Agricultural Science and Technology, 3: 825-834.
[21] Landman, D., Georgescu, C., Martin, D.A., Quale, J., (2008): Polymyxins revisited. Clinical Microbiology Reviews, 21(3): 449-465.
[22] Leelasuphakul, W., Sivanunsakul, P., Phongpaichit, S., (2006): Purification, characterization and synergistic activity of [beta]-1,3-glucanase and antibiotic extract from an antagonistic *Bacillus subtilis* NSRS 89-24 against rice blast and sheath blight. Enzyme and Microbial Technology, 38: 990-997.
[23] Lounaci, L., Athmani-Guemouri, S., (2014): Action de *Paenibacillus polymyxa* SGK2 sur quelques champignons de la fusariose du blé dur (*Triticum durum*) en Algérie. Algerian Journal of Natural Products, 2(2): 35-42.
[24] Miyadoh, S., (1993): Research on antibiotic screening in Japan over the last decade: a producing microorganism approach. Actinomycetes, 7(2): 100-106.
[25] Pal, K., Gardener, B.M., (2006): Biological control of plant pathogens. The Plant Health Instructor, pp. 1-6.
[26] Sicuia, O.A., Constantinscu, F., Petruta, C.C., (2015): Biodiversity of *Bacillus subtilis* group and beneficial traits of *Bacillus* species useful in plant protection. Romanian Biotechnology Lettters, 20(5): 10737-10750.
[27] Williams, G.E., Asher, M.J.C., (1996): Selection of rhizobacteria for the control of *Pythium ultimum* and *Aphanomyces cochlioide*s on sugar-beet seedlings. Crop Protection, 15: 479-486.
[28] Zaim, S., Belabid, L., Bellahcene, M., (2013): Biocontrol of chickpea *Fusarium* wilt by *Bacillus* spp. rhizobacteria. Journal Plant Protection Research, 53(2): 178-183.

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