JJBS

Volume 17, Number 4,December 2024
ISSN 1995-6673
Pages 667 – 680
https://doi.org/10.54319/jjbs/170410

Jordan Journal of Biological Sciences

The Impact of Rhizosphere Bacterial Strains as Biofertilizers: Inhibiting Fungal Growth and Enhancing the Growth and Immunity of Sprouted Barley as an Alternative Livestock Feed

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Received: May 9, 2024; Revised: June 26, 2024; Accepted: July 4, 2024

Abstract

Egypt faces challenge in supplementing animal feed requirements, which add huge pressure on the budget and foreign currency reserves annually, making it necessary to find alternative solutions. The sprouted barley is considered one of these recent alternatives to animal feed. Sprouted barley faces challenges represented in fungal growth, which have strong competition for oxygen with the embryo and can inhibit seed germination in addition to producing aflatoxins, biofertilizers of plant growth-promoting bacteria (PGPB) are considered a practical and safe solution for these challenges. In this work, five tomato rhizobacterial strains were isolated and identified using the 16SrRNA gene and were found to belong to *Bacillus amyloliquefaciens*, *Peribacillus frigoritolerans*, *Pseudomonas flourescens*, *Bacillus pumilus*, and *Paenibacillus uliginis*, respectively. We reported here that most of these five isolates exhibited multiple PGP properties (PGPP), including the production of ACC deaminase, Indole-acetic acid (IAA), chelating siderophores and phosphate solubilization. *Bacillus amyloliquefaciens* BMG150 isolate exhibited the highest values for all the PGPP except siderophores production (1457 nmol, 37.4 µg/ ml, and 3.7 mg/ml, respectively). We also scanned the presence/ absence of the NRP gene clusters in the five isolates as an important PGPP using bioinformatics tools and NRPs degenerate primers. All five isolates showed NRPs gene clusters presence with the superiority of NRPs number for the strain *Bacillus amyloliquefaciens* BMG150 (surfactin, plipastatin, iturin and bacillibactin siderophore). Accordingly, we used *Bacillus amyloliquefaciens* BMG150, *Pseudomonas flourescens* PMG01 separately and a formula of the other three isolated strains as biofertilizers in sprouted barley cultivation which proved their efficiency in promoting their growth characteristics and reflected on protein pattern.

Keywords: Biofertilizers, Livestock feeding, NRPs, PGPB, Rhizosphere bacteria, Sprouted barley.

1. Introduction

The use of PGPB as biofertilizers is widely applied to improve the safety, quality and production of crops (Hussein *et al*., 2016). The rhizosphere is considered the wealthy source of a variety group of beneficial plant microorganisms. It has the prospective to improve plant growth, health and soil fertility which can be determined through the beneficial interactive relationship between roots and microbes (Parray *et al*., 2016; Kalam *et al*., 2017a). These beneficial interactive relationships possess several characteristics which can be determined and referred to plant growth-promoting bacteria (Dutta and Podile, 2010; Asriatno *et al*., 2023). Almost all important and abundant biofertilizers are Bacilli and Pseudomonas groups which are easily cultivable PGP, colonize rhizosphere intensely (Orozco-Mosqueda *et al*., 2020) and possess PGP properties (Zhou *et al*., 2016; Sansinenea, 2019). Among these PGP properties of Bacilli, their role in increasing minerals availability, chelating iron through siderophores production, fixing nitrogen and producing the phytohormones. Moreover, the ACC deaminase role in ethylene catabolism, detoxification and pathogens virulence factors degradation are considered among this PGP properties (Ahmad *et al*., 2008; Barea *et al*., 2015; Asriatno *et al*., 2023). Besides these latter PGP properties for the Bacilli group, there is the production of a group of highly diverse and effective secondary metabolites called non-ribosomal peptides synthesized by huge modular enzymes called non-ribosomal peptide synthetases (NRPs) (Süssmuth and Mainz, 2017). The high diversity of non-ribosomal peptides is due to their machine’s ability to incorporate several non-proteogenic amino acids with different modifications compared to ribosomal machine (Walsh *et al*., 2013). Among these Bacilli NRPS are the lipopeptide families; surfactin, fengycin or plipastatin, iturins and kurstakins known as biosurfactants and have antifungal, antibacterial and antiviral activities. They are used in biocontrol of plant diseases which showed antagonistic activities against various phytopathogens (Ongena and Jacques, 2008). Pseudomonas group also is considered one of the most important producers of a wide spectrum of NRPs natural products. Among Pseudomonas NRPs, the cyclic lipopeptides (CLPs) which are classified into amphisin, syringomycin, viscosin, syringopeptin, or

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tolaasin group (Gross and Loper, 2009). In addition, other CLPs have been identified, namely putisolvin, orfamide (Kuiper *et al*., 2004; Gross *et al.,* 2007; Vallet-Gely *et al*., 2010), and entolysin. Also, few linear lipopeptides have been characterized, namely syringafactin and peptin 31 (Berti *et al*., 2007; Fiore *et al*., 2008; Chaida *et al*., 2022).

Egypt faces difficulties in supplementing animal feed requirements; it produces only 20 % of its feed corn and soybean while importing 50 % of feed corn and 90 % of soybean as an annual requirement, which places huge pressure on budget and foreign currency reserves. Cattle feed consists of about 50 % corn feed and 30 % soybeans, while poultry feed requires larger ratios of these ingredients than cattle feed. There are local alternatives that can go into the feed mixes for livestock, such as sprouted grains that have multiple benefits represented in increasing both the quality and quantity of protein and increasing some nutrients (sugars, minerals and vitamins) (Cuddeford, 1989; Gebremedhin, 2015). Sprouted grains fodders are considered a wealthy source for enzymes able to improve animals' productivity, which is rich in chlorophyll and grass juice which improves the animal's performance (Finney, 1983).

Among barely seeds, microbiome communities are fungi which have strong competition for oxygen with the embryo and can inhibit seed germination. (Harper *et al*., 1981) reported that aspergilli and penicillia when colonize the seed under the husk, they can participate the seed for their limited oxygen content. When 100 pg fungus colonized 80 % of the seed under the husk, maximum reduction in germination percentage was recorded.

The SDS-PAGE tool can explain the effect of both environment and the interactions between genotypes genes on protein (Johansson *et al*., 2012; Ling *et al*., 2012) by determining the genetic diversity between various species of crops as barley and wheat (Miháliková *et al*., 2016; Banta *et al*., 2021). SDS-PAGE technique is commonly used in biological analysis for determination of shifting in protein bands (proteins or enzymes) caused under bio-stress, due to the hormonal changes (Ghasempour *et al*., 2001; Ghasempour and Kianian, 2002; Ghasempour and Maleki, 2003) which reflect on protein patterns by increasing bands upon transition from control to environmental stress (Vyomesh and Pitambara, 2018; Ramadan and Soliman, 2020).

This work aims to prove the efficiency of some *Bacillus* and *Pseudomonas* strains producers for NRPs as biofertilizers by inhibiting fungal growth, enhancing sprouted barely growth and immunity to use the sprouted barely as an alternative feeding for animals.

2. Materials and methods

2.1. Soil strains isolation and counting

Nine various soil samples from three different locations in three replicates were collected from tomato field located in the Faculty of Agriculture, Menoufia University, Shibin-Elkom, Egypt. Counting of rhizosphere microbiome communities was performed in two replicates. Microbial colonies different in shape, color and viscosity were selected for cultivation and identification.

2.2. Rhizosphere soil sampling

Three replicates from three zones of tomato field were used for rhizosphere community preparation. 10 ml of sterilized 0.5% NaCl was added to 1 g rhizosphere soil. Serial soil dilutions technique was prepared 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 100 µl of each dilution was plated on LB medium plates and incubated for 24-48 hours at 28 °C.

2.3. Plant samples

The Giza 126 barely seeds variety used in this study was obtained from the field of crops Research Institute, Agricultural Research Center (ARC), Egypt.

2.4. Isolation and identification barely seed associated fungi

Associated fungi were isolated from Giza 126 barely seeds by barely seeds surface sterilization (Hussein *et al*., 2018) and then placed on PDA plates for five days at 25°C. To purify the fungi, they were sub-cultured three times on the same medium PDA. Pathogen identification was firstly performed based on morphology and microscopy observation followed by molecularly identification using the ITS gene after DNA extraction using the Zymo fungal/bacterial DNA miniprep. ITS gene was amplified using the universal primers; ITS_1-Fwd (TCCGTAGGTGAACCTGCGG) /ITS_4- Rev (TCCTCCGCTTATTGATATGC) followed by sequencing. The obtained sequences were aligned using BLASTN (https:// blast.ncbi.nlm.nih.gov/Blast.cgi).

2.5. ACC Deaminase Activity determination

The rhizobacterial isolates from tomato soil were scanned for their ACC deaminase activity on MDFS media (Minimal Dworkin and Foster Salts) supplemented with 3 mM ACC) (Dworkin and Foster, 1958; Penrose and Glick, 2001). After 3 days of incubation at 28 °C, the grown colonies were considered ACC deaminase producers. ACC deaminase quantification was performed using a Carry 100 UV-Vis spectrophotometer by measuring α-ketobutyrate production levels at 540 nm and comparing the results to a standard curve (0.1 to 1.0 mmol) as previously described (Honma and Shimomura, 1978).

2.6. Production of IAA

The isolated strains were incubated on Lauria Bertani medium supplemented with (5 mM Tryptophan) for 7 days at 28 °C under 200 rpm of shaking. The IAA determination was performed by Salkowski reagent colorimetric method (0.5 M Ferric chloride, 70% perchloric acid). The Cell free culture supernatant was mixed with Salkowski reagent in a 4:1 ratio to form indolic compounds, which exhibited a red colour. The absorbance was measured at 530 nm using a Carry 100 UV–Vis spectrophotometer as previously described (Gordon and Weber, 1951). A standard curve of pure indole-3-acetic acid (0-100 mg/ml) was used to determine IAA concentrations.

2.7. Phosphate solubilisation determination

The determination of phosphate solubilization by the rhizobacterial isolates was performed using Pikovaskya's agar medium supplemented with 2% Ca_3PO_4. A 10 µl spot of each bacterial culture was placed on the medium and incubated for 4 days at 28 °C as described by (Nautiyal, 1999). Phosphate solubilization was measured by the development of a clear area formed around the colony.

Additionally, quantitative determination of phosphate solubilization was carried out in NBRIP medium as described by (Nautiyal, 1999). The phosphate concentration in the culture supernatant was calculated as described by (Olsen *et al*., 1982).

2.8. Siderophores production

Siderophores production was assessed by inoculation bacterial colonies on CAS (Chrome Azurol S) plates at 28 ^{o}C for 4 days. An orange-yellow halo around the growth indicated siderophores production. For quantification of siderophores production, 100 µl of rhizobacterial isolate culture (10^8 cfu/ml) was inoculated in King B liquid medium for 72 hours at 28 °C. The cultures were then centrifuged at 5000 rpm for 30 minutes. Subsequently, 500 µL of the supernatant was mixed with an equal volume of CAS solution (1:1). After 20 minutes of incubation, the colour change from blue to orange indicated siderophore production, which was measured using a Cary UV-Vis spectrophotometer at 630 nm. The percentage of siderophores produced was estimated using the following equation:

$$\text{Siderophores \%} = \text{RA–SA/RA} \times 100$$

Where RA refers to the blank absorbance (CAS reagent) and SA refers to the sample absorbance.

2.9. DNA extraction, primers and 16S rRNA PCR conditions

Bacterial DNAs extraction was carried out using the Wizard genomic DNA extraction kit, Promega. PCR amplification of the 16S rRNA gene was carried out using primers 27F (AGAGTTTGATCMTGGCTCAG) and 1525R (AAGGAGGTGWTCCARCC) (DeLong, 1992). The PCR protocol included an initial denaturation step at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 2 minutes.

Amplified fragments were purified using the Wizard® SV Gel and PCR Cleanup kit from Promega and subsequently sent for sequencing.

2.10. Non-ribosomal peptide synthetase genes detection using bioinformatics tools and degenerate primers

The complete genome sequences of the related species for the five-tomato rhizosphere bacterial isolates were retrieved from GenBank - microbial genomes database-NCBI. These complete genomes were analyzed for the secondary metabolites' genes using AntiSmash (https://antismash.secondarymetabolites.org) (Blin *et al*., 2023).

Two sets of degenerate primers were used; Serie 1 designed for the *Bacillus* group, previously described by (Tapi *et al*., 2010; Abderrahmani *et al*., 2011); and Serie 2, designed for the *Pseudomonas* group, as described by (Rokni-Zadeh *et al*., 2011) Table (1).

Table 1. Degenerate primers list used for detection of genes involved in NRPS.

Primer name	Primer sequence	Expected fragment size (bp)	NRLPs identified	References
Bacillus group				
AP1-F	AGMCAGCKSGCMASATCMCC	959, 929, 893	Plipastatin	(Tapi *et al*., 2010)
TP1-R	GCKATWWTGAARRCCGGCGG			
AS1-F	CGCGGMTACCGVATYGAGC	419, 422, 424, 431	Surfactin	
TS1-R	ATBCCTTTBTWDGAATGTCCGCC			
Af2-F	GAATAYMTCGGMCGTMTKGA	443, 452 455	Fengycins	
Tf1-R	GCTTTWADKGAATSBCCGCC			
Am1-F	CAKCARGTSAAAATYCGMGG	416, 419	Mycosubtilin	
Tm1-R	CCDASATCAAARAADTTATC			
Abl1-F	GATSAWCARGTGAAAATYCG	428, 431, 434	Bacillomycin	(Abderrahmani et al., 2011)
Tbl1-F	ATCGAATSKCCGCCRARATCRAA			
AKs-F	TCHACWGGRAATCCAAAGGG	1125, 1152, 1161, 1167, 1173	Kurstakin	
TKs-R	CCACCDKTCAAAKAARKWATC			
Pseudomonas group				
PGPRB-5045 C1Fwd	YTG ATY STY GAY GGY TGG GG	321, 325	Depend on strain	(Rokni-Zadeh et al., 2011)
PGPRB-5046 C1 Rev	RSA CRT RSA IBG CIG CCA GC			
PGPRB-4681 TE1 Fwd	TCI TTY GGY GGS GTI CTG GC	819, 825		
PGPRB-4682 TE2 Rev	SIC CIG GNG MYT CRC TGT CG			

2.11. Protein pattern determination.

Soluble proteins extraction from samples was carried out following the method described by (Laemmli, 1970) and subsequently analyzed as detailed by (Stegmann, 1979).

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Table 2: Bacterial inoculant used in soaking barely seeds treatment before planting

Sample code	Sample description
Inoculant 1	*Paenibacillus uliginis* PMG250, *Peribacillus frigoritolerans* PMG200 and *Bacillus pumilus* BMG300.
Inoculant 2	*Pseudomonas flourescens* PMG01.
Inoculum 3	*Bacillus amyloliquefaciens* BMG150
A	control 1: barely seeds washed with water and sterilized using sodium hypochlorite (after 9 days)
A’	control 1: barely seeds washed with water and sterilized using sodium hypochlorite (after 12 days)
B	control 2: barely seeds washed with water (after 9 days)
B’	control 2: barely seeds washed with water (after 12 days)
C	barely seeds washed with water, sterilized with sodium hypochlorite, and soaked overnight in inoculant 1 (after 9 days)
C’	barely seeds washed with water, sterilized with sodium hypochlorite, and soaked overnight in inoculant 1 (after 12 days)
E	barely seeds washed with water, sterilized with sodium hypochlorite, and soaked overnight in inoculant 2 (after 9 days)
E’	barely seeds washed with water, sterilized with sodium hypochlorite and soaked overnight in inoculant 2 (after 12 days)
G	barely seeds washed with water, sterilized with sodium hypochlorite and soaked overnight in inoculum 3 (after 9 days)
G’	barely seeds washed with water, sterilized with sodium hypochlorite and soaked overnight in inoculum 3 (after 12 days)

3. Results

3.1. Counting, isolation, and identification of rhizobacterial community

The rhizosphere bacterial communities were quantified by colony counting. The highest colony count was observed in zone 2 with 766 colonies, decreasing to 460 and 136 colonies in subsequent dilutions. A total of sixteen rhizobacterial strain were isolated, five of which revealed considerable values of PGP properties. These strains were identified using 16SrRNA gene, and their sequences closely matched *Bacillus amyloliquefaciens*, *Peribacillus frigoritolerans*, *Pseudomonas flourescens*, *Bacillus pumilus*, and *Paenibacillus uliginis*. The phylogenetic tree illustrated the genetic relationships between these isolated rhizosphere strains depending on 16SrRNA gene sequence (Figure 1). Similarity percentage and GenBank accession numbers for these isolated strains are shown in table 3.

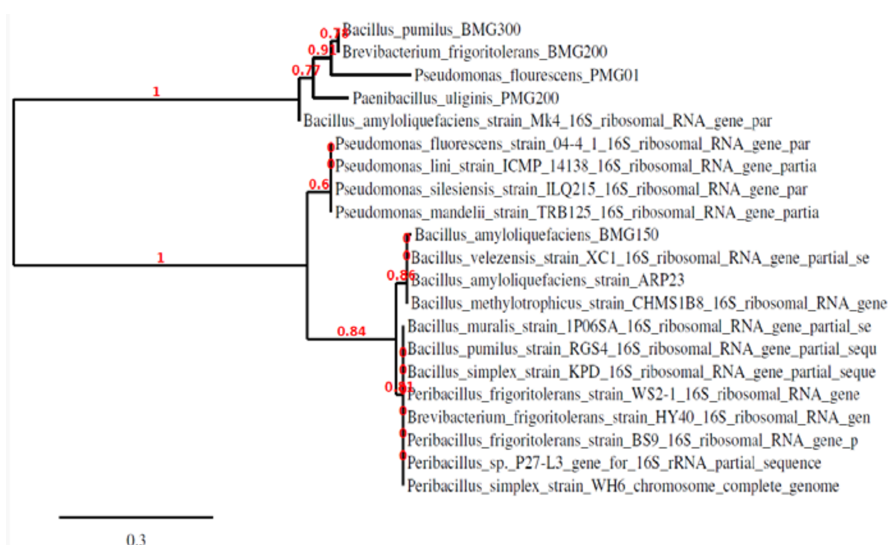


Figure 1. Phylogenetic tree of tomato rhizosphere bacterial isolates depending on 16SrRNA sequenced genes.

Table 3. Tomato rhizosphere bacterial isolates closest relativity.

Strain	Closest relativity	GenBank accession n°	Identity (%)
BMG150	*Bacillus amyloliquefaciens*	OR914616	99.57
PMG01	*Pseudomonas fluorescens*	OR914617	98.25
BMG300	*Bacillus pumilus*	OR914618	98.61
PMG200	*Peribacillus frigoritolerans*	OR914619	98.81
PMG300	*Paenibacillus uliginis*	OR914620	98.50

3.2. Identification of barely seeds associated fungi

Three phytopathogenic fungal strains were isolated from barley seeds and identified based on morphological traits and molecular analysis. Sequence alignment with the NCBI database using BLAST revealed 98.75% identity with *Rhizopus stolonifer*, 98.61% with *Aspergillus niger*, and 100% with *Alternaria alternata*. Thus, the isolated fungi were identified as *Rhizopus stolonifer*, *Aspergillus niger*, and *Alternaria alternata.* Fungal isolates and accession numbers are listed in Table 4.

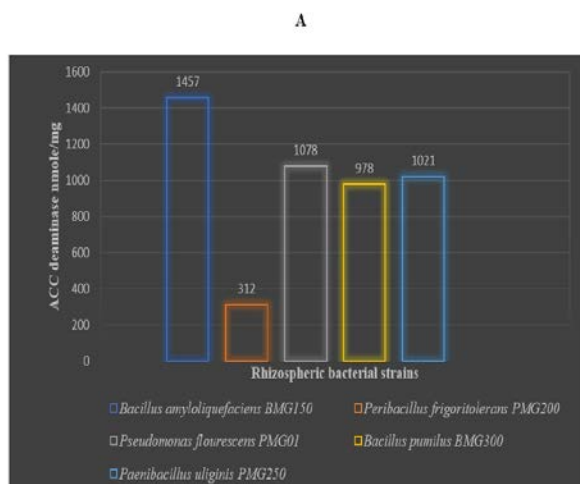
Table 4. Barely seeds fungal isolates closest relativity.

Strain	Closest relativity	GenBank accession n°	Identity (%)
AMG01	*Alternaria alternata*	PP495831	100
AMG02	*Aspergillus niger*	PP495830	98.61
RMG01	*Rhizopus stolonifers*	PP495829	98.75

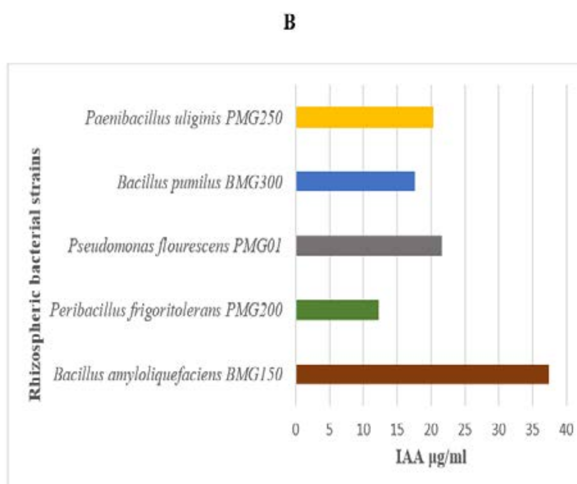
3.3. Quantification of ACC deaminase, IAA, phosphate solubilization, and siderophore activities

The activities of ACC deaminases for the five rhizobacterial isolates were quantified by measuring α-ketobutyrate produced from ACC deamination reaction on DF minimal salt broth media at 540 nm. The five bacterial isolates revealed activities of ACC deaminase ranged from 312–1457 nmol α-ketobutyrate/ mg protein. The *Bacillus amyloliquefaciens* BMG150 exhibited the highest activity (1457 nmol), followed by *Pseudomonas flourescens* PMG01 (1078 nmol), *Paenibacillus uliginis* PMG250 (1021 nmol), *Bacillus pumilus* BMG300 (978 nmol) and *Peribacillus frigoritolerans* PMG200 (312 nmol) (Figure 2A).

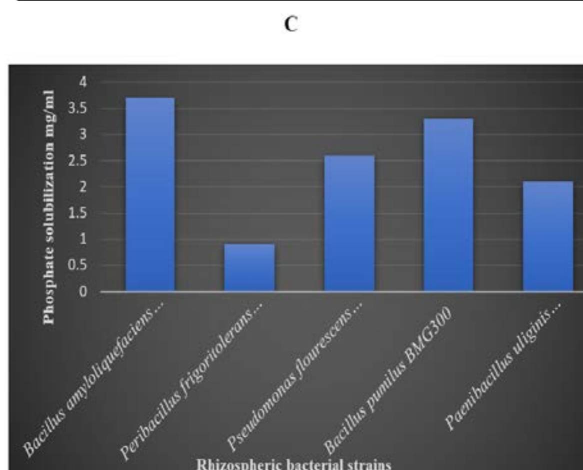
A



B



C



D

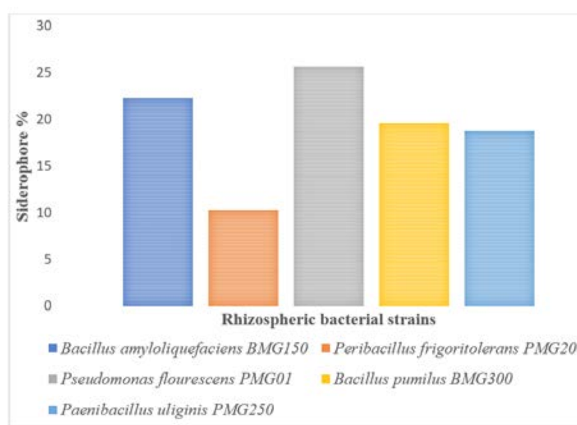


Figure 2. a. Activities of ACC deaminase; b. IAA production, c. phosphate solubilization (mg/ml); d. siderophores % of tomato rhizobacterial five isolates.

The IAA production by the five tomato rhizobacterial isolates was quantified by measuring the formation of indolic compounds at 530 nm. All isolates IAA production ranged between 12.3 µg/ml (*Peribacillus frigoritolerans* PMG200) and 37.4 µg/ml, whereas *Bacillus amyloliquefaciens* BMG110 displayed the highest IAA production followed by isolate *Pseudomonas flourescens*PMG01 (21.6 µg/ml), *Peribacillus frigoritolerans* PMG200 (20.3 µg/ml) and *Bacillus pumilus* BMG300 (17.6 µg/ml) (Figure 2B).

All the five tomato rhizobacterial isolates were solubilized phosphate in solid NBRIP medium by converting the inorganic form of phosphorous (Ca_3PO_4) into the solubilized form by development of yellow colour zone around the colonies on (Pikovaskya agar supplemented with 2% Ca_3PO_4). In liquid medium, *Paenibacillus uliginis* PMG250 displayed the highest solubilized phosphate (2.1 mg/ml) followed by *Peribacillus frigoritolerans* PMG200 (0.9 mg/ml) (Figure 2C). Siderophores production was proved for all the five isolates by development of greenish blue to yellow colour (CAS agar media) on both solid and liquid media. The maximum siderophore % showed for *Pseudomonas flourescens* PMG01 (25.7%) followed by *Bacillus amyloliquefaciens* BMG150 (22.3 %) (Figure 2D).

3.4. Non-ribosomal peptide synthetase genes detection using bioinformatics tools and degenerate primers

The detection of NRPs genes of the selected five tomato rhizobacterial isolates were performed using complete genome sequence accession number of the closest relativity strains available on GenBank: *Bacillus amyloliquefaciens* DSM7, *Peribacillus frigoritolerans, Pseudomonas flourescens*, *Bacillus pumilus* SAFR- 032 and *Paenibacillus uliginis* N3975. Using AntiSmash version 7.0, all these complete genome sequences have been analysed, and the most similar known clusters of different secondary metabolites were revealed. Only NRPs clusters from each genome were summarized in Table 4, whereas *Bacillus amyloliquefaciens* DSM 7 showed the largest number of NRPs clusters (5) represented in surfactin, fengycin, iturin, bacillibactin siderophore and bacillaene (polyketide-NRPs hybrid), followed by *Pseudomonas flourescens* which showed three NRPs clusters; viscosin, tolassin and lankacidin (hybrid polyketide-NRPs). *Bacillus pumilus* SAFR- 032 complete genome sequence analysis using AntiSmash detected two NRPs clusters of lichenysin (85% similarity), but when analyzed by PKS/NRPS analysis website, were identified as pumilacidin (surfactin family) and another cluster with 53 % identity to fengycin but with PKS/NRPS website analysis, no PKS/NRPS related domains were detected. *Peribacillus frigoritolerans* complete genome showed two NRPs clusters for koranimine and another cluster with 46 % similarity to fengycin which re-analyzed by PKS/NRPS analysis website and no PKS/NRPS related domains were detected. *Paenibacillus uliginis* N3975 complete genome showed the presence of two NRPs clusters with very low similarity 1% to pyoverdine with no PKS/NRPS related domains detected when analyzed by PKS/NRPS website, and 11% similarity to zwittermicin which was predicted as: (pks-x-Gly-x) by PKS/NRPS analysis website and might indicate the novelty of this NRPs cluster. All known NRPs clusters for the rhizosphere bacterial isolates were summarized in Table 5.

Table 5. AntiSmash Most similar known clusters detected for the five-tomato rhizosphere bacterial isolates.

Strain	Accession n	Region	Type	From	To	Similar known cluster AntiSmash	Similar known cluster PKS-NRPS& Norine	Similarity
Bacillus amyloliquefaciens DSM7	FN597644.1	1	NRP-Lipopeptide	314,040	378,185	Surfactin	Surfactin	82%
		5	Polyketide-NRP	1,766,333	1,867,399	Bacillaene	Bacillaene	100%
		6	NRP	1,948,676	2,058,873	Fengycin	Fengycin	93%
		9	NRP	2,506,988	2,551,970	Iturin	Iturin	100%
		10	NRP siderophore	3,033,649	3,085,384	Bacillibactin	Bacillibactin	100%
Bacillus pumilus SAFR-023	CP000813.4	1	NRP	323,520	403,989	Lichenysin	Pumilacidin	85%
		5	NRP	1,815,669	1,842,788	Fengycin	No NRP	53%
Pseudomonas flourescens	LT907842.1	1	NRP	114,934	144,184	Ambactin	No NRP	25%
		4	NRP	2,512,939	2,574,494	Viscosin		68%
		7	NRP	3,485,149	3,484,280	Pf-5 Pyoverdine	Pyoverdine partial	11%
		8	NRP	3,824,046	3,847,199	Fengycin	No NRP	13%
		9	NRP	3,951,152	3,996,195	Tolassin lipopeptide	Tolassin lipopeptide	70%
		11	NRP	4,325,050	4,377,946	Pf-5 Pyoverdine	Pyoverdine partial	9%
		14	NRP-polyketide	5,702,542	5,724,689	Lancacidin	No NRP	13%
Peribacillus frigoritolerans	CP091882.1	1	NRP	718,980	777,857	Koranimine	Koranimine	87%
		3	NRP	2,496,957	2,521,117	Fengycin	No NRP	46%
*Paenibacillus uliginis*N3975	LT840184.1	1	NRP	804,360	845,517	Pf-5 Pyoverdine	No NRP	1%
		2	NRP-polyketide	1,419,149	1,490,535	Zwittermicin	Unknown NRP-polyketide	11%

Degenerate primers Serie 1 were designed using the conserved nucleic acid sequences after the alignment of the adenylation and thiolation domains of different *Bacillus* strains for the NRPs lipopeptides known clusters; surfactin, fengycin or plipastatin, iturin (mycosubtilin and bacillomycin) and kurstakin (Tapi *et al*., 2010; Abderrahmani *et al*., 2011). Serie 2 of degenerate primers was designed before by the alignment of the amino acids sequence of the condensation domain (C) and the thioestrase domain (TE) of NRPs synthetases from *Pseudomonas* lipopeptides biosynthesis systems to detect the conserved sequences (Rokni-Zadeh *et al*., 2011). *Bacillus* degenerate primers amplified four fragments of the expected sizes for the presence of the NRPs lipopeptides surfactin, fengycin, plipastatin, mycosubtilin in strain *Bacillus amyloliquefaciens* BMG150, which is consistent with the AntiSmash analysis for the genome of the closest relativity strain *Bacillus amyloliquefaciens* DSM 7. Using surfactin primers, the Bacillus degenerate primers amplified a fragment from *Bacillus pumilus* BMG300 of the predicted size, which is also in agreement with the AntiSmash analysis of the genome of the nearest relativity strain, *Bacillus pumilus* SAFR-032.

On the other hand, *Pseudomonas* degenerate primers amplified two fragments of the expected sizes from *Pseudomonas flourescens* PMG01 isolate which often belongs to viscosin and tolassin NRPs clusters detected by AntiSmash analysis of the closest relativity strain *Pseudomonas flourescens*. On the contrary, both strains *Peribacillus frigoritolerans* PMG200 and *Paenibacillus uliginis* PMG250 amplified fragments of different sizes with fengycin primers (580 bp) which revealed 100 % similarity to Koranimine NRPs gene (Figure 3) as detected AntiSmash analysis for the closest relativity strains *Peribacillus frigoritolerans,* and revealed 100 % similarity to amino acid adenylate gene (684 bp) involved into the NRPs-PKS cluster (pks-x-Gly-x) predicted by AntiSmash and PKS/NRPS analysis website of the closest relativity strain *Paenibacillus uliginis* N3975 (Figure 4). All the positive amplification of degenerate primers for the rhizosphere bacterial isolates is listed in Table 6.

Table 6. Detected NRPs clusters for the tomato rhizosphere bacterial isolates using degenerate primers.

Isolated strains	Degenerate primers							
	***Bacillus* primers**							
	plipastatin	**surfactin**	**fengycin**	**mycosubtilin**	**bacillomycin**	**kurstakin**	***Pseudomonas* primers**	
	Ap, Tp	As, Ts	Af, Tf	Am, Tm	Abl, Tbl	Aks, Tks	C1	TE1, TE2
Bacillus amyloliquefaciens BMG150	+	+	+	+	-	-	-	-
Peribacillus frigoritolerans PMG200	-	-	+	-	-	-	-	-
Pseudomonas flourescens PMG01	-	-	-	-	-	-	+	+
Bacillus pumilus BMG300	-	+	-	-	-	-	-	-
Paenibacillus uliginis PMG250	-	-	+	-	-	-	-	-

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Score            Expect   Identities      Gaps        Strand
1072 bits(580)   0.0      580/580(100%)   0/580(0%)   Plus/Plus

Sequenced fragment P. frigoritolerans PMG200             GAGAATGGCGACCGAAATGATAGAGATAACCAATCAATCCAATATGGAGATTATGCTTTA  60
Koranimine synthetase gene P. frigoritolerans JHS1       GAGAATGGCGACCGAAATGATAGAGATAACCAATCAATCCAATATGGAGATTATGCTTTA  740885

Sequenced fragment P. frigoritolerans PMG200             TGGCaaaaaaaCTCACCAAATTATGTAACAGATAAAGATAATGAGTTCTGGAGTAATGAA  120
Koranimine synthetase gene P. frigoritolerans JHS1       TGGCAAAAAAACTCACCAAATTATGTAACAGATAAAGATAATGAGTTCTGGAGTAATGAA  740945

Sequenced fragment P. frigoritolerans PMG200             ATAACGGCATTACGGCAAAAGTCTTTTTTACAATATGATCATCAAAAAACCGGAGAAGGT  180
Koranimine synthetase gene P. frigoritolerans JHS1       ATAACGGCATTACGGCAAAAGTCTTTTTTACAATATGATCATCAAAAAACCGGAGAAGGT  741005

Sequenced fragment P. frigoritolerans PMG200             AAAAGCACGTCCGATATTATCAGCTTTTTGTTACCTAAAGAAATTCAAGACAACCTGGAA  240
Koranimine synthetase gene P. frigoritolerans JHS1       AAAAGCACGTCCGATATTATCAGCTTTTTGTTACCTAAAGAAATTCAAGACAACCTGGAA  741065

Sequenced fragment P. frigoritolerans PMG200             AGTCTTCATaaaaaaaCGAAATCGACTTTGTTCATGAGTTTACTTACCGCCTATCAAACC  300
Koranimine synthetase gene P. frigoritolerans JHS1       AGTCTTCATAAAAAAACGAAATCGACTTTGTTCATGAGTTTACTTACCGCCTATCAAACC  741125

Sequenced fragment P. frigoritolerans PMG200             TTTTTATCAGTTTACTTTGATGAAGAGGAAGTTGTCGTCGGCAGCCCTTTGGCGAAGAGA  360
Koranimine synthetase gene P. frigoritolerans JHS1       TTTTTATCAGTTTACTTTGATGAAGAGGAAGTTGTCGTCGGCAGCCCTTTGGCGAAGAGA  741185

Sequenced fragment P. frigoritolerans PMG200             AACCATGTGGATACTGAACAATTGATAGGATATTTCGTCAACACCTTGCCCTTTAAATTA  420
Koranimine synthetase gene P. frigoritolerans JHS1       AACCATGTGGATACTGAACAATTGATAGGATATTTCGTCAACACCTTGCCCTTTAAATTA  741245

Sequenced fragment P. frigoritolerans PMG200             CATGTATCCCAGCAAGATTCATTTGAAGGGATTTTGCGaaaaaacataaaaaaTATTGCA  480
Koranimine synthetase gene P. frigoritolerans JHS1       CATGTATCCCAGCAAGATTCATTTGAAGGGATTTTGCGAAAAAACATAAAAAATATTGCA  741305

Sequenced fragment P. frigoritolerans PMG200             GGTGTTTTTGACCATCAAAATTTACCTACTAAGGAGATTTTGAAATATTTATCGGCAGAA  540
Koranimine synthetase gene P. frigoritolerans JHS1       GGTGTTTTTGACCATCAAAATTTACCTACTAAGGAGATTTTGAAATATTTATCGGCAGAA  741365

Sequenced fragment P. frigoritolerans PMG200             AGAACCATGGAAAATACGCCATTGTTCGAAACAGTATTCG  580
Koranimine synthetase gene P. frigoritolerans JHS1       AGAACCATGGAAAATACGCCATTGTTCGAAACAGTATTCG  741405
```

Figure 3. BlastN sequence alignment of fragment amplified using fengycin degenerate primers from strain *Peribacillus frigoritolerans* PMG200.

```
Score            Expect   Identities       Gaps        Strand
1264 bits(684)   0.0      684/684(100%)    0/684(0%)   Plus/Plus

Sequenced fragment P. uliginis PMG250          GCCCCTGTTCAGCTGCCTGAATGGTATTTGCATCAACTGGATTCCGAAAGCACGAATTAC  60
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    GCCCCTGTTCAGCTGCCTGAATGGTATTTGCATCAACTGGATTCCGAAAGCACGAATTAC  1458600

Sequenced fragment P. uliginis PMG250          AACATTCCTATTGAGTTAATGTTTAGAGGTAATTTAAACTTGAAGGCATTTGAGAAGGCT  120
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    AACATTCCTATTGAGTTAATGTTTAGAGGTAATTTAAACTTGAAGGCATTTGAGAAGGCT  1458660

Sequenced fragment P. uliginis PMG250          TGGAACAGTTTGATTGAGAAAAATAGTGTGTTTAGAACTACTTTCGATATAACGAACGGA  180
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    TGGAACAGTTTGATTGAGAAAAATAGTGTGTTTAGAACTACTTTCGATATAACGAACGGA  1458720

Sequenced fragment P. uliginis PMG250          GAACCAATTCAAATCATACATGAGGAGATCAAGTTTGAACTAAGTGAAGTCTATTTTGAT  240
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    GAACCAATTCAAATCATACATGAGGAGATCAAGTTTGAACTAAGTGAAGTCTATTTTGAT  1458780

Sequenced fragment P. uliginis PMG250          TATTCAGATCTACCTAAATATGAGGCATTGAAAAAAGCGGAAGAACTAGCTTTATCTCAT  300
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    TATTCAGATCTACCTAAATATGAGGCATTGAAAAAAGCGGAAGAACTAGCTTTATCTCAT  1458840

Sequenced fragment P. uliginis PMG250          GCACATCAAGTTTTTGATTTTACGAATGGACCTATGTTTAGTGTTCAGCTAGTCCAAATA  360
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    GCACATCAAGTTTTTGATTTTACGAATGGACCTATGTTTAGTGTTCAGCTAGTCCAAATA  1458900

Sequenced fragment P. uliginis PMG250          GATCGTGATCATCACTTGTTCTTATTTGCTACCCATCATATTTTATGGGATGAAGTATCT  420
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    GATCGTGATCATCACTTGTTCTTATTTGCTACCCATCATATTTTATGGGATGAAGTATCT  1458960

Sequenced fragment P. uliginis PMG250          TCAATTAATCTCATCAGTGAATTATCCAGACTGTACAATTCCTTTAATCAGGATATCAAT  480
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    TCAATTAATCTCATCAGTGAATTATCCAGACTGTACAATTCCTTTAATCAGGATATCAAT  1459020

Sequenced fragment P. uliginis PMG250          AATCAAGTCATTTCCCAGTCTTCTGAAATCGACTACATCGATTATGTAGAATGGGTGAAT  540
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    AATCAAGTCATTTCCCAGTCTTCTGAAATCGACTACATCGATTATGTAGAATGGGTGAAT  1459080

Sequenced fragment P. uliginis PMG250          TCTTCGCTTGAAAAAGGATTATTTCACAGACAAAGAGACTATTGGTTGGAAAAATTCAAA  600
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    TCTTCGCTTGAAAAAGGATTATTTCACAGACAAAGAGACTATTGGTTGGAAAAATTCAAA  1459140

Sequenced fragment P. uliginis PMG250          ACGGTTCCAGAACCATTACAATTACCTACTGATTATGTGCGCCCAGAAATTCAAACATTT  660
                                               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    ACGGTTCCAGAACCATTACAATTACCTACTGATTATGTGCGCCCAGAAATTCAAACATTT  1459200

Sequenced fragment P. uliginis PMG250          GAAGGGGCAACAATTTTCGAGGTC  684
                                               ||||||||||||||||||||||||
amino acid adenylate gene P. uliginis N3975    GAAGGGGCAACAATTTTCGAGGTC  1459224
```

Figure 4. BlastN sequence alignment of fragment amplified using fengycin degenerate primers from strain *Paenibacillus uliginis* PMG250.

3.5. Sprout barely invivo experiment

In this experiment and after 12 days, results were interpreted depending on two main points: (1) production efficiency and (2) seedling length. For the production efficiency, it was remarked that the sample G (soaked in *Bacillus amyloliquefaciens* BMG150) recorded the highest values (7 folds), and seedling length (22.4 cm) followed by the sample E (soaked in *Pseudomonas flourescens* PMG01) (3.8 folds and 16.2 cm), respectively. On the other hand, the lowest production efficiency, and seedling length was observed for the samples B (control 2: washed only with water) (1.3 folds and 11.2 cm), followed by A (control 1: washed and sterilized using Sodium hypochlorite) (1.6 folds and 13.3 cm), C (soaked in *Paenibacillus uliginis* PMG250, *Peribacillus frigoritolerans* PMG200 and *Bacillus pumilus* BMG300,) (3 folds and 15.8 cm), respectively (Figure 5 and Table 7). These results underscore the importance and the efficiency of the use of strain *Bacillus amyloliquefaciens* BMG150 in enhancing sprouted barely seedling germination ratio, strength and length followed by the strain *Pseudomonas flourescens*PMG01.

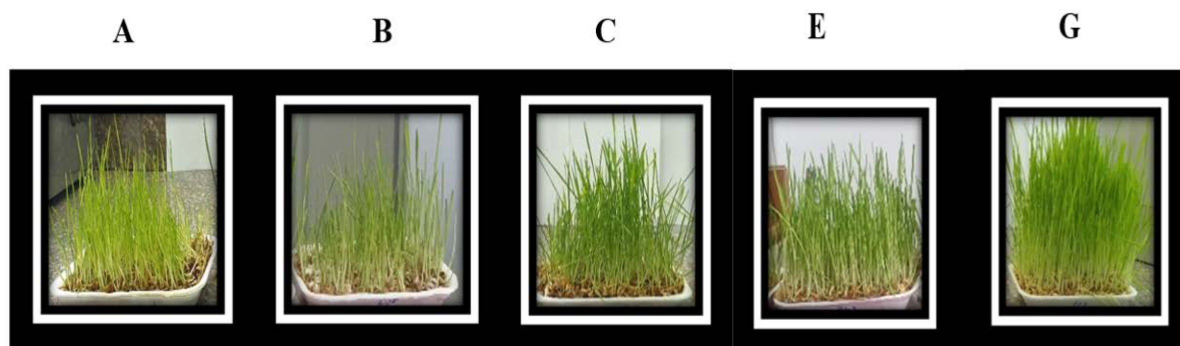

Figure 5. Sprouted barely cultivated for 12 days, (A) control 1: washed and sterilized using Sodium hypochlorite, (B) control 2: washed only with water, (C) barely seeds washed by water, sterilized by Sodium hypochlorite, and soaked in *Paenibacillus uliginis* PMG250, *Peribacillus frigoritolerans* PMG200 and *Bacillus pumilus* BMG300, (E) barely seeds washed by water, sterilized by Sodium hypochlorite and soaked in *Pseudomonas flourescens* PMG01, (G) barely seeds washed by water, sterilized by Sodium hypochlorite and soaked in *Bacillus amyloliquefaciens* BMG150.

Table 7. Sprout barley characteristics after 12 days of plantation using tomato rhizobacterial biofertilizers and fungal inoculants.

Characteristics	A	B	C	E	G
Seed weight	125g	125g	125g	125g	125g
Sprout barely weight	200g	168g	386g	478g	883g
Production efficiency	1.6 folds	1.3 folds	3 folds	3.8 folds	7 folds
Leave length	13.3 cm	11.2 cm	15.8 cm	16.2 cm	22.4 cm

3.6. Soluble protein banding patterns

Protein profile determination by SDS-PAGE for barley samples showed different banding patterns between the treatments. Many alterations in protein patterns were recorded for barley leaves *soaked in different rhizobacterial strains* (*Paenibacillus uliginis* PMG250, *Peribacillus frigoritolerans* PMG200 and *Bacillus pumilus* BMG300, *Pseudomonas flourescens* PMG01 and *Bacillus amyloliquefaciens* BMG150) under two controls. There is a number of bands totalling 21; ten of them were monomorphic bands with 47.6 %, while eleven polymorphic bands appeared with 52.4 %. There is one unique band at MW 15 KDa appeared in samples G (treated with *Bacillus amyloliquefaciens* BMG150 after 9 days). Also, there is one unique band that appeared at MW 40 KDa in samples C (treated with *Paenibacillus uliginis* PMG250, *Peribacillus frigoritolerans PMG200* and *Bacillus pumilus BMG300* after 9 days) *and G* (treated with *Bacillus amyloliquefaciens* BMG150 *after 9 days),* respectively. The highest number of bands was revealed in sample C *(treated* with *Paenibacillus uliginis* PMG250, *Peribacillus frigoritolerans* PMG200 and *Bacillus pumilus* BMG300 after 9 days) and sample G with 20 and 19 bands, respectively. On the other hand, sample B**'** (control 2 at 12 days) were the lowest number of bands (10) as shown in (Table 8 and Figure 6). These results were in harmony with barely invivo experiment**.** The density, strength and length of the weakest seedlings in growth were observed for the sample B (control 2: washed only with water).

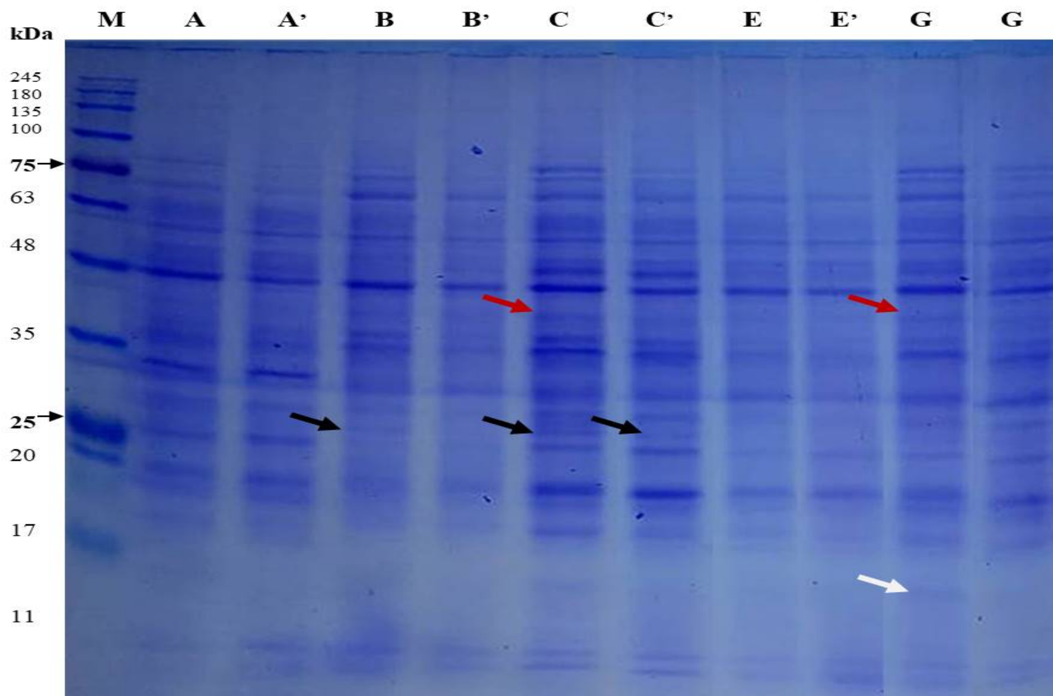

Figure 6. Electrophoretic patterns soluble proteins of the sprouted barely leaves treated with rhizobacterial isolates performed by SDS-PAGE. M: is standard protein.

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Table 8. Densitometric analysis water soluble protein patterns for sprouted barley plants treated with rhizobacterial strains. (+) presence of bands; (-) absence of bands.

		Samples										
Band NO.	**size KDa**	**A**	**A'**	**B**	**B'**	**C**	**C'**	**E**	**E'**	**G**	**G'**	
1	90	-	-	-	-	+	-	-	-	+	+	3
2	85	+	-	+	-	+	+	+	-	+	+	7
3	70	+	+	+	+	+	+	+	+	+	+	10
4	65	+	+	+	-	+	+	-	-	+	+	7
5	60	+	+	+	+	+	+	+	+	+	+	10
6	55	-	-	-	-	+	+	-	-	+	+	4
7	48	+	+	+	+	+	+	+	+	+	+	10
8	40	-	-	-	-	+	-	-	-	+	-	2
9	38	-	-	+	-	+	+	-	-	+	+	5
10	36	+	+	+	+	+	+	+	+	+	+	10
11	32	+	+	+	+	+	+	+	+	+	+	10
12	30	+	+	+	+	+	+	+	+	+	+	10
13	27	-	-	+	-	+	+	-	-	-	-	3
14	25	+	+	-	-	+	+	+	+	+	+	8
15	20	+	+	+	+	+	+	+	+	+	+	10
16	19	+	+	-	-	+	+	-	-	+	+	6
17	18	+	+	+	+	+	+	+	+	+	+	10
18	15	-	-	-	-	-	-	-	-	+	-	1
19	11	+	+	+	+	+	+	+	+	+	+	10
20	10	+	+	+	+	+	+	+	+	+	+	10
21	8	-	+	-	-	+	+	-	+	-	+	5
Total bands		**14**	**14**	**14**	**10**	**20**	**18**	**12**	**12**	**19**	**18**	**151**

4. Discussion

Plant-microbe interactions type is often responsible for plant growth and development in the rhizosphere, whereas various studies reported the presence of various Bacillus species in the soil rhizosphere and as PGPR (Kumar *et al*., 2012; Singh *et al*., 2014) according to their promoting plant growth role or suppressed several phytopathogens (Mumtaz *et al*., 2017; Akinrinlola *et al*., 2018).

Here, we highlighted the potential of some tomato rhizosphere beneficial bacteria isolates which are considered the first line defense against pathogens by choosing the more efficient growth promoting characteristics bacteria. In this work, a total of 16 tomato bacterial isolates were evaluated for their PGP characteristics. Only five strains of which revealed considerable values for ACC deaminase, IAA, siderophores and solubilizing phosphate. The five rhizobacterial isolates showed their ACC deaminase production ability in accordance with (Singh *et al*., 2019) who confirmed this ability for rhizobacterial isolated from various crop plants. Furthermore, the ACC deaminase activity quantification ranged from 312–1457 nmol α-ketobutyrate/ mg protein and the isolate *Bacillus amyloliquefaciens* BMG150 exhibited the highest ACC deaminase activity; therefore, ACC deaminase values determination is vital (Singh *et al*., 2019) and the rhizobacteria that can combine both the activity of ACC deaminase and other PGP properties is considered an amended symbiotic associate for the host plants (Tiwari *et al*., 2018; Gowtham *et al*., 2020).

The five isolates are positive producers of IAA which is considered growth regulator of different plant growth stages (Etesami *et al*., 2015). Furthermore, the isolates were assessed for their potential for producing siderophores which enhance iron availability to the plant and reduce iron availability for phyto-pathogens, respectively (Saha *et al*., 2016; Sansinenea, 2019). Phosphate solubilization ability was also tested by the five isolates, which can be interpreted by the production of several components capable of transforming insoluble phosphates into easily absorbed substances by plants (Rodríguez and Fraga, 1999; Chen *et al*., 2006; Patel *et al*., 2008; Brígido and Glick, 2017). Among the selected isolates, the high values were recorded to isolates *Bacillus amyloliquefaciens* BMG150, *Pseudomonas flourescens* PMG01 and *Bacillus pumilus* BMG300. The five efficient rhizosphere bacterial isolates were identified with the 16SrRNA gene sequences, which were similar by 99–100% with genera: Bacillus, Pseudomonas and Peribacillus. The results agree with the finding of (Hariprasad, 2014).

Here, we demonstrated the efficiency of using degenerate primers in detecting NRPs synthetase genes, whereas it empowered us to take insight view about several genes involved in this mechanism side by side with bioinformatics using AntiSmash and PKS-NRPS analysis websites. The use of degenerate primers in detecting NRPs genes became commonly applied. Several studies designed series of these primers, and (Marahiel, 1997) designed a set of primers depending on the motif A2 (KAGGAY) LV P which are highly conserved for peptide synthetases. The second set was designed by (Neilan *et al*., 1999; Viscaino *et al*., 2005) depending on the conserved motif of the adenylation domain (A) aligned from various bacteria and fungi. Recently, the design of NRPs degenerate primers became more specialized depending on bacteria genera; Bacillus, for instance, is considered as one of the first genera for which this kind of NRP degenerate primers was

designed. *Bacillus* non-ribosomal lipopeptide synthetase genes degenerate primers were designed depending on the extraction of the conserved nucleic acids sequence of the (A) and the (T) domains after their alignment from different Bacillus members (Tapi *et al.,* 2010; Chen *et al*., 2006), while Pseudomonas degenerate primers were designed before by the alignment of the amino acids sequence of the condensation domain (C) and the thioestrase domain (TE) of NRPs synthetases from Pseudomonas lipopeptides biosynthesis systems to detect the conserved sequences (Rokni-Zadeh *et al*., 2011). In our study, *Bacillus* degenerate primers (Tapi *et al*., 2010; Chen *et al*., 2006) proved their efficiency in detecting NRLPs clusters of surfactin (As1-F/Ts2-R primers), fengycin (Af2-F/Tf1-R primers), plipastatin (Ap1-R/Tp1-R primers), mycosubtilin (Am1-F/Tm1-R primers) in strain *B. amyloliquefaciens* BMG150, pumilacidin (As1-F/Ts2-R primers) from *B. pumilus* BMG300, koranimine (Af2-F/Tf1-R primers) from *Peribacillus frigoritolerans* PMG200 and unknown NRPs (Af2-F/Tf1-R primers) from *Paenibacillus uliginis* PMG250. The previous results agree with (Tapi *et al*., 2010) who amplified fragments of expected sizes with As1-F/Ts2-R primers from *B.s* 168 and *B. licheniformis* ATCC 14580 (99%) similarity with surfactin and lichenysin (surfactin family), respectively, amplified fragments of expected sizes with plipastatin (Ap1-R/Tp1-R) primers from *Bs* 168 similar (99%) to plipastatin and detected mycosubtilin gene by (Am1-F/Tm1-R) primers in *Bs* ATCC6633. (Tapi *et al*., 2010) also confirmed the amplification of fragments of different sizes with Af2-F/ Tf1-R primers from *Bs* ATCC6633, similarly to bacillaene polyketide synthase of *B. amyloliquefaciens* FZB42 (88%) and of *B.s* 168 (Chen *et al*., 2009; Al-shebly, 2022). The fengycin primer pairs can detect unexpected NRPs genes. These results agree with our finding for the isolates *Peribacillus frigoritolerans* PMG200 and *Paenibacillus uliginis* PMG250 amplified fragments corresponding to another NRPs gene rather than fengycin (Koranimine and unknown NRPs), respectively. On the other hand, *Pseudomonas* degenerate primers amplified two fragments of the expected sizes from *Pseudomonas flourescens* PMG01 isolate similar to viscosin and tolassin, as reported (Rokni-Zadeh *et al*., 2011) who amplified fragments similar to viscosin and tolassin with the expected size from three maize rhizosphere *Pseudomonas florescent* isolates (PGSB3962, PGSB7828, and PGSB8273).

The usage of rhizobacterial strains with PGP characteristics proved their efficiency in promoting barely growth and reduced fungal growth during sprout barely plantation. The production efficiency and leaves length decreased in control 1 and control 2; these results agree with (Paul and Nair, 2008) who confirmed that the presence of *Aspergillus flavus* decreased barley seeds germination and seedlings growth. We suggest that the use of microbial biofertilizers overcome the negative effects of salt by producing osmolytes and salt stress-induced proteins and therefore enhance sprouted barley production efficiency and leave length, which agrees with (Ryu *et al*., 2004). Also, our results agree with (Murphy *et al*., 2003) who described the capability of *Bacillus subtilis* GBO3 in inducing defense-related pathways like, salicylic acid (SA) and jasmonic acid (JA). These results agree with (Aliasgharzad, 2006) who confirmed the enhancement of tomato immunity against tomato mottle virus by the application of *Bacillus amyloliquefaciens* 937b and *Bacillus pumilus* SE-34 as PGPR.

The effect of different bacterial treatments appears in the expression of some genes encoding proteins by switching on or off. Based on our results, we found different bacterial treatments by combination formula of the three strains *Paenibacillus uliginis* PMG250, *Peribacillus frigoritolerans* PMG200 and *Bacillus pumilus* BMG300 and the use of *Bacillus amyloliquefaciens* BMG150 separately revealed the highest number of bands with 20 and 19 bands, respectively. These findings are in accordance with (Murphy *et al*., 2003; Aliasgharzad, 2006) who reported that PGPR strains can induce defense-related pathways like, Salysalic Acid and Jasmonic Acid and enhance tomato immunity. The findings might reflect on protein patterns by the appearance of newly synthesized bands and the absence of others. (Boston *et al*., 1996; El-Saber, 2021) observed that under different stress conditions, molecular chaperones are involved in various cellular functions which agrees with our findings of the low molecular weight proteins accumulation.

5. Conclusion

The use of biofertilizers isolated from soil rhizosphere is considered an alternative to chemical substances and enhances plant growth. The soaking of barely seeds before planting in these rhizosphere isolates suspension enhances the growth of sprouted barely.

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