Global Science Research Journals

ISSN: 2505-001X Vol. 5(1), pp. 313-322, January, 2017.

http://www.globalscienceresearchjournals.org/

Global Journal of Biological and Biomedical Research

Full Length Research Paper

A study on the isolates of *B. cereus* from soil towards potential plant growth promoting (PGP)

Ferhat Mosteghanemi Arkoun

Department of Biology, Faculty of Natural Sciences and Life, Tlemcen University, P. O. Box 119, 08000 - Algeria.

Accepted 23 December, 2016

Bacillus cereus **is a group of bacteria frequently found in soil, widely distributed in the environment. They are a group of ubiquitously facultative anaerobic spore-forming Gram-positive rods and are of health and economic benefits. The present study was conducted to identify, characterize about 36 *B. cereus* and examined for their potential plant growth promoting (PGP), which was tested *in vitro*. Parameters assessed were indole acetic acid (IAA) production, phosphate solubilization, starch hydrolysis, proteolytic activity and biofilm formation. Multiple *B. cereus* were isolated from several soil plots from South-Western region of Algeria and characterized by using phenotypic methods including fatty acid methyl ester. Ten bacterial isolates were examined in this study. Fatty acid profiles showed that bacterial isolates were classified into *B. cereus* group, three isolates were *B. cereus* Subgroup "A" and seven isolates were *B. cereus* Subgroup "B". Temperature effect on the maximal specific growth rate was studied in *B. cereus* between 10 and 50°C, no growth was observed in 10°C, all *B. cereus isolates* grown from 15 to 45°C and no *grown* observed in 20 to 50°C. This study demonstrates adaptation of isolates of the *B. cereus* group to different habitats. The ability to solubilize precipitated phosphate was positively exhibited by three isolates, five isolates showed ability to produce IAA. All the isolated bacterial isolates had amylolytic and proteolytic activity. All isolates did not form a biofilm in the microtiter plate assays, while all *B. cereus* in our study formed biofilm in tubes at air-liquid interfaces.**

Key words: *Bacillus cereus*, soil, identification, plant growth promoting rhizobacteria (PGPR), phosphate solubization, biofilm

INTRODUCTION

In soil, microbes are especially active in the rhizosphere, which can contain more than a million microorganisms per gram of soil. Microbial community structures are variable and depend on factors such as temperature, pH,

*Corresponding author: E-mail: ferhat.arkoun@hotmail.com

or geographic location, but are able to tolerate environmental change. Microorganisms found in this environment use energy substrates released by roots such as and necessary to their metabolism: sugars, amino acids, organic acids, hormones. Some of these microorganisms, mainly bacteria, are able to effectively colonize the root systems and stimulating plant growth and / or protecting against infections by plant pathogens. These rhizosphere bacteria are sometimes called Plant Growth-Promoting rhizobacteria (PGPR) (Kloepper et al., 1986).

The beneficial effects of rhizobacteria on plant growth are the result of either direct or indirect mechanisms by PGPR. Indirect mechanisms occur outside of the plant, while direct mechanisms are those that occur inside of the plant and directly affect their metabolism. These mechanisms can function simultaneously or sequentially at different stages of plant growth. Many strains of PGPR can affect plant growth directly by solubilizing phosphates, nitrogen fixation and mineral nutrients, making these foods available for the plant, by producing phytohormones such as indole-3-acetic acid (IAA), and repressing of soil pathogenic microorganisms (production of hydrogen cyanide, siderophores, antibiotics, and / or competition for nutrients). In addition, PGPR can contribute to the improvement of plant resistance to biotic and abiotic stress such as salinity, drought and heavy metal toxicity (Canbolat et al., 2006).

Bacillus cereus sensu lato (s.l.) are Gram-positive endospore forming bacteria that are abundant in different ecological environments. The growth temperature range varies from 5 to 50°C (Guinebretiere et al., 2008).

This group of bacteria includes seven species that are genetically very similar. *B. cereus* sensu stricto (*B. cereus* s.s.) which is associated with food borne illness but is also involved in some cases of local super infections (Logan, 2012). *Bacillus thuringiensis* is an entomopathogen used with success in agriculture as a biocontrol agent against insects (De Maagd et al., 2001). *Bacillus anthracis* is the agent of anthrax (Mock and Fouet, 2001). This group (*B. cereus sensu lato (s.l.)* also species (Lechner et al., 1998). *Bacillus cytotoxicus* characterized by its high toxicity and its particular thermotolerance (Guinebretière et al., 2013), and *Bacillus mycoides* and *Bacillus pseudomycoides* species that are characterized by the formation of rhizoids colonies (Nakamura, 1998).

This study was aimed to characterized thirty six isolates of *B. cereus* from soil for a better appreciation for their potential plant growth promoting (PGP), that is, IAA production, phosphate solubilizer, starch hydrolysis, proteolytic activity and biofilm formation.

MATERIALS AND METHODS

Isolation of strains

Samples were isolated from several soil plots in the Naama region (South-Western region of Algeria) (Figure 1) over ten months (March till December 2014), from four different areas (about 100 m^2 each). 1 g of each sample of soil was added to a tube containing 9 mL of sterile distilled water. Serial dilutions were heated at 80°C for 10 min to eliminate vegetative cells and to select for spores. Each dilution (0.1 ml) was spread on Luria-Bertani (LB) agar plates. A total of thirty-six isolates of *B. cereus* group, were isolated according to the International Organization for Standardization (ISO, 1993).

Morphological, physiological and biochemical characterizations of isolates

It is possible to differentiate *B. cereus* from other *Bacillus* species because they share similar morphology mobility, hemolytic activity, inability to utilize mannitol, and the production of an extremely active lecithinase , on Mossel culture media (MYP agar) *B. cereus* forming rough and dry colonies with a violet pink background surrounded by egg yolk precipitation. Hemolytic activity is tested using on Columbia blood agar incubated at 30°C for 24 h and is manifested by a characteristic lysis area near the bacterium (Fricker et al., 2008). To characterize the effect of temperature on the growth of *Bacillus cereus* group, isolates, were incubated on LB plates at 10, 15, 20, 30, 37, 45 and 50°C for 72 h. For long term conservation, obtained isolates were stored in Eppendorf tubes containing LB with 20% glycerol at -20ºC (Guinebretiere et al., 2008). Other biochemical characterizations of isolates are tested using the API 20E.

Extraction and analysis of fatty acid methyl ester (FAME) profiles

We used gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, USA) to separate FAME. We identified FAME profiles of each bacterial strain by comparing the commercial databases (TSBA 6) with the MIS software package (Sherlock 6.0 MIDI, Inc., Newark, DE, 2005).

Each samples is processed in a single test tube; a summary of the processing activities associated with each step includes:

- Harvest bacteria from third quadrant.
- Saponification: Combine water and methanol. Add NaOH pellets to the solution while stirring and stir until the pellets dissolve.
- Methylation: add acid to methanol while stirring.
- Extraction: add the methyl tert- butyl ether (MTBE) to the hexane and stir well.
- Wash: add NaOH pellets to the water while stirring and stir the pellets are dissolved.

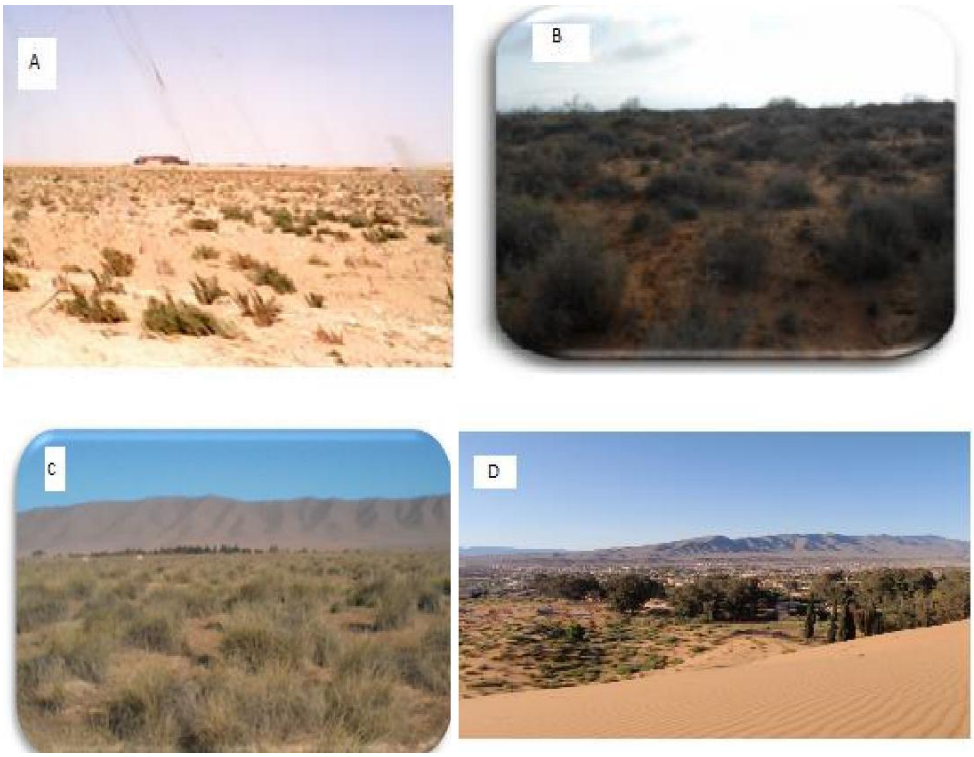

Figure 1. Locations of soil sampling (A) Abdelmoula (B) Mekmen Ben Amar (C) Mechria (D) Ain Sefra.

Plant growth promotion activities for isolates

Production of Indole acetic acid

40 µg/ml of L-tryptophan was added to LB medium to assure growth of our isolates. They were incubated at 30°C for 48 h. Cultures of bacteria were centrifuged for 15 min at 10000 rpm, and 1 ml of culture filtrate was mixed with 1 ml of Salkowski's reagent and the mixture incubated at room temperature for 30 min. A pink color was indicative to indole acetic acid production (Gordon and Weber, 1951).

Phosphate solubilization

Prepared GYA according to Beneduzi et al. (2008): 10 µL aliquot of each culture was plate on GYA and incubated at 28ºC for 7 days, presence of clear halos around their colonies indicate that isolate can solubilize phosphate.

Starch hydrolysis

This test was performed by cultivating the isolates on a nutrient agar containing 1% starch. After incubation at 30° for 48 h, cultures bacteria were covered with Lugol's solution. Hydrolysis of starch is well demonstrated by the appearance of a clear zone around the colony when placed against a negative result revealing a brown color around culture (Gupta et al., 2004).

Proteolytic activity: Search for caseinase

The hydrolysis of casein was studied on an agar medium containing 5% skim milk. After incubation at 30°C for 48 h, a clear halo around the streak indicated the hydrolysis of casein by against a negative result showed no hydrolysis zone around culture (Marchand et al., 2009).

Formation of biofilm in microtiter plate

Polystyrene microtiter plates were filled with 200 µl cultures grown in LB and inoculated. The plates were incubated at 30°C. After 24 h, we washed wells three times with 200 µl of phosphate buffered saline, and stained with 0.1% (wt/vol) crystal violet for 30 min to color biofilm. The cultures were distained twice with 200 µl sterile deionized water. The remaining crystal violet was dissolved in 200 µl 96% ethanol, and we measured at 595 nm the absorbance (Djordjevic et al., 2002).

Biofilm formation in tube

A loopful of isolates was inoculated in 10 mL LB medium in test tubes at 30ºC for 24 h. After incubation, tubes were decanted and washed with phosphate buffer saline and dried. Tubes were then colored with crystal violet (0.1%). Excess isolate was washed with deionized water. Tubes were dried in inverted position. Isolates which showed a visible film lined the wall of the tube were considered as positive biofilm formation (Christensen et al., 1982).

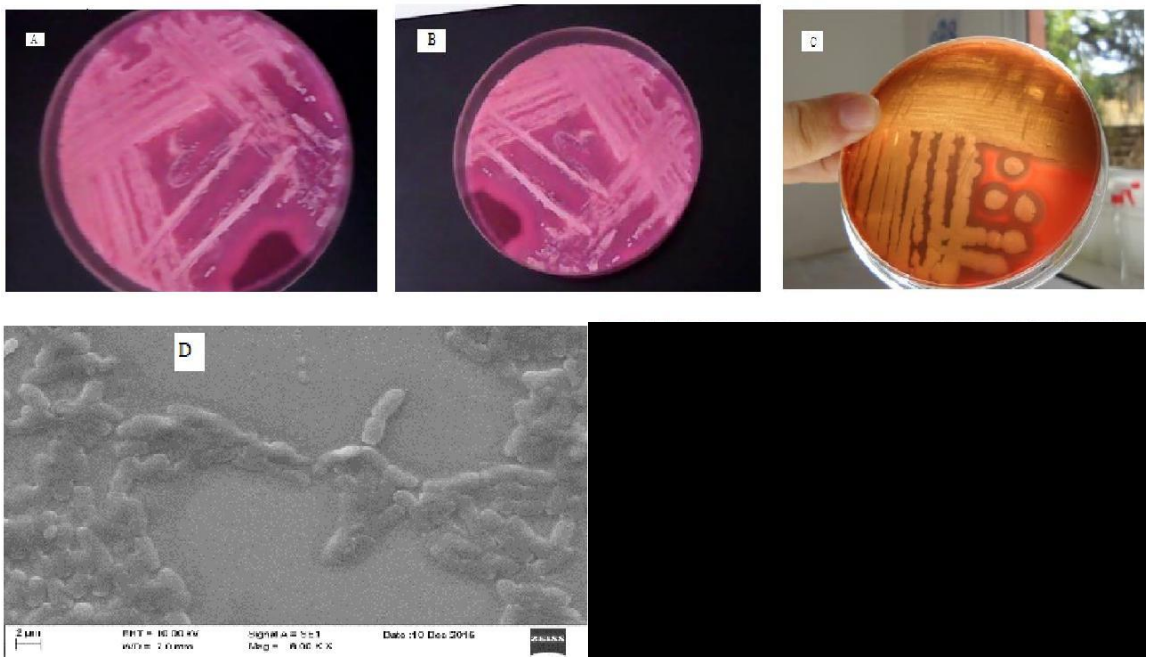

Figure 2. Morphological characteristics of two isolates A: *B. cereus* (Bact 2) in MYP; B: *B. cereus* (Bact 3) in MYP; C: hemolytic activity by *B. cereus* (Bact 2); D: Scanning Electron Microscopy (SAM) picture of *B. cereus* (Bact 2) E: phase contrast microscopy picture of *B. cereus* (Bact 3).

Hydrophobicity

The hydrophobicity of 10 *B. cereus* isolates was determined according to the microbial adhesion to solvents (MATS) (Bellon-Fontaine et al., 1996). Hydrophobicity is expressed as percentage of adhesion to hexadecane. The prepared suspension was adjusted at 595 nm of 0.6-1 (A_0). Samples of each suspension (2 ml) were added to 400 µL of hexadecane mixed on a vortex mixer for 10 S for 15 min. the absorbance of the aqueous phase was measured at 595 nm (A_1).The percentage of hydrophobicity (%h) was determined from the absorbance of the initial bacterial cell, or spore suspensions (A_0) and the absorbance of the aqueous phase after mixing with hexadecane (A_1) using the following equation: %h = [(A_0 - A_1)x 100]/ (A_0. Spores are very hydrophilic (%h < 20%), hydrophilic (20 > %h < 40%), moderately hydrophobic (40 > %h < 60%) and highly hydrophobic (%h > 60%).

RESULTS AND DISCUSSION

Identification and characterization of bacterial strains

We obtained thirty six *B. cereus* group isolates from various localities in Naama south-western Algeria. All isolates showed typical colony morphology with a zone of precipitation. All of the isolates were Gram positive, rod shaped, endospore forming, catalase positive, lecithinase positive and hemolytic (Figure 2). All isolates were able to grow in the range of temperature tested 15 to 45°C (Table 1). To clarify the existing diversity of *B. cereus* group and its special ability to adapt to widely diverse habitats, Guinebretière et al. (2008) showed that the genetic structure of *B. cereus* group belong the same phylogenetic group, describe more that found. All isolates of this study affiliated to group III. This genetic group includes isolates whose growth temperature is between 15 to 45°C. Temperature is one of the most important environmental factors to which microorganisms tolerate different kinds of environmental changes. In the API 20 E, all isolates were VP, Citrate, ADH, Gelatine positive, except three isolates were ADH negative, however the results given by other tests are always negative (Table 1).

FAME analysis

Ten bacterial isolates were examined in this study (Figure 3). They were classified into *B. cereus* groups. The characterization studies based on FAME analysis showed that total 31 different FAMEs were present in 10 bacterial isolates tested in the present study (Table 2). The data of fatty acid analysis showed that three isolates (bact3, bact4, bact7) were *B. cereus* Subgroup A, remaining isolates were *B. cereus* Subgroup B.

According to the results based on fatty acid profiles we can deduce a low diversity among the isolates. The ten samples were isolated from several soil plots, both the origin of the isolates and the pressure exerted by

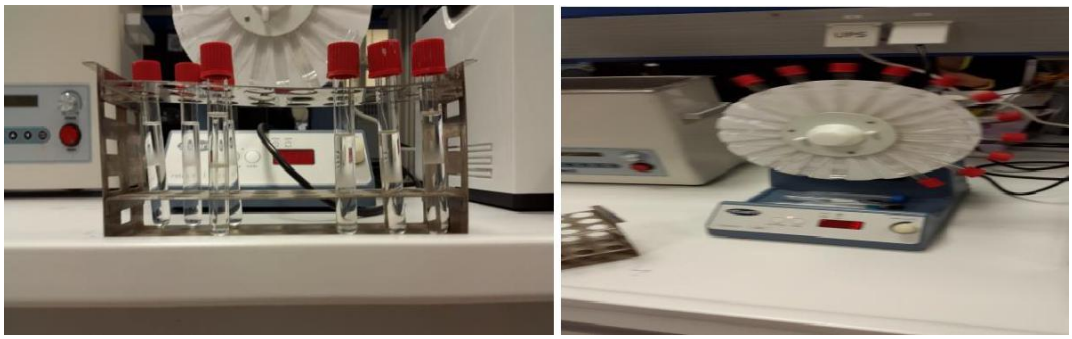

Figure 3. Fatty acid methyl ester (FAME) analysis.

Table 1. Biochemical and physiological characteristic of 36 *B. cereus* isolates.

Number of strains	Origin	Positif biotype character	Thermic profile of growth
5	Abdelmoula	Biotype I (VP, Citrate, ADH, Gelatine)	Mesophilic 15 to 45°C
6	Mekmen Ben Amar		
4	Mechria		
18	Ain Sefra		
1	Mechria	Biotype II (VP, Citrate, Gelatine)	
2	Ain Sefra		

environmental conditions in the soil can explain the low diversity identified by our results, it has been reported that temperature is assumed to select some bacterial groups (Von Stetten et al., 1999). Major environmental variables that may influence bacterial-community composition include vegetation type and temperature, plant species variables influence the composition of bacterial communities (Kuske et al., 2002), agricultural growing practice (Buckley and Schmidt 2001), temperature (Ward et al., 1998), nutrient status (Broughton and Gross, 2000), salinity (Nubel et al., 2000), and other environmental variables.

Plant growth promotion activities for isolates

Five isolates of *B. cereus* showed IAA production (Table 3). According to Mirza et al. (2001), IAA by the production of microorganisms may vary between different species and strains of the same species. The culture conditions and substrate stage growth conditions may also cause variations in production. Also, Idris et al. (2007) showed that reduction in IAA concentration in *B. amyloliquefaciens* FZB42 was caused by inactivating of gene responsible for IAA biosynthesis consequently causing in decrease plant growth promotion activity. The level of IAA production was affected also by two genetic factors, the location of auxin biosynthesis genes in the bacterial genome and the mode of expression.

Among environmental stress factors, acidic pH, osmotic and matrix stress, and carbon limitation modulate the IAA biosynthesis in different bacteria (Spaepen et al., 2007). The ability to produce heat resistant endospores is one of the main features of *Bacillus*, tailoring it to design and marketing, since these microorganisms can be stored for a longer period and may stay longer in the soil (Kokalis-Burelle et al., 2006). The isolation efficient of bacteria depends on some factors of the interaction of plant-soil-climatic conditions (Chagas-Junior et al., 2012). There are few studies related to quantification of IAA by bacteria of the species *B. thuringiensis* and *B. cereus*. Gomes et al. (2003) demonstrated that both bacteria, *B. thuringiensis* and *B. pumilus*, isolated from the cabbage plant, increased the growth of lettuce in the greenhouse. *B. cereus* is known for its ability to produce gibberellic acid, IAA, zeatin and gibberellins (Karadeniz et al., 2006). In addition, Tilak et al. (2006) confirmed the involvement of *B. cereus* in promoting the growth of *Cajanus cajan* (L.) Mill sp., while Bullied et al. (2002) found that this promotes the growth of bacteria soybean (Glycine max). Three isolates showed capability to solubilize inorganic phosphate (Table 3). *Bacillus* species have been reported for their capacity to increase availability of phosphorus in soil (Bhattacharyya and Jha, 2012) and

Table 2. Fatty acid composition (%w/w) of isolates.

Fatty acids	bact1	Bact2	Bact3	Bact4	Bact5	Bact6	Bact7	Bact8	Bact9	Bact10
12 :0 iso	0.97	1.33	0.48	1.04	0.73	0.88	0.48	1.04	1.25	1.17
12 :0	0.74	0.80	0.38	0.81	0.56	0.66	0.51	0.57	0.71	0.70
13 :0 iso	8.16	7.71	6.57	9.49	7.61	7.46	8.78	8.93	11 .32	8.96
13 :0 anteiso	0.94	1.45	0.79	1.17	1.42	1.62	0.65	1.04	2.16	1.24
13 :0	-	0.31	-	-	-	0.33	-	0.17	-	0.36
14 :0 iso	5.99	7.15	4.01	5.10	5.52	5.08	3.71	6.58	5.21	6.34
14 :1 w5c	--	-	-	-	-	-	0.22	-	-	-
14 :0	5.06	6.21	3.29	5.84	5.50	5.02	4.67	4.69	4.43	5.36
15 :0 iso	27.49	24.08	30.99	30.98	29.89	29.16	31.91	28.15	25.28	29.44
15 :0 anteiso	4.29	5.14	4.21	4.05	6.85	6.73	3.36	4.63	6.35	4.75
15 :1 w5c	-	0.27	-	0.28	-	0.27	-	0.27	-	0.26
15 :0	-	-	-	-	-	-	-	-	-	-
16 :1 w7c alcohol	0.44	0.55	0.59	0.44	0.50	0.70	0.44	0.49	0.91	0.95
16 :0 iso	9.59	10.72	9.76	7.33	7.89	8.12	6.05	9.77	7.39	9.00
16:1 w11c	0.36	0.34	0.43	0.28	0.40	0.45	0.22	0.29	1.97	0.54
16 :1 w5c	-	0.26	-	0.27	0.43	0.37	1.00	-	-	-
16 :0	11.01	10.03	8.12	8.30	9.13	5.90	5.77	10.27	10.51	8.41
15 :0 2OH	-	-	0.30	-	-	0.25	0.34	-	-	-
17 :1 iso w10c	0.94	1.15	1.96	1.29	1.43	1.31	-	0.90	1.93	1.84
17 :1 iso w5c	1.67	1.53	2.59	2.32	2.03	3.16	2.90	1.71	1.19	1.47
17 :1 anteisoA	0.48	0.54	0.81	0.54	0.70	1.39	0.70	0.52	0.49	0.48
17 :0 iso	9.19	7.69	11.71	8.62	7.09	5.84	8.54	8.30	7.91	8.81
17 :0 anteiso	1.87	2.12	2.78	1.52	2.39	2.36	1.40	1.84	2.57	1.84
17 :1 w6c	-	-	-	-	-	0.24	0.36	-	-	-
17 :0	0.40	0.41	0.29	0 .27	-	0.24	-	0.32	-	0.36
18 :0 iso	0.26	0.37	-	-	-	-	-	0.20	-	-
18 :1 iso H	-	--	-	-	-	-	0.29	-	-	-
18:1 w9c	1.62	0.76	1.28	0.31	0.40	0 .23	0.35	0.31	0.79	0.46
18 :1 w5c	-	--	-	-	-	--	0.17	-	-	-
18 :0	0.69	0.43	0.59	0.34	0.30	0.19	0.23	0.46	0.49	0.33
18 :1 2OH	-	-	-	0.29	0.29	-	-	0.19	-	0.31

many environmental factors such as pH and soil type can affect highly the phenomena of fixation and precipitation of P in soil. Thus, according to Jones et al. (1991) in alkaline soils it is fixed by calcium, causing a low efficiency of soluble P fertilizers.

From the present study, pH of soil isolates was alkaline (8.5), Hwangbo et al. (2003) reported that the inverse relationship between pH and soluble phosphate. However, P-solubilizing bacteria (PSB) are widely distributed in soil; environmental stress factors can affect establishment and performances of these bacteria (Ahemad and Khan, 2012).

Furthermore, obtained results showed that all *Bacillus cereus* in our study were starch hydrolysis and proteolytic activity (Figure 4A and 4B). There are many reports (Sellami-Kamoun et al., 2008) indicating that *Bacillus sp.* were able to produce a large variety of extracellular enzymes, such as amylases and proteases are the most significant industrial. It has been confirmed that variation of amylases and proteases not only in type but also in pH and optimum temperature (Gupta et al., 2003).

Role of hydrophobicity of spores in attachment

The hydrophobicity of *B. cereus* spores was determined and expressed a percentage and illustrated in Table 4. Surface hydrophobicity of untreated *B. cereus* spores(ranged between 18.5 and 57%). In this study, it is interesting to note that hydrophobicity of spores varied among the analyzed *B. cereus* soil isolates which displayed either a hydrophilic or moderate hydrophobic character. Three isolates were moderately hydrophobic, adherence to hexadecane range between 43, 37 and

Table 3. IAA production, phosphor solubilization, amylolytic and proteolytic activity and biofilm-forming capacities in tube and microtiter plate of the thirty six *B. cereus* strains from four different sites. (A): Abdelmoula; (B): Mekmen Ben Amar; (C): Mechria; (D): Ain Sefra.

Strains	IAA production[a]	Phosphor Solubilization[b]	Amylolytic activity[c]	Proteolytic activity[c]	Microtiter plate biofilm formation[d]	Biofilm-forming capacities in tube[d]	Sites
Bact1	-	-	+	+	-	+	B
Bact2	+	-	+	+	-	+	D
Bact3	+	+	+	+	-	+	D
Bact4	+	+	+	+	-	+	D
Bact5	-	-	+	+	-	+	A
Bact6	-	-	+	+	-	+	D
Bact7	+	-	+	+	-	+	D
Bact8	-	-	+	+	-	+	B
Bact9	+	+	+	+	-	+	C
Bact10	-	-	+	+	-	+	D
Bact11	-	-	+	+	-	+	D
Bact12	-	-	+	+	-	+	D
Bact13	-	-	+	+	-	+	C
Bact14	-	-	+	+	-	+	C
Bact15	-	-	+	+	-	+	C
Bact16	-	-	+	+	-	+	A
Bact17	-	-	+	+	-	+	A
Bact18	-	-	+	+	-	+	D
Bact19	-	-	+	+	-	+	D
Bact20	-	-	+	+	-	+	D
Bact21	-	-	+	+	-	+	D
Bact22	-	-	+	+	-	+	D
Bact23	-	-	+	+	-	+	C
Bact24	-	-	+	+	-	+	A
Bact25	-	-	+	+	-	+	A
Bact26	-	-	+	+	-	+	B
Bact27	-	-	+	+	-	+	D
Bact28	-	-	+	+	-	+	D
Bact29	-	-	+	+	-	+	D
Bact30	-	-	+	+	-	+	B
Bact31	-	-	+	+	-	+	B
Bact32	-	-	+	+	-	+	D
Bact33	-	-	+	+	-	+	D
Bact34	-	-	+	+	-	+	D
Bact35	-	-	+	+	-	+	B
Bact36	-	-	+	+	-	+	D

([a]): +: able to produce IAA, -: not able to produce IAA; ([b]): +: able able to solubilize Phosphor, - : not able to solubilize; ([c]): +: amylolytic and proteolytic activity; ([d]): +: forms a biofilm, - : does not form a biofilm.

57%. Remaining isolates were hydrophilic character, with hydrophobicity values between 18.5% and 36%.

According to the literature, spores of *B. cereus* groups generally considered hydrophobic. On the other hand, the hydrophilic character of the soil isolates of *B. cereus* could also be related to an adaptation to alkaline pH conditions. This notice is an accordance with those mentioned by Seale et al. (2008). They stated that hydrophobicity increased at acidic pH while it decreased at alkaline pH (Giotis et al., 2009). Decreased hydrophobicity was observed for the spores of a strain of *B. cereus* alkali-tolerant (Bernardes et al., 2010). These data are confirmed by the results of Hamadi et al. (2004) who found that the hydrophilicity of strains of *S. aureus*

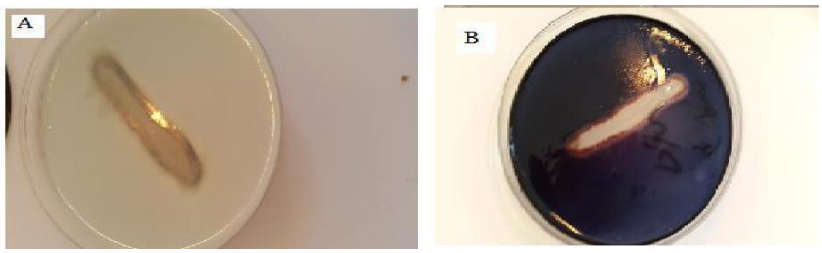

Figure 4. Starch hydrolysis, proteolytic activity by *B cereus* (Bact2). A: proteolytic activity B: starch hydrolysis.

Table 4. Hydrophobicity percentage of the ten *Bacillus cereus* selected isolates.

Isolates	Hydrophobicity (%)
Bact1	19
Bact2	36
Bact3	44.15
Bact4	18.5
Bact5	26
Bact6	35.13
Bact7	32.55
Bact8	57
Bact9	43.37
Bact10	20

Figure 5. Biofilms formed by *B. cereus* on LB at 30°C for 48 h at air-liquid interfaces. *B. cereus* Bact2 (lane 1), *B. cereus* Bact3 (lane 2).

and *E. coli* is low apparent for neutral to alkaline pH (> 5 to 11) so that the hydrophobicity is more apparent at acidic pH (2-3). In our results, pH of soil isolates was alkaline between (8 to 9) and hydrophobicity associated with very moderate hydrophobicity is an argument for adaptation at pH alkaline conditions.

Biofilm formation

In our study, we use a tube method and microtiter plate assay to test the capacity of thirty six isolates to form biofilms. The tube test showed significantly different results from the results obtained by the standard microtiter-plate test. All isolates did not form a biofilm in the microtiter plate assays, while in the Tube assays, formation of biofilm took place preferentially at the point of the interface between the liquid and air for all *B. cereus* (Figure 5). According (Deighton and Balkau, 1990) the adherence capability of Staphylococci was influenced by many factors include different mechanisms of adherence for plastic and glass surfaces. Number of spores attached to the polystyrene surface was dramatically affected by hydrophobicity. Auger et al. (2006) assured same observations and reported that *B. cereus* ATCC14579 did not produce biofilm in the microtiter plate.

The dynamic condition had negative effect not only on attachement of *B. cereus* cells to polystyrene surface but also on biofilm development, where all tested isolates were no biofilm producer. According to Carpentier and Cerf (2011), the conditions for growth are not always favorable for the adhesion of bacteria. The choice of material in which these microtiter are produced, also remains imperative to avoid negative interference with the adhesive properties of the tested microorganisms. This illustrates the need for reliable and reproducible techniques to culture and biofilm development. It is recognized that biofilms are formed in response to environmental signals which differ from one species to another. These signal nutrient availability and quorum-sensing involved in the regulation of maturation of the biofilm (Stanley and Lazazzera, 2004). Factors which influence structure and development of biofilm, and the composition of the microbial community include nutrient availability and type of sugar provided (Stoodley et al., 2001). Finally, the results indicate that the bacteria tested in this study may be promising in promoting plant growth.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the laboratories of the Veterinary Center of Tlemcen, the LAMAABE of the Biology Department, University of Tlemcen, Algeria and the Head of LASNABIO for their assistance.

REFERENCES

Ahemad M, Khan MS (2012). Effect of fungicides on plant growth promoting activities of phosphate solubilizing *Pseudomonas putida* isolated from mustard (*Brassica compestris)* rhizosphere. Chemosphere 86:945-950.

Bellon-Fontaine MN, Rault J, Van Oss CJ (1996). Microbial adhesion to solvents, a novel method to determine the electron–donor/electron–acceptor or Lewis acid–base properties of microbial cells. Colloid. Surface. B. 7:47-53.

Beneduzi A, Peres D, Vargas LK, Bodanese-Zanettini MH, Passaglia LMP (2008). Evaluation of Genetic Diversity and Plant Growth Promoting Activities of Nitrogen-Fixing Bacilli Isolated from Rice Fields in South Brazil. Appl. Soil Ecol. 39(3):311-320.

Bernardes PC, NJ de Andrade, SO Ferreira, JP N de Sá, EA Araújo, DM Z Delatorre, LM Pinheiro Luiz (2010). Assessment of hydrophobicity and roughness of stainless steel adhered by an isolate of Bacillus cereus from a dairy plant. Braz. J. Microbiol. 41:984-992.

Bhattacharyya PN, Jha DK (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J. Microbiol. Biotechnol.* 28:1327-1350.

Buckley D & Schmidt T (2001). The structure of microbial communities in soil and the lasting impact of cultivation. Microb. Ecol. 42:11-21.

Bullied WJ, TJ Buss and J K Vessey (2002). *Bacillus cereus* UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes: field studies. Canadian J. Plant Sci. 82:291-298.

Canbolat M, Bilen S, Cakmakcı R, Fiahin F, Aydın A (2006). Effect of plant growth promoting rhizobacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol. Fertil. Soils 42:50-357.

Carpentier B, Cerf O (2011). Review-Persistence of Listeria monocytogenes in food industry equipment and premises. *Int. J. Food Microbiol.* 145:1-8.

Chagas-Junior AF, GR Santos, RCL Melo,AG Oliveira, B Vizioli, LF Borges-Chagas and JL Costa (2012). Effect of natural nodulation in the development of leguminous trees on soils of cerrado in Tocantins. J. Biotechnol. Biodivers. 3:38-44.

Christensen GD, Simpson WA, Bisno AL and Beachey EH (1982). Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect. Immun. 37:318-326.

De Maagd RA, Bravo A, Crickmore N (2001). How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends Genet 17:193-199.

Deighton MA, Balkau B (1990). Adherence measured by microtiter assay as a virulence marker for *Staphylococcus epidermidis* infections. J. Clin. Microbiol. 28:2442-2447.

Djordjevic D, M Wiedmann and LA McLandsborough (2002).Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Appl. Environ. Microbiol. 68:2950-2958.

Fricker M, R Reissbrodt (2008). Evaluation of standard and new chromogenic selective plating media for isolation and identification of *Bacillus cereus*. Int. J. Food Microbiol. 121(1):27-34.

Giotis ES, Blair IS, McDowell DA (2009). Effect of short-term alkaline adaptation on surfaces properties of *Listeria monocytogenes* 10403S. Open Food Sci. J. 3:62-65.

Gomes AMA, RLR Mariano, E B Silveira and JCP Mesquita (2003). Isolamento, selecao de bacterias e efeito de *Bacillus spp*. na producao de mudas organicas de alface. Hortic. Bras. 21:701-705.

Gordon SA, Weber RP (1951). Colorimetric Estimation of Indoleacetic Acid. Plant Physiol. 26(1):192-195.

Guinebretière MH, Auger S, Galleron N, Contzen M, De Sarrau B (2013). *Bacillus cytotoxicus sp.* nov. is a novel thermotolerant species of the *Bacillus cereus* group occasionally associated with food poisoning. Int. J. Syst. Evol. Microbiol. 63:31-40.

Guinebretière MH, Thompson FL, Sorokin A, Normand P, Dawyndt P, Ehling-schulz M, Svensson B, sanchis V, Nguyen-The C, Heyndrickx M, De Vos P (2008). Ecological diversification in the *Bacillus cereus* group. Environ. Microbiol. 10:851-865.

Gupta R, Gigras P, Mohapatra H, Goswami VK, and Chauhan B (2003). Microbial α-amylases: A biotechnological perspective. Process Biochem. 38(11):1599-1616.

Gupta R, Gupta N and Rathi P (2004). Bacterial lipases: an overview of production, purification and biochemical properties. Microbiol. Biotechnol. 6:763-81.

Hamadi F, H Latrache, A El Ghmari, M El louali, M Mabrrouki, N Kouider (2004). Effect of pH and ionic strength on hydrophobicity and electron donor and acceptor characteristics of *Escherichia coli* and *Staphylococcus aureus*. Annal Microbiol. 54:213-225.

Hwangbo H, Park RD, Kim YW, Rim YS, Park KH, Kim TH, Suh JS, Kim KY (2003). 2-Ketogluconic production and phosphate solubilization by Enterobacter intermedium. Curr. Microbiol. 47:87-92.

Idris EE, Iglesias DJ, Talon M, Borriss R (2007). Tryptophan-Dependent Production of Indole-3-Acetic Acid (Iaa) Affects Level of Plant Growth Promotion by *Bacillus Amyloliquefaciens* Fzb42. Mol. Plant Microbe. 20(6):619-626.

International Organization for Standardization (1993). 2nd Ed., Geneva, Switzerland, Method ISO. P 793.

Jones DA, Smith BFL, Wilson MJ, Goodman BA (1991). Solubilizator fungi of phosphate in rise soil. Mycol Res. 95:1090-1093.

Karadeniz A, SF Topcuoglu and S Inan (2006). Auxin, gibberellin, cytokinin and abscisic acid production in some bacteria. World J. Microbiol. Biotechnol. 22:1061-1064.

Kloepper JW, Scher FM, Laliberte M, Tipping B (1986). Emergence promoting rhizobacteria: description and implications for agriculture. In: Swinburne, T.R. (Ed.), Iron, Siderophores and Plant Disease. Plenum Publishing Co, New York. pp. 155-164.

Kokalis-Burelle N, Kloepper JW, Reddy MS (2006). Plant growth-promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Appl. Soil Ecol. 31:91-100.

Kuske CR, Ticknor LO, Miller ME, Dunbar JM, Davis JA, Barns SM & Belnap J (2002). Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Appl. Environ. Microbiol. 68:1854-1863.

Lechner S, Mayr R, Francis KP, Pru¨b BM, Kaplan T(1998) *Bacillus weihenstephanensis sp.* nov. is a new psychrotolerant species of the *Bacillus cereus* group. Int. J. Syst. Bacteriol. 48:1373-1382.

Logan NA (2012). *Bacillus* and relatives in foodborne illness. J. Appl. Microbiol. 112:417-429.

Marchand S, Heylen K, MessensW, Coudijzer K, De Vos P, Dewettinck K, Herman L, De Block J, Heyndrickx M (2009). Seasonal influence on heat-resistant proteolytic capacity of *Pseudomonas lundensis* and *Pseudomonas fragi*, predominant milk spoilers isolated from Belgian raw milk samples. Environ. Microbiol. 11(2):467-482

Mirza MSW Ahmad, F Latif, J Haurat, R Bally, P Normand and KA Malik (2001). Isolation, partial characterization, and the effect of plant growth-romoting bacteria (PGPB) on micro-propagated sugarcane in vitro. Plant Soil. 237:47-54.

Mock M, Fouet A (2001). Anthrax. Ann. Rev. Microbiol. 55:647-671.

Nakamura LK (1998). *Bacillus pseudomycoides sp. nov.* Int. J. Syst. Bacteriol. 48:1031-1035.

Nubel U, Garcia-Pichel F, Clavero E, Muyzer G (2000). Matching molecular diversity and ecophysiology of benthic cyanobacteria and

diatoms in communities along a salinity gradient. Environ. Microbiol. 2:217-226.
Seale RB, Flint SH, McQuillan AJ, Bremer PJ (2008). Recovery of spores from thermophilic dairy bacilli and effects of their surface characteristics on attachment to different surfaces. Appl. Environ. Microbiol. 74:731-737.
Sellami-Kamoun A, Haddar A, El-Hadj Ali N, Ghorbel-Frikha B, Kanoun S, Nasri M. (2008). Stability of thermostable alkaline protease from *Bacillus licheniformis* RP1 in commercial solid laundry detergent formulations. Microbiol. Res. 163(3):299-306.
Spaepen S, Vanderleyden J, Remans R (2007). Indole- 3-acetic acid in microbial and microorganism-plant signaling FEMS. Microbiol. Rev. 31:425-448.
Stanley NR, Lazazzera BA. (2004). Environmental signals and regulatory pathways that influence biofilm formation. Mol. Microbiol. 52:917-924.

Stoodley P, Hall-Stoodley L, Lappin-Scott HM (2001). Detachment, surface migration, and other dynamic behavior in bacterial biofilms revealed by digital time-lapse imaging. Methods Enzymol. 337:306-319.

Tilak KVBR, Rauganayaki N, Manoharachari C (2006). Synergistic effects of plant-growth promoting rhizobacteria and Rhizobium on nodulation and nitrogen fixation by pigeonpea (Cajanus cajan). Eur. J. Soil Sci. 57:67-71.

Von Stetten F, Mayr R, Scherer S (1999). Climatic influence on mesophilic *Bacillus cereus* and psychrotolerant *Bacillus weihenstephanensis* populations in tropical, temperate and alpine soil. Environ. Microbiol. 1(6):503-515.
Ward DM, Ferris MJ, Nold SC & Bateson MM (1998). A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. Microbiol. Mol. Biol. 62(4):1353-1370.