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Potentialities of Wheat-Associated Bacterial Diversity as Growth Promoter of Wheat (*Triticum aestivum* L.)

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Abstract

Context: Some microorganisms stimulate plant growth. Accordingly, this study focused on wheat-associated bacterial diversity to select promising strains for farming.
Aim: To select promising strains that stimulate wheat growth, with a wheat-associated bacterial diversity.
Methods: The bacteria from the rhizosphere and interior of wheat plants were isolated (cultivars Cuba C 204 and I 399). Its nitrogen fixing potential was characterized *in vitro*, along with the microorganisms' capacity to solubilize nutrients and antagonistic activity against Fusarium sp and Curvularia lunata. The strains were introduced in wheat seeds under semi-controlled conditions, and their effect on growth indicators were evaluated.
Results: Several microorganisms were isolated, such as four strains classified as Bacillus, four Azotobacter, and two of them as Azospirillum, depending on their morphology. All the microorganisms were capable of fixing the nitrogen from the atmosphere. Except for one strain, they solubilized nutrients, and showed antagonistic activity against F. graminearum, F. chlamydosporum, F. oxysporum, and C. lunata. Its inoculation in wheat demonstrated the feasibility of using bacterial diversity associated with the plant species to stimulate 21-day-old plantlet growth from cultivars Cuba C 204 and I 399.
Conclusions: There is a microbial diversity associated with the wheat plants with a potential to stimulate *in vitro* and *in vivo* growth. Some of these microorganisms have promising features to obtain a new product for cropping, which can increase yields in the Cuban conditions.

Keywords: *gramineous, microorganisms, biofertilizer.*

Introduction

Cuba invests large amounts of funds to import grains for human and animal consumption, at ever-increasing costs (Martínez et al., 2016). Hence, the country uses different strategies to increase yields, such as greater cropland area and the inclusion of varieties with a higher yielding potential. However, the production of grains still falls short to meet the people's demands. One helpful tool could be the plant growth promoting bacteria (PGPB), which might constitute active principles of farming bioproducts identified as soil and environmentally-friendly technologies (González & Fuentes, 2017).

The plant microbiome comprises phylospheric, endophyte, and rizhospheric microorganisms. Among them are the PGPB, bacteria that stimulate crop growth by activating mechanisms such as biological nitrogen fixing, nutrient solubilization, plant-hormone production, the control of phytopathogenic organisms, and so on (Yadav, 2017). Several research studies have demonstrated the positive effect of PGPB application on wheat (*Triticum aestivum* L.) (Alves et al., 2017). However, in Cuba, none of the biofertilizers registered is biospecific for this plant species (Department of Soils and Fertilizers, 2018), which limits the practical use of microorganisms as part of the agrotechnology recommended for this crop.

This research looked to create a new bioproduct for wheat farming; hence, the bacterial strains associated with this plant species were isolated, from which the most promising microorganisms in plant growth promotion, were selected. That way, there is a microbial diversity associated with this crop that has favorable characteristics to become the active principle of farming bioproducts for wheat production.

Materials and Methods

Isolation and selection of the microorganisms: 30-day-old wheat plants (Cuba C 204 and I 399), and the associated rhizosphere soil, were included. The samples were handled at the Bacterial Preservation Laboratory, Department of Microbial Genetic Resources and Bioactive Products, at the Alejandro de Humboldt Institute for Major Research on Tropical Agriculture (INIFAT). The plant material consisted in 1 cm fragments from the roots, stems, and leaves, which were disinfected with 4% sodium hydrochloride, and then were crushed. The sap from the plant material was added to the culture media (semisolid LGI) (Cavalcante & Döbereiner, 1988), and Nutrient Agar (BIOCEN). The soil samples (10 g) were dissolved in 90 mL of sterile distilled water to make serial dilutions (Madigan et al., 2018), which were later added to the culture media Nutrient Agar (BIOCEN), Asbhy, NFB, and YMA (the last three were semisolid), cited by Martínez et al., 2006). Both the sap and soil samples were stored at 100 °C for 10 minutes, and then were added to the Nutrient Agar medium. The purification of microorganisms was performed by depleting the culture media for isolation. The incubation temperature varied between 28 and 30 °C, and time depended on the culture medium (24h for Nutrient Agar, 48-72h for Asbhy and YMA, and 120h for LGI and NBF).

The selection of microorganisms was based on the morpho-physiological characteristics of *Azotobacter*, *Azospirillum*, *Rhizobium*, *Gluconacetobacter* and the *Bacilli* class. The morphological and tinctorial characteristics were verified through Gram-staining (Madigan et al., 2018). Meanwhile, the colonies were observed with a stereoscopic microscope (10X CARLZEISS JENA). The microorganisms isolated from the culture media NFB and YMA were also added to the Congo red dye from the medium for *Azospirillum*, and Red Congo for YMA (both cited by Martínez et al., 2006), respectively. The physiological indicators for all the isolates were the presence of catalase and cytochrome oxidase, the utilization of citrate as a carbon source, and glucose, according to the growth test in Kliger medium, the starch and gelatin hydrolysis capacity, as well as motility (Harrigan & McCance, 1968). The incubation temperature always varied between 28 and 30 °C.

Characterization of microorganisms as plant growth promoters: their capacity to fix atmospheric nitrogen was determined qualitatively, according to the criteria of Baldani et al. (2014). Accordingly, the strains were added to the Asbhy culture medium (cited by Martínez et al., 2006), without combined nitrogen, then the possible presence of bacterial growth under these conditions was evaluated. The culture medium was the negative control. The microorganism potential to solubilize calcium phosphate, iron and aluminum, and potassium was quantified as well, based on measurements using a gauge caliper (0.05 mm error) of the solubilization halo formed in the Pikosvkaya culture medium (cited by Martínez et al., 2006), and supplemented with those nutrients. Lastly, the antagonistic activity of the bacterial strains against *Fusarium graminearum* (cepa 3991), *Fusarium chlamydosporum* (strain 2022), *Fusarium oxysporum* (strain 3114), and *Curvularia lunata* (strain 3835), was determined. The determination method was the one described by Grobelak et al. (2014). Bacterial cell suspensions in sterile distilled water were used in the cases where they showed 10^8 UFC mL^{-1} concentration. A volume of 1 mL of the suspension was used for deep inoculation of 10 mL of the culture medium (PDA, BIOCEN). After 4 h in the medium, every fungal strain (Pure Crop Fungi from INIFAT collection 853 of the World Crop Collection Federation (WFCC)) was placed in a 7 mm diameter disc. A control treatment was included, which only received the fungal strains. The mycelium diameter of the fungus (DM) was measured in all the treatments, using a gauge caliper (0.05 mm error), at 7 days of incubation, and the percentage of mycelial growth inhibition (IM) was calculated (IM (%) = dmc–dt/dmc x 100, where, dmc: Diameter of the control mycelium, and dt: Diameter of the mycelium of the treatments). The incubation temperature varied between 28 and 30 °C.

Evaluation of the effect of microorganism utilization in two wheat cultivars: the study took place between January and March, 2021. Wheat seeds from cultivars Cuba C 204 and I 399, from the INIFAT Germplasm Bank, were used. The bioassay was practiced to the 40-alveolus root balls containing red lixiviated, gleyed ferritic, and ferruginous soil (A. Hernández et al., 2015). The wheat seeds were disinfected with 4% sodium hypochlorite solution, and were rinsed with plenty of sterile distilled water before plating. Then, the bacterial strains were added separately by washing away the soil above the seed, at 1 mL per alveolus, of a cell suspension of sterile distilled water at 10^8 UFC mL^{-1}. Plant growth was recorded daily for 21 days, with irrigation and observation of their conditions daily. Then, the plantlets were removed, and the following growth indicators were measured: height, stem diameter, root length, leaf number, and plant fresh and dry mass. A cm ruler was used to determine height and root length; the stem diameter was determined with a gauge caliper (0.05 mm error); while mass was determined using a balance (DS 3K0.01S, 0.01 g error).

Experimental design and statistical analysis: all the trials relied on a completely randomized design. Five replicas per strain or treatment were made in the lab, whereas the bioassay used 20 plants in each treatment (strains and control) in the two replicas made. The statistical analysis included every measurement, after corroborating the absence of differences between replicas. The variance normality and homogeneity of the results was corroborated through the Kolmogorov-Smirnov, Cochran C, Hartley, and Bartlett tests; then analyses of variance were performed, and the means were compared using Duncan's Multiple Rank Comparison Test (5% error probability). STATGRAPHICS Plus, 5.0 was used for statistical processing.

Results and Discussion

According the sample analysis conducted, a total of 40 microorganisms were collected from the wheat plants and the soil (10 isolates), more from cultivar Cuba C 204 than the I 399. There was greater representativeness from the microorganisms purified in the NFB culture medium, though, with Asbhy standing out in the case of Cuba C 204. From these microorganisms, 10 were selected for the study of their morph-physiological characteristics described for genera *Azotobacter*, *Azospirillum*, and *Bacillus* (Table 1).

Table 1. Distribution of the microorganism isolates from wheat plant fragments (*Triticum aestivum* L.), and the corresponding rhizosphere soil

	Isolated microorganisms		Selected microorganisms	
Culture medium	Cultivar Cuba C 204	Cultivar I 399	Cultivar Cuba C 204	Cultivar I 399
Asbhy	11	3	3	1
NFB	11	6	1	1
Nutrient Agar	1	3	1	3
LGI	0	0	0	0
YMA	2	3	0	0
Total	25	15	5	5

Bacillus, Azotobacter, Azospirillum, Burkholderia, Pseudomonas, Clostridium and *Gluconacetobacter* stood out as PGPB, due to their association with gramineous (de la Fé et al., 2015). Therefore, the isolate focused on some of these genera, using recommended selective media for *Azotobacter, Azospirillum* (Martínez et al., 2006) and *Gluconacetobacter* (Cavalcante & Döbereiner, 1988). Although Rhizobiaceae have historically associated with leguminous, their association with gramineous has been demonstrated in recent years, thus favoring their growth (Vital et al., 2015), the reason for their inclusion in this paper.

The isolation of more microorganisms associated with a particular cultivar rather than another, is explained by the presence of a complex relation between the plant and microorganisms, and their specificity, where the plant regulates the microbial population through physiological changes and root exudates produced along their growth, then released to the surrounding (Chinakwe et al., 2019). Therefore, even when the cultivars belong to the same plant species, they may have a different associated microbiota.

The selection of microorganisms included the assessment of their morph-physiological characteristics, and their correspondence to the genera targeted by this research. As to Asbhy, the isolates with circular colonies, beige-translucent mucosa, with cells like short Gram-negative bacilli, and a positive oxidase and catalase response (*Azotobacter*) (Brenner et al., 2005). In turn, the selection criterion for the isolated microorganisms from NFB, was a micromorphology similar to short Gram-negative bacilli, in white colonies, positive oxidase and catalase response, forming scarlet-red colonies in the Congo red dye culture medium (*Azospirillum*) (Piña et al., 2016). The other group included in the final selection was *Bacillus*. Although its taxonomic classification is very complex today due to insufficient gene 16 S rRNA sequencing. Other genes must be sequenced to determine the identity of the genus and species (Ruiz-Sánchez et al., 2016). The primary identification criteria for positive response to Gram staining was the presence of spores and catalase (Rojas et al., 2016), which coincide with the results of the tests performed to the microorganisms purified in Nutrient Agar.

No purified isolate was selected in YMA, as they showed a Gram-positive response, alkalizing the culture medium with bromothymol blue, or absorbed the Congo red dye, which did not coincide with the description of genus *Rhizobium* (J.L. Hernández et al., 2012; Saldaña, 2017). Neither were the microorganisms purified in LGI, as they showed a positive response to Gram staining, or white colored colonies, contrary to the characteristics of genus *Gluconacetobacter*, which includes short Gram-negative bacilli that form yellow-orange colonies in LGI (Cavalcante & Döbereiner, 1988).

The morph-physiological characteristics of the microorganisms selected and their classification depending on these results are shown in Tables 2 and 3. The diversity between them can be observed, even when they are microorganisms associated with a single taxonomic group.

The 10 bacterial strains isolated from wheat were selected due to their morph-physiological characteristics, with direct mechanisms of plant growth stimulation. The biological fixation of nitrogen from the atmosphere was a common feature of microorganisms grown in the culture media lacking combined nitrogen. Eight strains solubilized calcium

phosphate, three iron phosphate, and one was capable of solubilizing potassium, with *Azotobacter* strains T10 and T12, and strain T16 from *Bacillus to solubilize more than a single nutrient (Table 4).*

The atmospheric nitrogen fixating capacity and phosphate solubilization by genera *Azotobacter* and *Azospirillum* was demonstrated in the research of Pérez-Pazos & Sánchez (2017). Likewise, the phosphate solubilization and the FBN by *Bacillus* potential have been described in other papers, such as Zahid et al. (2015), and there are even references of these characteristics for strains associated with gramineous like maize (*Zea mays* L.) (Rojas et al., 2016).

The strains from isolated bacteria in wheat also showed antagonistic activity. Fig 1 shows the assay results in species *Fusarium* (*F. graminearum, F. chlamydosporum, F. oxysporum*), and *C. lunata.*

Table 2. Morphological characteristics of the microorganisms selected and isolated from wheat cultivars Cuba C 204 and I 399 (*Triticum aestivum* L.)

Strain	Micro-morphology	Shape	Edges	Raised area	Consistency	Color
T1	Short Gram-negative bacillus	Irregular	Whole	Slightly raised	Dry	White
T3	Short Gram-negative bacillus	Irregular	Whole	Flat	Dry	White
T7	Short Gram-negative bacillus	Circular	Whole	Convex	Mucosa	Beige
T10	Short Gram-negative bacillus	Circular	Whole	Convex	Mucosa	Translucent
T 11	Gram-negative bacillus	Circular	Whole	Convex	Mucosa	Translucent
T12	Short Gram-negative bacillus	Circular	Whole	Convex	Mucosa	Beige
T13	Long, chain Gram-positive bacillus	Irregular	Irregular	Slightly raised	Dry with granules	White
T14	Gram-positive bacillus with spores	Circular	Whole	Convex	Mucosa	Beige
T15	Gram-positive bacillus with spore	Irregular	Whole	Slightly raised	Dry	Yellow-white
T16	Gram-positive bacillus with spore	Irregular	Whole	Slightly raised	Dry with granules	White

Table 3. Physiological characteristics and classification recommended for the microorganisms selected and isolated from wheat cultivars Cuba C 204 and I 399 (*Triticum aestivum* L.)

	Physiology									Classification recommended
Strain	Ox	Cat	Mot	Gel	Alm	Cas	Klig	Cit	RM	
T1	+	+	+	-	-	-	+	+	+	*Azospirillum*
T3	+	+	+	-	+	-	-	-	+	
T7	+	+	+	-	-	-	-	+	-	*Azotobacter*
T10	+	+	+	-	-	-	+	+	+	
T 11	+	+	+	-	-	-	-	+	-	
T12	+	+	+	-	-	-	-	+	+	
T13	+	+	+	-	+	+	+	-	+	*Bacillus*
T14	+	+	+	-	+	-	-	-	+	
T15	+	+	+	-	-	-	+	-	+	
T16	+	+	+	-	-	+	-	-	-	

Legend: Ox: oxidase test; Cat: catalase test; Mot: motility; Gel: gelatin hydrolysis; Alm: starch hydrolysis; Cas: casein hydrolysis; Klig: growth changing to yellow in Kliger Agar; Cit: citrate use as a carbon source; RM: red methyl test

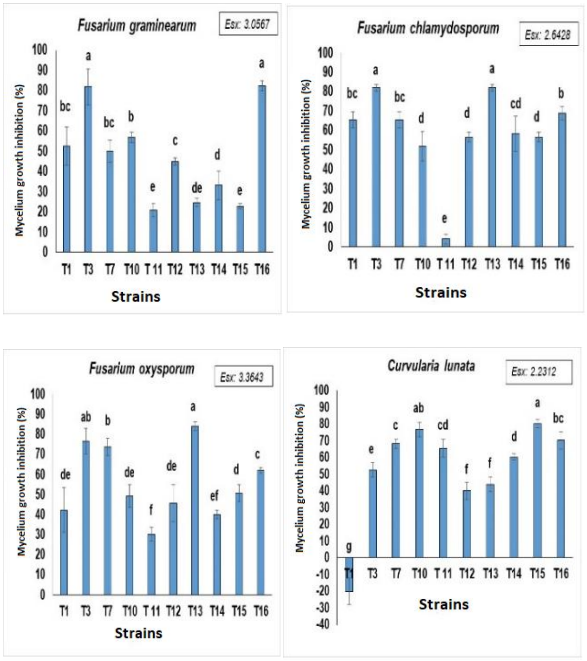

Fig. 1. Antagonistic activity of the microorganisms selected and isolated from wheat cultivars Cuba C 204 and I 399 (*Triticum aestivum* L.), against four phytopathogenic species

Note: Means on the same column with different scripts differ statistically p (0.05). n=10

Table 4. Direct plant growth stimulation mechanisms evaluated for the strains selected and isolated from wheat cultivars Cuba C 204 and I 399 (*Triticum aestivum* L.)

Strain	Growth in Asbhy culture medium without nitrogen	Solubilization (Halo cm) Calcium	Iron	Aluminum	Potassium
T1	+	-	-	-	-
T3	+	0.08	-	-	-
T7	+	0.12	-	-	-
T10	+	-	0.1	-	0.1
T 11	+	0.16	-	-	-
T12	+	0.09	0.1	-	-
T13	+	0.08	-	-	-
T14	+	0.18	-	-	-
T15	+	0.17	-	-	-
T16	+	0.10	0.1	-	-

Against *F. graminearum*, the 10 strains showed mycelial inhibition percentages over 20%, though strains T3 (*Azospirillum*) and T16 (*Bacillus*) stood out with positive results, over 80%. *F. chlamydosporum* was also affected by all the bacteria, which, somehow inhibited fungal growth. Again, genera *Azospirillum* and *Bacillus stood out for their* effectiveness, particularly strain T3 and then strain T13. These two bacterial strains also stood out for their antagonistic activity against *F. oxysporum*, though *Azotobacter* strain T7 showed significant results. However, none of the microorganisms studied was effective against *C.*

lunata. In this case, *Bacillus* strains T15 and *Azotobacter* strains T10 sowed the highest control percentages (76.7 and 80.1%, respectively).

Azotobacter strain T11 was the microorganism with the lowest antagonistic effect against the three *Fusarium* species, though it was very active as nitrogen fixing and calcium phosphate solubilizer. Another interesting result of the study was observed in *Azospirillum* strain T1, which promoted *C. lunata* growth, causing a negative mycelial inhibition percentage. Moreover, the same bacterial genus has strains with antagonistic activity against a phytopathogenic species, while others do not, thus evidencing the specificity of this bacterium-fungus interaction, since the effect is not ensured by the genus *per se*, but by the strain. A similar result was reported by Rojas et al. (2017), who found that though most *Bacillus* strains used showed a similar mycelial growth inhibition percentage against *F. oxysporum* (above 80%), others did not show such activity, or were capable of controlling fungal growth between 20 and 40%.

Research done by Ruiz-Sánchez et al. (2016) demonstrated that *Bacillus* species, especially *B. subtilis* inhibited *C. lunata* growth between 59 and 78%, depending on the strain. Rojas et al. (2017) proved that different strains of this bacterial genus are active against *F. oxysporum* and *F moniliforme*, with over 80% mycelial inhibition for most strains. *B. subtilis*, in the study conducted by Mahmoud (2016), limited *F. graminearum* growth significantly. There are also references of the antagonistic potential of *Bacillus* against *C. lunata*, reaching 20-70% mycelial inhibition, depending on the species (Toledo-Hernández et al., 2021). These results were similar to the behavior of some *Bacillus* strains in this research, corroborating the antagonistic character of this bacterial group.

Among the *Bacillus* mechanisms that provide biological control are the production of antimicrobial substances, such as bacteriocins, surfactins, iturins, and fengycins (the last one has an effect against *Fusarium*), competition, and colonization, with the mediation of biofilm formation, and the production of siderophores, as well as the production of lytic bio enzymes like kinases, proteases, and glucanases, and volatile compounds like aldehydes and ketones (Villarreal-Delgado et al., 2017; Cesa-Luna et al., 2020; Pedraza et al., 2020). Any of these mechanisms or their combination might have caused the response of *Bacillus* strains against the pathogens studied.

Although genera *Azospirillum* and *Azotobacter* are more widely known for their characteristics as nitrogen fixing and plant-hormone producer (Pérez-Pazos & Sánchez, 2017; Vega-Celedón et al., 2016), other studies have demonstrated their antagonistic potential. J. J. Hernández et al. (2015) proved that *Azospirillum* strains were *Fusarium* sp and *Sclerotium* sp antagonists, while *Azotobacter* Azospirillu improved its capacity to produce siderophores, such as aminochelin, azotochelin, protochelin, and azotobactin, with effects against pathogens, like *F. oxysporum*.

The addition of bacterial strains to wheat cultivars Cuba C 204 and I 399 demonstrated the possibility of increasing the value of growth indicators by using microorganisms, though the plant response was conditioned by the cultivar. The number of leaves and the diameter of the stem did not respond to this addition, and showed no significant differences among the control and inoculated treatments, just like for plant fresh mass for cultivar I 399 (Tables 5 and 6).

Cultivar I 399, strains T11 and T14 promoted plant height and root length, respectively, whereas the dry mass increased after the application of strains T3, T10, and T13. However, the effect on Cuba C 204 was more remarkable, with the inclusion of more strains. In this case, the indicators producing the best responses were plant height and fresh mass, both influenced by the presence of the plant-microorganism interaction of five of the seven microorganisms used (T3, T7, T10, T11, and T13 for plant height, and T1, T3, T7, T11, and T12 for the fresh mass). Strains T3, T7, and T11 stood out, stimulating both indicators, particularly T11, whose application also increased root length and dry mass.

The assays showed that the strain isolated from a cultivar is not necessarily effective as a growth promoter of this plant material. For instance, strain T14, with a very active role in I 399, is an endophyte *Bacillus* previously isolated from Cuba C 204, whereas strain T3, which raised growth indicators in Cuba C 204, is an *Azospirillum* isolated from the stem of cultivar I 399.

The presence of growth stimulation mechanisms in *Azospirillum*, *Azotobacter*, and *Bacillus*, was previously discussed. As to the effectiveness of these genera interacting with wheat, Alves et al. (2017) demonstrated that the addition of this plant species along with *Azospirillum*, increases plant weight, as occurred with T3 and Cuba C 204. In other studies, the application of compost and mycorrhizae combined with *Azotobacter* and *Bacillus*, respectively, also increased wheat yields in the fields (Andrade et al., 2021). These references provide more practical value to the outcome of these studies, since more effective strains interacting with plants could maintain the growth stimulating effect in real production conditions.

Table 5. Growth indicators in wheat plants (*Triticum aestivum* L.), cultivar I 399, with and without the application of plant growth promoting bacteria

Treatments	Plant height (cm)	Root length (cm)	Number of leaves	Stem diameter (mm)	Fresh mass (g)	Dry mass (g)
T1	32.83 b	3.77 cd	3	0.2	0.91	0.02 e
T3	34.39 ab	3.5 d	3	0.2	1	0.03 abc

T7	35.0 ab	4.03 bcd	3	0.2	1	0.02 bcde
T10	34.82 ab	4.05 bc	3	0.2	0.99	0.04 a
T11	36.1 a	4.16 bc	3	0.2	0.99	0.03 ab
T12	34.42 ab	3.81 cd	3	0.2	0.96	0.03 abcd
T13	34.49 ab	4.54 ab	3	0.2	0.96	0.03 ab
T14	32.86 b	4.76 a	3	0.2	1	0.04 a
T15	33.87 ab	4.51 ab	3	0.2	0.98	0.02 cde
T16	34.01 ab	4.03 bcd	3	0.2	0.98	0.03 abcd
CONTROL	33.49 ab	4.17 bc	3	0.2	0.95	0.02 de
Esx	*1.1358*	*0.201*	*0.0000 ns*	*0.000 ns*	*0.0452 ns*	*0.0034*

Note: Means on the same column with different scripts differ statistically p (0.05). n=30

Table 6. Growth indicators in wheat plants (*Triticum aestivum* L.), cultivar Cuba C 204, with and without the application of plant growth promoting bacteria

Treatments	Plant height (cm)	Root length (cm)	Number of leaves	Stem diameter (mm)	Fresh mass (g)	Dry mass (g)
T1	33.47 cd	3.15 f	3	0.2	1.25 a	0.02 d
T3	36.74 a	4.17 def	3	0.2	1.14 ab	0.03 abc
T7	36.06 ab	3.79 ef	3	0.2	1.11 ab	0.03 abcd
T10	35.55 bc	4.59 bcde	3	0.2	1.08 bc	0.02 d
T11	35.58 bc	5.65 ab	3	0.2	1.11 ab	0.04 a
T12	34.23 abcd	4.61 bcde	3	0.20	1.09 b	0.02 bcd
T13	34.72 abc	5.04 bcd	3	0.2	1.04 bc	0.02 cd
T14	33.59 bcd	6.49 a	3	0.2	1.02 bc	0.03 abcd
T15	32.91 cd	5.58 abc	3	0.20	0.98 bc	0.03 ab
T16	33.8 bcd	5.37 bc	3	0.2	1.01 bc	0.02 abcd
CONTROL	31.37 d	4.50 cde	3	0.2	0.93 c	0.02 bcd
Esx	*1.0371*	*0.3934*	*0.0000 ns*	*0.000 ns*	*0.0542*	*0.0042*

Note: Means on the same column with different scripts differ statistically p (0.05). n=30

Conclusions

There is a large microbial diversity associated with the wheat plants, which includes genera *Azotobacter*, *Azospirillum* and *Bacillus*, with the potential to fix nitrogen from the atmosphere, solubilize nutrients, and develop antagonistic activity against *Fusarium graminearum*, *Fusarium chlamydosporum*, *Fusarium oxysporum* and *Curvularia lunata*. Some of these strains also share a growth promoting effect when interacting with wheat cultivars Cuba C 204 and I 399; hence, they offer a promising choice for a novel bioproduct for agricultural use that can enhance wheat yields under the Cuban conditions.

Author contribution statement

Yoania Ríos Rocafull: research planning, design, data collection, analysis, interpretation and writing of the article.

Marisel Ortega García: analysis of results, design and final review.

Janet Rodríguez Sánchez: literature search, analysis of results, final review.

Bernardo Dibut Álvarez: analysis of the results.

Conflict of interest statement

Not declared.

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References

Alves, C.J., Arf, O., Ramos, A.F., Galindo, F.S., Nogueira, L.M., & Rodrigues, R.A.F. (2017). Irrigated wheat subjected to inoculation with *Azospirillum brasilense* and nitrogen doses as top-dressing. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *21* (8), 537-542. https://doi.org/10.1590/1807-1929/agriambi.v21n8p537-542

Andrade, F. C., Fernandes, F., Oliveira, A., Rondina, A.B.L., Hungria, M., & Nogueira, M.A. (2021). Enrichment of organic compost with beneficial microorganisms and yield performance of corn and wheat. *Brazilian Journal of Agricultural and Environmental Engineering*, *25* (5), 332-339. http://dx.doi.org/10.1590/1807-1929/agriambi.v25n5p332-339

Baldani, I., Massena Reis, V., Videira, S., & Boddey, L. H. (2014). The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. *Plant Soil*, *384*, 413–431 (2014). https://doi.org/10.1007/s11104-014-2186-6

Brenner, D.J., Krieg, N.R., & Stanley, J. T. (Eds.) (2005). Volume 2: The Proteobacteria. Part B. The Ganmaproteobacteria. En *Bergey's Manual os Sytematic Bacteriology.* (pp. 384-402). Springer US.

Cavalcante, V.A., & Dobereiner, J. (1988). A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. *Plant Soil*, *108*, 23–31. https://doi.org/10.1007/BF02370096

Cesa-Luna, C., Baez, A., Quintero-Hernández, V., de La Cruz, J., Castañeda-Antonio, M. D., & Muñoz-Rojas, J. (2020). Importancia de compuestos antimicrobianos producidos por bacterias benéficas en el biocontrol de fitopatógenos. *Acta Biológica Colombiana*, *25*(1), 140-154. http://dx.doi.org/10.15446/abc.v25n1.76867

Chinakwe, E.C., Ibekwe, V.I., Nwogwugwu, U.N., Onyemekara, N.N., Ofoegbu, J., Mike-Anosike, E., Emeakaraoha, M., Adeleye, S., & Chinakwe P.O. (2019). Microbial population changes in the rhizosphere of tomato *Solanum lycopersicum* varieties during early growth in greenhouse. *Malaysian Journal of Sustainable*

Agriculture (MJSA), *3*(1), 23-27. http://doi.org/10.26480/mjsa.01.2019.23.27

de la Fé, Y., Díaz de la Osa, A., Restrepo, G. M., Diván- Baldani V. L., & Hernández, A. (2015). Diversidad de bacterias diazotróficas asociativas potencialmente eficientes en cultivos de importancia económica. *Revista Cubana de Ciencias Biológicas*, *4*(1), 17-26. http://www.rccb.uh.cu/index.php/RCCB/article/view/85

Departamento de Suelos y Fertilizantes. (2018). *Manual Práctico para el Uso de Bioproductos.* Órgano Central del Ministerio de la Agricultura.

Grobelak, A., Napora, A., & Kacprzak, M. (2014). The impact of plant growth promoting bacteria (PGPB) on the development of phytopathogenic fungi. *Folia Biologica et Oecologica*, *10*, 107-112. http://doi.org/10.2478/fobio-2014-0008

González, H., & Fuentes, N. (2017). Mecanismo de acción de cinco microorganismos promotores de crecimiento vegetal. *Revista de Ciencias Agrarias*, *34*(1), 17-31. http://dx.doi.org/10.22267/rcia.173401.60

Harrigan, W.F., & Mc Cance, M. (1968). *Métodos de Laboratorio en Microbiología.* Ed. Academia.

Hernández, A., Pérez, J.M., Bosch, D. & Castro, N. (2015). *Clasificación de los suelos de Cuba. Instituto Nacional de Ciencias Agrícolas, Instituto de Suelos.* Ediciones INCA.

Hernández, J. J., Morales, P.G., Rodríguez, R.F., & Sánchez, J.M. (2015). Inoculación de *Burkholderia cepacia* y *Gluconacetobacter diazotrophicus* sobre la fenología y biomasa de *Triticum aestivum* var. Nana F2007 a 50% de fertilizante nitrogenado. *Scientia Agropecuaria*, *6*(1), 7–16. https://doi.org/10.17268/sci.agropecu.2015.01.01

Hernández, J.L., Cubillos, J.G., & Milian, P.E. (2012). Aislamiento de cepas de *Rhizobium spp*., asociados a dos leguminosas forrajeras en el Centro Biotecnológico del Caribe. *Revista Colombiana de Microbiología Tropical*, *2*(2), 51-62. https://www.researchgate.net/publication/298069900_Aislamiento_de_cepas_de_Rhizobium_spp_asociados_a_dos_leguminosas_forrajeras_en_el_Centro_Biotecnologico_del_Caribe_Isolation_of_Rhizobium_spp_associated_two_forage_leguminous_in_the_Caribbean_Biotech

Madigan, M.T., Bender, K.S., Buckley, D.H., Sattley, W. M., & Sthal, D.A. (2018). *Brock Biology of Microorganisms.* (15th edition). Published by Pearson.

Mahmoud, A.F. (2016). Genetic variation and biological control of *Fusarium graminearum* isolated from wheat in Assiut-Egypt. *Plant Pathology Journal*, *32*(2),145-156. https://doi.org/10.5423/PPJ.OA.09.2015.0201

Martínez, V. R., López, M., Brossard, F. M., Tejeda, G. G., Pereira, A. H., Parra, Z. C., Rodríguez, S. J., & Alba, A. (2006). *Procedimientos para el estudio y fabricación de Biofertilizantes Bacterianos.* Ed. INIA.

Martínez, S., Gómez-Kosky, R., Rodríguez, G., Veitia, N., Saucedo, O., & Gil, V. (2016). Caracterización morfoagronómica de plantas de sorgo granífero variedad CIAP 132R-5 regeneradas vía embriogénesis somática en condiciones de campo. *Centro Agrícola*, *43*(3), 73-79. http://cagricola.uclv.edu.cu/descargas/pdf/V43-Numero_3/cag09316.pdf

Pedraza, L. A., López, C. E., & Uribe-Vélez, D. (2020). Mecanismos de acción de *Bacillus* spp. (Bacillaceae) contra microorganismos fitopátogenos durante su interacción con planta. *Acta Biológica Colombiana*, *25*(1), 112-125. http://dx.doi.org/10.15446/abc.v25n1.75045

Pérez-Pazos, J.V., & Sánchez, D.B. (2017). Caracterización y efecto de *Azotobacter*, *Azospirillum* y *Pseudomonas* asociadas a *Ipomoea Batatas* del Caribe Colombiano. *Revista Colombiana de Biotecnología*, *19*(2), 35-46. https://doi.org/10.15446/rev.colomb.biote.v19n2.69471

Piña, J., García, V., Herrera, H., & Flores, J. A. (2016). Valoración de cepas silvestres de *Azospirillum* sp. y *Gluconacetobacter* sp. como promotoras de crecimiento vegetal. *Revista Mexicana de Ciencias Agrícolas*, *7*(7), 1613-1623. http://www.scielo.org.mx/pdf/remexca/v7n7/2007-0934-remexca-7-07-1613.pdf

Rojas, M. M., Sánchez, D., Rosales, K., & Lugo, D. (2017). Antagonismo de *Bacillus* frente a hongos fitopatógenos de cultivos hortícolas. *Revista de Protección Vegetal*, *32*(2), 1-9. http://scielo.sld.cu/scielo.php?pid=S1010-27522017000200005&script=sci_arttext&tlng=pt

Rojas, M. M., Tejera, B., Bosh, D.M., Ríos, Y., Rodríguez, J., & Heydrich, M. (2016). Potentialities of Bacillus strains for promoting growth in maize (*Zea mays* L.) *Cuban Journal of Agricultural Science*, *50*(3), 485-496. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S2079-34802016000300016

Ruiz-Sánchez, E., Mejía-Bautista, M.Á., Serrato-Díaz-Serrato, A., Reyes-Ramírez, A., Estrada-Girón, Y., & Valencia-Botín, A.J. (2016). Actividad antifungica e identificacion

molecular de cepas nativas de *Bacillus subtilis*. *Agrociencia*, *50*, 133-148. http://www.scielo.org.mx/pdf/agro/v50n2/1405-3195-agro-50-02-00133.pdf

Saldaña, J.M. (2017). Aislamiento e identificación de cepas nativas de *Rhizobium phaseoli* de Suelo de la Presa de la Juventud de Marín, Nuevo León. *Revista Iberoamericana de Producción Académica y Gestión Educativa*, *4*(7). https://www.pag.org.mx/index.php/PAG/article/view/659/826

Toledo-Hernández, E., Peña-Chora, G., Toribio-Jiménez, J., Romero-Ramírez, Y., Bolaños-Dircio, A., Velázquez-del Valle, M. G., Hernández-Lauzardo, A.N., León-Rodríguez, R., & Vero, S. (2021). Isolated rhizobacteria of *Jatropha curcas* L.: antagonistic activity of phytopathogens and plant growth promoter. *Revista Chapingo Serie Ciencias Forestales y del Ambiente*, *27*(2), 181-198. https://doi.org/10.5154/r.rchscfa.2020.04.029

Vega-Celedón, P., Canchignia, H., González, M., & Seeger, M. (2016). Biosíntesis de ácido indol 3-acético y promoción del crecimiento de plantas por bacterias. *Cultivos Tropicales*, *37* (No. especial), 33-37. http://dx.doi.org/10.13140/RG.2.1.5158.3609

Villarreal-Delgado, M.F., Villa-Rodríguez, E.D., Cira-Chávez, L.A., Estrada-Alvarado, M.I., Parra-Cota, F.I., & de los Santos-Villalobos, S. (2017). The genus *Bacillus* as a biological control agent and its implications in the agri-cultural biosecurity. *Revista Mexicana de Fitopatología*, *36*(1), 95-130. https://doi.org/10.18781/R.MEX.FIT.1706-5

Vital, L., Cruz, M.A., Fernández, S., & Mendoza, A. (2015). Diversidad bacteriana en raíces de maíz híbrido convencional y genéticamente modificado. *ΦYTON. Revista Internacional de Botánica Experimental*, *84*, 233-243. http://www.scielo.org.ar/pdf/phyton/v84n1/v84n1a30.pdf

Yadav, A. N. (2017). Agriculturally Important Micro biomes: Biodiversity and Multifarious PGP Atributes for Amelioration of Diverse Abiotic Stresses in Crops for Sustainable Agriculture. Biomedical *Journal of Scientific and Technical Research*, *1*(4),1- 4. https://doi.org/10.26717/BJSTR.2017.01.000321

Zahid, M., Kaleem, M., Hameed, S., & Rahim, N. (2015). Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.). *Front. Microbiol.*, *6*, 207, 1-10. https://doi.org/10.3389/fmicb.2015.00207