Assessment of bacterial populations associated with banana tree roots and development of successful plant probiotics for banana crop

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A B S T R A C T
Two hundred and sixty-one bacterial isolates associated with banana tree roots in organic systems were isolated from 19 farms located in four different provinces of the Dominican Republic. The isolates were analysed by means of ARDRA plus RAPD, and as a consequence 114 of them were selected for identification by means of complete 16S rRNA sequencing and phylogenetic reconstruction. The 114 isolates belonged to 20 different genera, with Bacillus, Pseudomonas, Enterobacter and Stenotrophomonas the prevailing genera. Of these, 65 isolates showed more than 99.5% similarity with a type strain, and they were assigned to 34 different species from 16 genera; 29 isolates could constitute new species and the remaining 20 isolates belonged to groups containing more than one species with identical 16S rRNA genes, and therefore they could not be assigned to any species. This result showed a higher number of bacterial taxa associated with banana tree roots than previously described. Additionally, we found seven bacterial species with significant in vitro plant growth promoting (PGP) activity, which had not been previously described as PGP bacteria in any crop. Field trials with isolates pre-selected based on their in vitro PGP activity showed that one strain of the species Pseudomonas plecoglossicida improved fruit yield and controlled the incidence of the disease black sigatoka caused by Mycosphaerella fijiensis. This activity was tentatively attributed to induced systemic resistance mechanisms. Bacterial diversity was analysed among the 261 isolates based on the Shannon index of diversity (H), calculated from the ARDRA profiles. Interestingly, the majority of the bacterial diversity was found within farms (86% of the total), being higher than the bacterial diversity between farms (14%). Moreover, the differences in the average H Index between provinces were very low. Consequently the biodiversity of the bacterial communities was little influenced by the soil characteristics. These results could work in favour of the efficient adaptation of the bacterial strains selected for use as plant probiotics in a range of soils in the region analysed.

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1. Introduction

Banana is a key crop for the economy in tropical regions (De la Torre-Gutiérrez et al., 2008). Moreover, it is ranked the eighth crop worldwide for its importance as a foodstuff, and the fourth, after rice, wheat and maize when only developing countries are considered (Arias et al., 2004). Banana is a “dual crop” in many tropical countries because it is cultivated as a subsistence crop but is also one of the most important crops for export. Banana export is a technological and economic activity, different from its production as subsistence crop (Arias et al., 2004). The preferences of the international market make bananas produced in certified organic systems more competitive than conventionally produced bananas. In the Dominican Republic, organic bananas represented more than 50% of the total banana exports on average during the last five years, and over 85% of the organic bananas produced in Dominican Republic are exported (CEI-RD, 2013).

Nowadays, there is no doubt about the important role of the beneficial interactions between microbes and plants in improving agricultural production. The exploitation of the intrinsic biological potential of rhizosphere processes improves nutrient use efficiency.
(Pi et al., 2015) and plant health by different mechanisms of action (Babalola, 2010; Cummings, 2009; Lugtenberg and Kamiova, 2009; Maheshwari, 2011). In organic systems, where the use of traditional chemical products is limited and strictly regulated, the exploitation of plant–microbe interactions to improve the nutritional and health status of the plant is especially important.

The use of seeds or soil inoculants consisting of products based on soil microorganisms called bioprotectants, biofertilisers or bio-stimulants (Viveros et al., 2010; Prashar et al., 2014) or more general plant probiotic microorganisms (PPM) (Lugtenberg and Kamiova, 2009), is becoming more popular in agriculture. What it is expected from such inoculants is that the crops show a yield increase and a better defence against pathogens, with reduced or eliminated chemical inputs (Bhardwaj et al., 2014). These results are a consequence of a complex mix of different modes of action involving: improvement of plant nutrition through better nutrient uptake, improved nitrogen fixation, and phosphate, potassium and iron solubilisation or mineralisation; control of diseases by several mechanisms; and increase of chlorophyll content and photosynthetic activity (Mantelin and Touraine, 2004; Glick et al., 2007; Esitken et al., 2010; Singh et al., 2011; Adesemoye et al., 2009; De Souza et al., 2013; Sinha et al., 2010).

However, there are still inconsistencies in the performance of the inoculants at the field scale (Morrisey et al., 2004). To tackle this issue, research is being undertaken to learn how to prepare the rhizosphere environment for PGPR rhizosphere colonization by means of rhizosphere engineering (Ryan et al., 2009). Nevertheless, at present, the best strategy to improve the performance of the microorganisms at the field scale is to search for region-specific microbial strains, to be used as the inocula to achieve the desired effects in the crop (Deepa et al., 2010).

Knowledge of the native bacterial population, its characterisation, and identification is required to understand the distribution and diversity of indigenous bacteria in the rhizosphere of specific crops in a specific region (Keating et al., 1995; Chahlboune et al., 2011).

Therefore, the present work analyses the culturable bacterial communities associated with the roots of banana trees in the Dominican Republic, with the objective of designing plant probiotics based on microorganisms for such crops. As each plant species selects the bacteria associated with them by means of several mechanisms, it is important to know the regional bacterial strains associated with a given crop in order to design successful inoculants. Consequently the specific objectives were: i) to identify the cultivable bacterial populations associated with banana tree roots; ii) to test the effect of strains with in vitro PGP traits in field conditions; iii) to investigate the biodiversity of the bacteria associated with the banana tree roots and their determinant factors; iv) to work out practical considerations for the design of successful plant probiotics based on microorganisms for banana crops.

2. Materials and methods

2.1. Isolation of bacteria

The isolation of bacteria associated with the roots of banana trees (Musa AAA cv. ‘Dwarf Cavendish’) was carried out in 19 farms under organic management, from the four provinces that are the main producers of banana in the Dominican Republic (Fig. 1). The farmers planted the crop from plants produced in vitro. The sampling was made in the third, fourth or fifth ratoon depending on the farm, at the phenological stage of bunch emergence (E). A brief summary of the soil characteristics is Table 1. The complete soil analyses are in Table S1. For the isolation of endophytic bacteria, the roots were washed with tap water to eliminate adhered particles of soil, following sterilization of the outer part of the roots with 70% ethanol for 1 min and 7% NaClO for 5 min, and three successive washings with sterile distilled water. Five grams of the surface-sterilised roots were macerated with 45 ml of sterile saline solution and dilution series were prepared with the saline solution. Aliquots of 100 μl from 10^-1 to 10^-4 dilutions were plated onto petri dishes with TSA medium (SIGMA Cat. No. 22091) supplemented with 1 mg l^-1 cycloheximide to prevent fungal growth. For rhizospheric bacteria extraction, roots were brushed with a No. 4 brush, and 1 g of the soil obtained was placed in 9 ml of sterile saline solution and dilution series were prepared as indicated above, and plated onto petri dishes with the same medium. In both cases, individual colonies were tested for purity in other petri dishes with TSA medium, and used for the next steps.

2.2. Amplified 16S rRNA restriction analysis (amplified ribosomal DNA restriction analysis, ARDRA)

DNA bacterial extraction was carried out as indicated by Álvarez-Martínez et al. (2009). Amplification of the 16S rRNA gene was performed from bacterial genomic DNA by PCR. The primers 5′-AAGGAGGTGATCCANCCRCA-3′ (5′-AAGGAGGTGATCCANCCRCA-3′) and 8F (5′AGAGTTTGATCCTGGCTCAG-3′) were used to amplify almost the full length of the 16S rRNA gene. The primers were purchased from ISOCEN, and the kit used for the PCR reaction from Promega Corporation (USA) (“Go Taq Flexi DNA Polymerase”). Each PCR mixture (50 μl) contained a reaction mix of 1x buffer, 200 μM of each dNTP, 0.12 μM of each primer, 2 mM MgCl₂, 0.85 U of the polymerase and approximately 100 ng template DNA. PCR amplification was performed under the following conditions: 5 min at 95 °C for initial denaturation; 35 cycles of 1 min at 94 °C for denaturation; 30 s at 45 °C for annealing; 1 min 30 s at 72 °C for extension; and 7 min at 72 °C for a final extension. Five microlitres of the PCR product was used for electrophoresis in a 1.5% agarose gel in TBE buffer (100 mM Tris, 83 mM boric acid, 1 mM EDTA, pH 8.5) at 6 V/cm, stained in a solution containing 0.5 g/ml ethidium bromide, and photographed under UV light. Standard VI (Roche, USA) was used as a size marker. One single band corresponding to the 16S rRNA gene was typically visualised.

After PCR amplification of the 16S rRNA, 6 μl of each product was digested separately by the restriction enzymes (RE) BanI, EcoRI, HindIII and Stul (Thermo Fisher Scientific, USA) and Sau3AI (New England Biolabs Inc. USA) following the manufacturer’s instructions, and subjected to electrophoresis on a 2% (w/v) agarose gel prepared in the same way as indicated above. The electrophoresis conditions, the staining procedure and the photography process were also the same as above.

The band pattern obtained from the ARDRA, was coded in a presence/absence matrix in order to calculate the Shannon index of diversity (H index) (Shannon and Weaver, 1949). A principal component analysis (PCA) was carried out for the 18 soil physico-chemical characters analysed (Table S1) plus the H index, for the 11 farms (cases) with a complete soil analysis (the rest of the farms had to be discarded because several parameters of the soil analysis were missing). The data matrix was log10 transformed for standardisation, and then the variance-covariance matrix was obtained and the PC (principal components) extracted, and finally rotated using the varimax method. A biplot was drawn for the two first PC for the 11 farms and the 19 characters analysed. All the analyses were carried out by means of the software SPSS Statistics (IBM Corp. Released, 2010).

Additionally, the band pattern model for each isolate and each RE was recorded.
2.3. RAPD fingerprinting

Bacterial DNA isolated as described above was used to obtain the RAPD patterns following the procedure described by Rivas et al. (2006) using the primer M13 (5' - GAGGGTGCGGTTCT - 3') (2 mM final concentration) purchased from ISOGEN, and the “Dream Taq Green PCR Master Mix” from Thermo Fisher Scientific, USA. PCR conditions were: preheating at 95 °C for 9 min; 35 cycles of denaturing at 95 °C for 1 min; annealing at 45 °C for 1 min; extension at 75 °C for 2 min; and a final extension at 72 °C for 7 min. Seventeen microlitres of each PCR product were used in electrophoresis in a 1.5% agarose gel in TBE buffer and photographed using the conditions described above. Standard VI (Roche, USA) was used as a size marker. For all the isolates included in a restriction group (RG) (see Section 3.2 for definition) the band patterns were coded as presence/absence and Jaccard’s similarity coefficient was used to

Fig. 1. Locations of the isolation of bacteria.
were inferred using the neighbour-joining (Saitou and Nei, 1987) Kimura's two-parameter model (Kimura, 1980). Phylogenetic trees were assembled using the Seqman program (Madison, Wisconsin, USA) and compared with those from EzTaxon-e server, that contains the type strains of all described bacterial species (Kim et al., 2012). The sequences were aligned using Clustal W software.

2.5.1. Determination of IAA production

For each isolate, a suspension of 3 × 10⁸ cfu ml⁻¹ in sterile saline solution was prepared from a bacterial mass growth obtained in a Petri dish with TSA medium. Five hundred microlitres were transferred into a test tube containing 4 ml of TS (SIGMA Catalogue No. T8907), and incubated for 3 day at 28 °C with continuous shaking. Subsequently, 500 µl of the suspension was transferred to a test tube containing 4 ml of JMM medium (O'Hara et al., 1989) supplemented with 0.167 g l⁻¹ of L-Tryptophan, and incubated for 4 day at 28 °C with continuous shaking. After the incubation period, the optical density (600 nm) was measured to estimate the bacterial concentration, and 1.5 ml of suspension was centrifuged (2500 g) every 10 min. One millilitre of Salkowsky agent (FeCl₃ 0.01 M and perchloric acid 34.3%) was added to 1 ml of the supernatant and incubated for 30 min in dark. The IAA production was estimated from the OD at 535 nm by comparison with a standard curve prepared for known concentrations of IAA, and expressed as μg IAA ml⁻¹.

2.5.2. Determination of ACC deaminase activity

ACC-deaminase activity of the isolated strains was detected on plates with Dworkin-Foster (DF) minimal medium (Dworkin and Foster, 1958) containing 1-aminoacyclopropane-1-carboxylate (ACC) as the sole source of nitrogen (Penrose and Glick, 2003). The plates were incubated at 28 °C in the dark for three days. The growth of the isolates on DF agar medium amended with ACC was taken as an indicator of potential ACC deaminase activity, as the growing bacteria are able to utilize ACC as N source. For quantitave determination of the ACC deaminase activity, the protocol described by El-Tarabily (2008) was carried out. After determining the amount of protein and α-Ketobutyrate (α-KB), the enzyme activity was expressed as micromoles of α-KB per milligramme of protein per hour of the active isolates.

2.5.3. Siderophore production

Siderophore production was estimated following the Alexander and Zuberer (1991) methodology, using the chrome azurol S (CAS) agar medium (Swinn and Neilands, 1987). CAS agar plates were inoculated with cells and incubated at 28 °C for 72 h. Orange halos around the colonies on blue agar were indicative of siderophore excretion. The result was coded as 0 for the absence of halo, 1 for a halo of less than 5 mm, 2 for halo between 5 and 10 mm, and 3 for halo >10 mm.

2.5.4. Growth in N-free medium

The ability to grow in N-free medium was assessed following the methodology described by Wertz et al. (2012) and the inoculation was carried out following the procedure suggested by E. Velázquez (University of Salamanca, Spain, pers. comm.). For each isolate, a suspension of 3 × 10⁸ cfu ml⁻¹ in sterile saline solution was obtained as indicated above. One hundred microlitres were used to inoculate a test tube containing 5 ml of sterile Norris Glucose Nitrogen-free medium and another 100 µl was used to construct a similarity matrix. For each RG, a dendrogram was drawn from the similarity matrix using unweighted pair group with arithmetic mean (UPGMA) for clustering.

2.4. Identification based on 16S rRNA sequencing

The amplification of the 16S rRNA gene was performed using the primer pair 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R (5’-TACGACTTGGGCTCAG-3’) of the gene sequencing was carried out using the primers 518F (5’-CCAGCAGCCGCGGTAATACGACTT-3’) and 800R (5’-TACGACTTGGGCTCAG-3’) (Lane, 1991; Weisburg et al., 1991) by Macrogen (The Netherlands) that yield a product around 1400 bp or more. The obtained sequences were assembled using the Seqman program (Madison, Wisconsin, USA) and compared with those from EzTaxon-e server, that contains the type strains of all described bacterial species (Kim et al., 2012). The sequences were aligned using Clustal W software (Thompson et al., 1997) and distances were calculated according to Kimura’s two-parameter model (Kimura, 1980). Phylogenetic trees were inferred using the neighbour-joining (Saitou and Nei, 1987) and maximum likelihood (Rogers and Swoford, 1998) models, and the MEGA5 package (Tamura et al., 2011) was used for all analyses.

2.5. Determination of PGP traits in vitro

Table 1

<table>
<thead>
<tr>
<th>Province</th>
<th>Location</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Texture</th>
<th>pH</th>
<th>OM (%)</th>
<th>Phosphorus Olsen (mg kg⁻¹)</th>
<th>Potassium (cmol(+)) kg⁻¹</th>
<th>Electric conductivity (dS m⁻¹)</th>
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<td>IDIAF</td>
<td>N18 23,405 W70 50.375 Loam</td>
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<td>12.42</td>
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<td>2.02</td>
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<td>3.58</td>
<td>0.23</td>
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<td>N19 16.897 W73 33.990 Silt</td>
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<td>0.34</td>
<td>0.04</td>
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<td>2.12</td>
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<td>0.51</td>
<td>0.21</td>
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<td>1.79</td>
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<td>3.09</td>
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<td>Field experiment plot</td>
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<td>3.45</td>
<td>20</td>
<td>0.84</td>
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inoculate another test tube containing 5 ml of the same medium supplemented with 2 g 1⁻¹ of (NH₄)₂SO₄ as a positive control. Uninoculated tubes were used as control. The test tubes were statically incubated for 3 day at 28 °C. At the end of the period the bacterial concentration was estimated by measuring the optical density at 600 nm. The growth in the N-free medium was expressed as percentage of the growth observed in the N-supplemented medium.

2.5.5. Phosphate solubilisation

Inorganic phosphate solubilisation was estimated following the methodology of Nautiyal (1999). Each pure isolate was grown in a petri dish containing YED-P medium amended with 0.5% of tri-calcium phosphate at 30 °C for 10 days. Four different isolates were grown per petri dish. The presence of a clearing halo around bacterial colonies is indicative of phosphate solubilisation. The result was coded as 0 for no solubilisation and 1 for the presence of a halo.

2.5.6. Selection of isolates for the field experiment

The selection of the isolates for the field experiment was based on a PCA of the in vitro PGP traits. A PCA was carried out for the numerical results of the five PGP traits, and 50 isolates fulfilling the following conditions were selected: endophytic, fully identified on the 24th h 16S rRNA gene, and non-pathogenic for humans that there was no phosphate solubilisation but was grown per petri dish. The data matrix was standardised by subtracting the mean value of each trait and dividing the result by the standard deviation. For the PCA, a similarity matrix among traits was calculated based on the Pearson product–moment correlation. The PC were extracted and then rotated using the varimax method. A biplot was drawn for the two first PC for the 50 isolates and the five PGP traits. All the analyses were carried out by means of the software SPSS Statistics (IBM Corp. Released, 2010).

2.6. Field experiment

The field experiment was located in the province of Azua, in the Dominican Republic (18° 23’ 38” N; 70° 50’ 47” W). The chemical characteristics of the soil are shown in Table 1. The soil was medium in phosphorous and magnesium content, and high in potassium. The crop was fertilised with 5000 kg per ha of vermicompost (from cattle manure (N 3.1%, P 1.7%, K 1.6%) one month after transplanting. Transplanting was carried out on June 6th 2012.

The crop was fertilised with 5000 kg per ha of vermicompost from cattle manure (N 3.1%, P 1.7%, K 1.6%) one month after transplanting, and drip irrigated to keep the soil humidity near to the field capacity. The crop was kept free from weeds manually. Flowering started at the beginning of January 2013, and the routine management practices were carried out (deleafing which consists of the removal of the leaves interfering with the bunch development, and cutting off male buds). Cropping time started in mid-March 2013.

In order to estimate the effect of the treatments on the crop, the two central plants in each plot were subjected to measurements of the typical parameters for an agronomic study using a banana crop. At regular time intervals during the vegetative growth the number of developed leaves was recorded, as was the first necrotic leaf as a consequence of endemic black sigatoka (Mycosphaerella fijiensis Morelet) infection. In the same plants, at flowering time, the plant height and the diameter of the pseudostem were also scored. At cropping time, the number of leaves, the yield and the yield components (number of hands per bunch, number of fruits per bunch, and average weight per fruit) were also measured. Analysis of variance appropriate to randomised complete block design was performed using the software SPSS Statistics (IBM Corp. Released, 2010).

3. Results

3.1. Number of isolates

From the 261 isolates, 185 were endophytic and the rest rhizospheric. Eighty-eight were isolated in the province of Azua (five farms), 41 in Dajabón (three farms), 61 in Mao (five farms) and 72 in Monte Cristi (six farms) (Table 2 and Table S2).

3.2. Amplified 16S rRNA restriction analysis (amplified ribosomal DNA restriction analysis, ARDRA)

We used five different RE to obtain the ARDRA profiles for the 261 strains isolated and each RE produced a different number of band patterns in this collection (Fig. 2). The results were analysed in two different ways. Firstly, the band patterns were converted into a presence/absence matrix (matrix not shown) for all the isolates and the five RE. This was in order to calculate the Shannon index of diversity. In this way we could assess the biodiversity of the culturable bacteria associated with banana roots in the four different analysed provinces of the Dominican Republic. Secondly, for each isolate, we recorded the model for each RE. Combining the models for each RE in the 261 isolates, we found 40 restriction groups (RG) (Table 3), and we assigned each isolate to a RG. Since this grouping of the isolates was based on the 16S rRNA gene, it was used to select isolates for further detailed taxonomic study, consisting of the complete sequencing of this gene in some representative isolates.

3.3. Biodiversity of the bacteria associated with the roots of banana trees, estimated from ARDRA analysis

The H index was calculated for 218 isolates from 15 farms (five from Azua, two from Dajabón, three from Mao and five from Montecristi), out of the 261 total isolates from 18 farms. The other three farms were not included because of the lack of soil analysis. The H index showed values ranging from 0.19 in the farm Clavellina (Montecristi province) to 0.34 in the farm Amina 1 (Mao province) (Table 4). For each province, we calculated the average value of the H index for all the farms in the province, and we also compared the average values of the four provinces by ANOVA, obtaining an F value of 0.563 and a p value of 0.63. This indicates that there was no significant difference between the provinces in terms of the average biodiversity of the bacterial populations associated with the roots of banana trees.
Moreover, the global value of the H index for the collection of bacteria isolated in the 15 mentioned farms was 0.325, and the average value of the H index within the farms (intra-farm average value of the H index) was 0.280, thus it can be deduced that most of the global biodiversity (86%) is intra-population diversity (intra-farms) and only 14% is among farms (Table 4).

In order to analyse the influence of the soil properties on the bacterial diversity, we carried out a PCA with the soil parameters and the H index. The first two components accounted for 71.3% of the total variability and the first three for 84.5% (Table 5). The parameters with the highest influence in the first principal component were the lime content, and the contents of Fe, Ca, and K. For the second principal component the contents of organic matter, Ca, Mg and Fe had the greatest influence (Table 6). The first component distinguished between the farms in the Azua province on the one hand and those in Montecristi and Dajabón provinces on the other, the main difference being in the lime content (high Azua and low in Montecristi and Dajabón) (Fig. 3). The second component distinguished between the farm of Carrizal in Dajabón on the one hand and the farms in Mao, Montecristi and Azua on the other. The soil at Carrizal had a very low Ca content, and was high in Fe and organic matter, with the other farm from the province of Dajabón (La Rosa) in an intermediate position. In the biplot representing in the first two PC the farms, the soil parameters and the H index (Fig. 3), this last was plotted very near to the coordinate origin. This is a consequence of the small differences in biodiversity between farms, and reveals the small influence of the soil properties on the H value.

3.4. Identification of 114 bacterial strains isolated from the rhizosphere of banana trees, based on 16S rRNA gene sequences

The entire collection of 261 isolates was divided into 40 different RG (Table 3 and Table S2). In order to establish a correspondence between the RG and their taxonomic identity, several representatives from each RG were identified by 16S rRNA sequencing. In order to select the isolates for sequencing, a RAPD analysis with the primer M13 was carried out within each RG, and a dendrogram obtained for each RG. The dendrogram was cut at the similarity level of 0.30 and one representative of each group or branch was sequenced (Fig. S1) as an example the dendrogram obtained for the RAPD analysis of the RG IV with 33 isolates, in which 11 of them were selected. After the RAPD analysis 114 isolates were selected (Table 3 and Table S2) and the complete 16S rRNA gene sequences were obtained for each. The GenBank accession numbers (Benson et al., 2005) are given in Table S2.

The identification of the 114 isolates was based on the sequence similarity of the 16S rRNA gene with respect to those of the type strains deposited in the EzTaxon database and the closest related species according this database were used for the construction of the phylogenetic trees. We obtained four phylogenetic trees (Figs. 4–7), each one corresponding to a different bacterial group: β-proteobacteria, gram positive, γ-proteobacteria, and α-proteobacteria, respectively. For the construction of the phylogenetic trees, we used the 16S rRNA gene sequences of the isolates plus the sequence of the type strain closest to each isolate, as well as an outgroup.

The general criterion used to identify the isolates at species level was the existence of a similarity of 99.5% or higher with respect to a type strain in the EzTaxon database. The 114 isolates belonged to 20 different genera. Sixty five isolates were identified at the species level, and belonged to 34 species from 16 genera (Table 3 and Figs. 4–7). Twenty-nine strains showed a similarity level lower than 99.5% with respect to any type strain in the database, and therefore could represent new species, further research based on the sequencing of other genes would be needed to clarify their identity. The remaining 20 strains belonged to groups in which identification at species level is not possible on the basis of the 16S rRNA gene sequences because the sequence is identical among several species. This is the case for the “Bacillus cereus group”, including the species B. cereus, Bacillus thuringiensis and Bacillus anthracis, which contained 16 strains isolated in this study, and for the “Bacillus pumilus group”, including the species Bacillus safensis, Bacillus altitudinis and Bacillus aerophilus, which contained four strains isolated in this study (Fig. 5 and Table 3).

3.5. In vitro assessment of PGP traits of the isolates

The 261 isolates were analysed for five in vitro plant growth promoting traits: IAA production; ACC deaminase activity; siderophore production; phosphate solubilisation; and the ability to grow in the absence of a source of combined nitrogen. The values

<table>
<thead>
<tr>
<th>Province</th>
<th>Farm</th>
<th>Code given to the bacteria isolated</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azua</td>
<td>IDAF</td>
<td>RD_AZIDI</td>
<td>12</td>
</tr>
<tr>
<td>Azua</td>
<td>Proyecto 2C</td>
<td>RD_AZP2C</td>
<td>16</td>
</tr>
<tr>
<td>Azua</td>
<td>Proyecto Isura</td>
<td>RD_AZPIS</td>
<td>9</td>
</tr>
<tr>
<td>Azua</td>
<td>Pueblo Viejo</td>
<td>RD_AZPVI</td>
<td>12</td>
</tr>
<tr>
<td>Azua</td>
<td>Los Tramajos</td>
<td>RD_AZLITR</td>
<td>11</td>
</tr>
<tr>
<td>Dajabón</td>
<td>Carrizal</td>
<td>RD_DACAR</td>
<td>5</td>
</tr>
<tr>
<td>Dajabón</td>
<td>Rabial</td>
<td>RD_DARAB</td>
<td>14</td>
</tr>
<tr>
<td>Dajabón</td>
<td>La Rosa</td>
<td>RD_DAROS</td>
<td>9</td>
</tr>
<tr>
<td>Mao</td>
<td>Amina 1</td>
<td>RD_MAAAMIA</td>
<td>19</td>
</tr>
<tr>
<td>Mao</td>
<td>Amina 2</td>
<td>RD_MAAAMIB</td>
<td>5</td>
</tr>
<tr>
<td>Mao</td>
<td>Tierra Fía 1</td>
<td>RD_MATFA</td>
<td>9</td>
</tr>
<tr>
<td>Mao</td>
<td>Tierra Fía 2</td>
<td>RD_MATFB</td>
<td>4</td>
</tr>
<tr>
<td>Mao</td>
<td>El Charco</td>
<td>RD_MAELEC</td>
<td>7</td>
</tr>
<tr>
<td>Montecristi</td>
<td>La Pinta</td>
<td>RD_MOLAP</td>
<td>6</td>
</tr>
<tr>
<td>Montecristi</td>
<td>Pepillo Salcedo</td>
<td>RD_MOPEP</td>
<td>12</td>
</tr>
<tr>
<td>Montecristi</td>
<td>Gozuela</td>
<td>RD_MOGOZ</td>
<td>10</td>
</tr>
<tr>
<td>Montecristi</td>
<td>Santa María 1</td>
<td>RD_MOSAA</td>
<td>9</td>
</tr>
<tr>
<td>Montecristi</td>
<td>Santa María 2</td>
<td>RD_MOSAB</td>
<td>4</td>
</tr>
<tr>
<td>Montecristi</td>
<td>Clavellina</td>
<td>RD_MOCLA</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>185</td>
</tr>
</tbody>
</table>

obtained for the 261 isolates are given in Table S2. Each trait varied widely among the isolates. In order to aid the interpretation of the distribution of this variation, we defined quartiles for each trait. IAA production ranged from 0.66 to 17.94 μg ml⁻¹. The isolates in the last three quartiles, produced between 0.66 and 3.24 μg ml⁻¹ of IAA, and those in the first quartile produced 3.24–17.94 μg ml⁻¹. Of

Fig. 2. Band patterns obtained after the restriction analysis of the 16S rRNA gene with the five restriction enzymes indicated.
these only five isolates produced more than 10 μg ml⁻¹ and belonged to the species *Pseudomonas otitidis*, *Pseudochrobactrum asaccharolyticum*, *Pseudomonas saccharolyticum* and *Enterobacter\n\ncancerogenus*. 

ACC deaminase activity was estimated by the production of α-Ketobutyrate from 1-aminocyclopropane-1-carboxylic acid, with 217 isolates showing some degree of α-KB production, which ranged from 0.01 to 165.69 μM α-KB mg of protein⁻¹ h⁻¹. The isolates in the last quartile produced from 0.01 to 1.38 μM α-KB mg of protein⁻¹ h⁻¹, and the isolates in the third and second quartiles from 1.38 to 13.23 μM α-KB mg of protein⁻¹ h⁻¹. On the other hand, the isolates in the first quartile showed a range of α-KB production from 13.23 to 165.69 μM mg of protein⁻¹ h⁻¹, although only seven of them exceeded 100 μM mg of protein⁻¹ h⁻¹. They were *Rhizobium radiobacter*; *Enterobacter absuriae*; three *Bacillus* sp. phylogenetically close to *B. sonorense*, *B. aerophilus* and *B. thuringiensis* respectively; *Pseudomonas taiwanensis* and *Serratia\n\ncematodiphila*.

The isolates with a significant growth rate in N-free medium belonged to the genus *Bacillus*: *B. circulans*, *B. kochii*, *B. cereus* group, and a *Bacillus* spp. phylogenetically close to *B. gottheilii*. Also one *Pseudomonas beteli* strain and a *Pseudoxanthomonas* spp. strain grew in N-free medium.

One hundred and forty-two isolates produced an orange halo in the medium indicative of siderophore production, belonging to the genus *Pseudomonas*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Leclercia*, *Serratia*, *Brevundimonas*, *Pantoea*, *Stenotrophomonas*, *Capriavidus*, *Pseudochrobactrum*, *Ochrobactrum*, *Comamonas* and *Rhizobium*. The

<table>
<thead>
<tr>
<th>Restriction group (RG)</th>
<th>Total number of isolates in each RG</th>
<th>No. of isolates selected for the complete 16S rRNA sequencing (based on RAPD)</th>
<th>Genera included in the RG</th>
<th>Species identified. The number corresponds to the number of isolates belonging to a given species</th>
</tr>
</thead>
<tbody>
<tr>
<td>A A A A A</td>
<td>4</td>
<td>2</td>
<td><em>Pseudomonas</em></td>
<td><em>P. otitidis</em> (1)</td>
</tr>
<tr>
<td>A A A A D</td>
<td>1</td>
<td>1</td>
<td><em>Pseudomonas</em></td>
<td><em>P. aeruginosa</em> (1)</td>
</tr>
<tr>
<td>A A I E A</td>
<td>3</td>
<td>1</td>
<td><em>Pseudomonas</em></td>
<td><em>P. pleomorpha</em> (1)</td>
</tr>
<tr>
<td>A A I E C</td>
<td>11</td>
<td>1</td>
<td><em>Pseudomonas</em></td>
<td><em>P. pleomorpha</em> (1)</td>
</tr>
<tr>
<td>A B A K D</td>
<td>4</td>
<td>2</td>
<td><em>Acinetobacter</em></td>
<td><em>A. pittii</em> (2)</td>
</tr>
<tr>
<td>A B B B A</td>
<td>3</td>
<td>1</td>
<td><em>Enterobacter</em></td>
<td><em>E. cancerogenus</em> (1)</td>
</tr>
<tr>
<td>A B B B B</td>
<td>4</td>
<td>2</td>
<td><em>Pantoea</em></td>
<td><em>P. dispersa</em> (1)</td>
</tr>
<tr>
<td>A B B B E</td>
<td>8</td>
<td>4</td>
<td><em>Enterobacter Leclercia</em></td>
<td><em>E. asburiae</em> (1)</td>
</tr>
<tr>
<td>A B D D B</td>
<td>1</td>
<td>1</td>
<td><em>Achromobacter</em></td>
<td><em>A. flava</em> (1)</td>
</tr>
<tr>
<td>A B F K D</td>
<td>6</td>
<td>2</td>
<td><em>Acinetobacter</em></td>
<td><em>A. flava</em> (1)</td>
</tr>
<tr>
<td>A B H B B</td>
<td>1</td>
<td>1</td>
<td><em>C. youngiae</em></td>
<td><em>C. youngiae</em> (1)</td>
</tr>
<tr>
<td>A B A E C</td>
<td>11</td>
<td>1</td>
<td><em>Pseudomonas</em></td>
<td><em>P. koreensis</em> (1)</td>
</tr>
<tr>
<td>A B D I A</td>
<td>3 widespread</td>
<td>3</td>
<td><em>Brevundimonas</em></td>
<td>*B. oelei (3)</td>
</tr>
<tr>
<td>A B B I A</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Bacillus</em></td>
<td><em>B. kochii</em> (1)</td>
</tr>
<tr>
<td>A B B B B</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Enterobacter</em></td>
<td><em>E. cloacae subsp. dissolvens</em> (1)</td>
</tr>
<tr>
<td>A B C D B</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Comamonas</em></td>
<td><em>C. aquatica</em> (1)</td>
</tr>
<tr>
<td>A B D C A</td>
<td>8 widespread</td>
<td>23</td>
<td><em>Bacillus</em></td>
<td><em>B. marisflavi</em> (2)</td>
</tr>
<tr>
<td>A B D H A</td>
<td>4 widespread</td>
<td>2</td>
<td><em>Lysinibacillus</em></td>
<td><em>L. xylanilyticus</em> (2)</td>
</tr>
<tr>
<td>A B D M A</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Lysinibacillus</em></td>
<td><em>L. xylanilyticus</em> (2)</td>
</tr>
<tr>
<td>A B J B A</td>
<td>4 widespread</td>
<td>2</td>
<td><em>Pantoea</em></td>
<td><em>P. dispersa</em> (1)</td>
</tr>
<tr>
<td>B B J J B</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Bacillus</em></td>
<td><em>B. amyoliqfaciensi subsp. plantarum</em> (2)</td>
</tr>
<tr>
<td>B B J C A</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Bacillus</em></td>
<td><em>B. amyoliqfaciensi subsp. plantarum</em> (2)</td>
</tr>
<tr>
<td>B B J D A</td>
<td>5 widespread</td>
<td>2</td>
<td><em>Bacillus</em></td>
<td><em>B. amyoliqfaciensi subsp. plantarum</em> (2)</td>
</tr>
<tr>
<td>B B J D D</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Burkholderia</em></td>
<td><em>B. diffusa</em> (1)</td>
</tr>
<tr>
<td>B B J F A</td>
<td>23 widespread</td>
<td>10</td>
<td><em>Lateomomas; P. beteli</em></td>
<td><em>P. beteli</em> (3)</td>
</tr>
<tr>
<td>C B J G A</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Pseudomonas</em></td>
<td><em>Salmophilus</em> (1)</td>
</tr>
<tr>
<td>C B J H A</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Pseudomonas</em></td>
<td><em>Salmophilus</em> (1)</td>
</tr>
<tr>
<td>C B J I A</td>
<td>7 widespread</td>
<td>4</td>
<td><em>Burkholderia</em></td>
<td><em>B. subtilis inaquosorum</em> (3)</td>
</tr>
<tr>
<td>B B K D D</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Burkholderia</em></td>
<td><em>B. subtilis inaquosorum</em> (3)</td>
</tr>
<tr>
<td>C B A G B</td>
<td>13 widespread</td>
<td>4</td>
<td><em>Lateomomas; P. beteli</em></td>
<td><em>P. beteli</em> (3)</td>
</tr>
<tr>
<td>C B A K B</td>
<td>15 widespread</td>
<td>4</td>
<td><em>Stenotrophomonas</em></td>
<td>*S. maltophilia (4)</td>
</tr>
<tr>
<td>C B J J D</td>
<td>3 widespread</td>
<td>1</td>
<td><em>Cupriavidus</em></td>
<td>*C. necator (1)</td>
</tr>
<tr>
<td>D A A J D</td>
<td>3 widespread</td>
<td>2</td>
<td><em>Pseudochrobactrum</em></td>
<td><em>P. saccharolyticum</em> (2)</td>
</tr>
<tr>
<td>D A J J D</td>
<td>3 widespread</td>
<td>3</td>
<td><em>Pseudochrobactrum</em></td>
<td><em>P. saccharolyticum</em> (2)</td>
</tr>
<tr>
<td>D B A J A</td>
<td>5 widespread</td>
<td>2</td>
<td><em>Ochrobactrum</em></td>
<td>*O. pseudogenes (2)</td>
</tr>
<tr>
<td>D B D K D</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Ochrobactrum</em></td>
<td>*O. intermedium (1)</td>
</tr>
<tr>
<td>D B G C D</td>
<td>6 widespread</td>
<td>5</td>
<td><em>Rhizobium</em></td>
<td><em>R. radiobacter</em> (5)</td>
</tr>
<tr>
<td>D B G J D</td>
<td>3 widespread</td>
<td>1</td>
<td><em>Rhizobium</em></td>
<td>*R. massiliae (1)</td>
</tr>
<tr>
<td>E A A G D</td>
<td>1 widespread</td>
<td>1</td>
<td><em>Pseudoxanthomonas</em></td>
<td>*P. massiliae (3)</td>
</tr>
</tbody>
</table>

Total number of isolates: 261, 114
isolates that produced a larger halo belonged to the genera Pseudomonas, Bacillus and Burkholderia.

Forty-four isolates solubilised tricalcium phosphate in vitro, mainly belonging to the genus Pseudomonas (P. plecoglossicida, P. taiwanensis, P. resinovorans, P. otitidis, Pseudomonas mosselii), and to the species Acinetobacter pittii. Also most of the beta proteobacteria isolated, plus two strains of Enterobacter, three of Pantoea, one of Citrobacter, and one of Pseudochrobactrum were able to solubilise tricalcium phosphate.

For each province, we calculated the average value of each of the PGP activities analysed in vitro, comparing the average values for the four provinces with an ANOVA, obtaining the following F values (p value in parenthesis): IAA production 2.246 (0.083); production of α-Ketobutyrate from 1-aminocyclopropane-1-carboxylic acid 0.982 (0.402); growth in N-free medium 1.467 (0.224); siderophore production 0.961 (0.412); inorganic phosphate solubilisation 5.571 (0.001). Therefore, there were no significant differences in the average values of the PGP traits between the provinces for four of the

Table 4
Shannon Diversity Index (H) for the bacteria isolated in the indicated farms, based on the 0/1 matrix from the ARDRA.

<table>
<thead>
<tr>
<th>Farm</th>
<th>H index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azua-IDIAF</td>
<td>0.2869</td>
</tr>
<tr>
<td>Azua-Proyecto 2C</td>
<td>0.3297</td>
</tr>
<tr>
<td>Azua-Proyecto Isura</td>
<td>0.2480</td>
</tr>
<tr>
<td>Azua-Pueblo Viejo</td>
<td>0.3219</td>
</tr>
<tr>
<td>Azua-Los Tramojos</td>
<td>0.2518</td>
</tr>
<tr>
<td>Dajabón-Carrizal</td>
<td>0.2885</td>
</tr>
<tr>
<td>Dajabón-La Rosa</td>
<td>0.3184</td>
</tr>
<tr>
<td>Mao-Amina 1</td>
<td>0.3360</td>
</tr>
<tr>
<td>Mao-Tierra Pía 1</td>
<td>0.2369</td>
</tr>
<tr>
<td>Mao-El Charco</td>
<td>0.2855</td>
</tr>
<tr>
<td>Montecristi-La Pinta</td>
<td>0.3076</td>
</tr>
<tr>
<td>Montecristi-Pepillo Salcedo</td>
<td>0.2383</td>
</tr>
<tr>
<td>Montecristi-Gozuela</td>
<td>0.2425</td>
</tr>
<tr>
<td>Montecristi-Santa María 1</td>
<td>0.3143</td>
</tr>
<tr>
<td>Montecristi-Clavelina</td>
<td>0.1909</td>
</tr>
<tr>
<td>Intra-farm average value</td>
<td>0.2798</td>
</tr>
<tr>
<td>Global value</td>
<td>0.3253</td>
</tr>
</tbody>
</table>

Table 5
Eigenvalues, percentages and cumulative variance corresponding to the Principal Component Analysis (PCA) of the 18 physico-chemical soil parameters, and the Shannon index of diversity (H) based on the 0/1 matrix from the ARDRA, evaluated in the 11 farms where the bacteria were isolated.

<table>
<thead>
<tr>
<th>Principal component</th>
<th>Eigenvalue</th>
<th>Percentage of variance</th>
<th>Cumulative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.382</td>
<td>50.727</td>
<td>50.727</td>
</tr>
<tr>
<td>2</td>
<td>0.155</td>
<td>20.603</td>
<td>71.330</td>
</tr>
<tr>
<td>3</td>
<td>0.099</td>
<td>13.111</td>
<td>84.461</td>
</tr>
<tr>
<td>4</td>
<td>0.067</td>
<td>8.955</td>
<td>93.416</td>
</tr>
<tr>
<td>5</td>
<td>0.018</td>
<td>2.383</td>
<td>95.799</td>
</tr>
<tr>
<td>6</td>
<td>0.013</td>
<td>1.790</td>
<td>97.589</td>
</tr>
<tr>
<td>7</td>
<td>0.010</td>
<td>1.370</td>
<td>98.959</td>
</tr>
<tr>
<td>8</td>
<td>0.004</td>
<td>0.588</td>
<td>99.546</td>
</tr>
<tr>
<td>9</td>
<td>0.003</td>
<td>0.372</td>
<td>99.918</td>
</tr>
<tr>
<td>10</td>
<td>0.001</td>
<td>0.082</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Table 6
Eigenvector values for the four first Principal Components of the 18 physic-chemical soil parameters plus the Shannon index of diversity (H), evaluated in the 11 farms where the bacteria were isolated.

<table>
<thead>
<tr>
<th>Character</th>
<th>P.C. 1</th>
<th>P.C. 2</th>
<th>P.C. 3</th>
<th>P.C. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>H index</td>
<td>−0.0001</td>
<td>−0.0034</td>
<td>−0.0068</td>
<td>0.0041</td>
</tr>
<tr>
<td>Si%</td>
<td>0.0098</td>
<td>0.0384</td>
<td>0.0186</td>
<td>0.1527</td>
</tr>
<tr>
<td>Clay %</td>
<td>0.0215</td>
<td>0.0065</td>
<td>0.1263</td>
<td>0.0332</td>
</tr>
<tr>
<td>pH</td>
<td>0.0323</td>
<td>0.0410</td>
<td>−0.0048</td>
<td>−0.0143</td>
</tr>
<tr>
<td>Lime %</td>
<td>0.4528</td>
<td>0.0472</td>
<td>0.0032</td>
<td>−0.0028</td>
</tr>
<tr>
<td>OM %</td>
<td>−0.0049</td>
<td>−0.0077</td>
<td>0.0109</td>
<td>0.0062</td>
</tr>
<tr>
<td>Total N %</td>
<td>−0.0049</td>
<td>−0.0077</td>
<td>0.0109</td>
<td>0.0062</td>
</tr>
<tr>
<td>Ratio of organic carbon to total nitrogen</td>
<td>−0.0248</td>
<td>−0.0098</td>
<td>0.0254</td>
<td>0.0255</td>
</tr>
<tr>
<td>P Olsen mg/kg</td>
<td>0.0187</td>
<td>0.0519</td>
<td>0.1794</td>
<td>−0.0279</td>
</tr>
<tr>
<td>Ca cmol(+)/kg</td>
<td>0.1225</td>
<td>0.3250</td>
<td>−0.0171</td>
<td>−0.0012</td>
</tr>
<tr>
<td>K cmol(+)/kg</td>
<td>0.1038</td>
<td>0.0478</td>
<td>0.1246</td>
<td>−0.0286</td>
</tr>
<tr>
<td>Mg cmol(+)/kg</td>
<td>−0.0086</td>
<td>0.2375</td>
<td>0.0862</td>
<td>0.0240</td>
</tr>
<tr>
<td>Na cmol(+)/kg</td>
<td>0.0671</td>
<td>0.0236</td>
<td>0.0416</td>
<td>−0.0025</td>
</tr>
<tr>
<td>Cation Exchange Capacity cmol(+)/kg</td>
<td>0.0052</td>
<td>0.0445</td>
<td>0.0057</td>
<td>0.0202</td>
</tr>
<tr>
<td>Cu mg/kg</td>
<td>0.0884</td>
<td>0.0225</td>
<td>0.0376</td>
<td>0.1157</td>
</tr>
<tr>
<td>Fe mg/kg</td>
<td>−0.1354</td>
<td>−0.0202</td>
<td>−0.0419</td>
<td>0.1837</td>
</tr>
<tr>
<td>Zn mg/kg</td>
<td>−0.0605</td>
<td>0.0215</td>
<td>0.0800</td>
<td>0.0880</td>
</tr>
<tr>
<td>B mg/kg</td>
<td>0.0446</td>
<td>0.0236</td>
<td>0.0344</td>
<td>−0.0086</td>
</tr>
<tr>
<td>Electric Conductivity dS/m</td>
<td>0.0002</td>
<td>0.0445</td>
<td>0.0152</td>
<td>0.0600</td>
</tr>
</tbody>
</table>
the five traits evaluated, in accordance with the lack of differences in the average genetic biodiversity between provinces. However, there was an exception in the case of phosphate solubilisation. In this case, the isolates from Azua province showed higher tricalcium phosphate solubilising activity than those from Dajabón and Mao provinces.

3.6. Field trials

The criteria used to select the isolates for the field trials were the results obtained from the assessment of the five in vitro PGP traits. Only the endophytic bacteria from isolates fully identified by means of 16S rRNA sequencing were considered for this trial. Endophytic bacteria likely have better performance from the agronomic viewpoint than the rhizospheric ones, as they are protected by the root tissues. From them we eliminated human pathogens: 14 isolates closely related with *B. anthracis* and *B. cereus*, four isolates closely related to *Burkholderia metallica*, *P. otitidis* and *Stenotrophomonas maltophilia* (the last one because it has been described as an opportunistic pathogen resistant to several antibiotics). Moreover, we also eliminated plant pathogens, five isolates closely related to *R. radiobacter*. Finally we eliminated five isolates close to *B. thuringiensis* because it is considered a biocontroller; this can be a problem for the registration of a bacterial strain as a plant probiotic. Consequently the collection was reduced to 50 isolates. With the values obtained for the PGP traits in the 50 pre-selected isolates, a PCA was carried out. Fig. 8 presents the biplot obtained after the PCA showing the five traits and the 50 isolates. The first two
principal components accounted for 52% of the total variability and with the first three the variability was 75% (data not shown). In the first component, the traits with the most influence on the differentiation of the isolates were siderophore production and growth in N-free medium, both being negatively correlated (Fig. 8). In the second component, the most important parameters were IAA production and ACC deaminase activity, also negatively correlated. Tricalcium phosphate solubilisation was positively correlated with

Fig. 5. Neighbour-joining phylogenetic tree based on 16S rRNA sequences of the isolated gram-positive strains and their closest relatives (type strains which GeneBank accession number are given in parenthesis). Bootstrap values over 50 expressed as percentages of 1000 replications are shown at the branch points. Asterisks denote nodes that were also recovered using the maximum likelihood method. Bar 0.02 represents substitutions per nucleotide position. RG means Restriction Group. The codes of the isolated strains give information about their origin according to Table 2. For more detailed information about each strain see Table S2.
Fig. 6. Neighbour-joining phylogenetic tree based on 16S rRNA sequences, of the isolated γ-proteobacteria strains, and their closest relatives (type strains which GeneBank accession number are given in parenthesis). Bootstrap values over 50 expressed as percentages of 1000 replications are shown at the branch points. Asterisks denote nodes that were also recovered using the maximum likelihood method. Bar 0.02 represents substitutions per nucleotide position. RG means Restriction Group. The codes of the isolated strains give information about their origin according to Table 2. For more detailed information about each strain see Table S2.
siderophore production, but had less influence on the separation of the isolates. The selection of bacteria was based on the biplot (Fig. 8). We did not consider growth in N-free medium, as it is not a standardised method for nitrogen fixation estimation, instead we prioritised other traits. Firstly we considered siderophore production, as it is related to competition with pathogens for Fe ions, but...
without a direct biocidal effect, and is also related to induced systemic resistance (ISR) mechanisms (García Gutiérrez et al., 2012; Djavaheri et al., 2012). Other important traits were IAA production and ACC deaminase activity, but unfortunately they were negatively correlated, therefore it was not possible to select a single isolate with a high expression of both characters. Therefore in the pre-selection of isolates, we chose two in the most positive section of the first component (AZPV_05 and MAAMIA_05 P. plecoglossicida), which showed the highest expression of siderophore production, but very low expression of the other traits; plus other three isolates with the highest possible expression of siderophore production, but also with IAA production (MOPEP_07 P. mossellii) and ACC deaminase activity (DARAB_12 and MAAMIB_01 P. tawanensis) (Fig. 8).

Banana plants were inoculated with the pre-selected bacteria one day before transplanting to the field (June 6th, 2012). During vegetative growth the number of developed leaves did not show significant differences between any of the sampling days (Table 7). It is worth mentioning that on the third sampling day (November 30th), there was a dramatic decrease in the number of leaves in all the treatments, as a consequence of black sigatoka infection. This disease is present in all the banana tree crops throughout the country, and therefore it can be considered as another production factor. The incidence of black sigatoka showed significant differences between treatments, on four of the five sampling dates. This was observed because the position of the first leaf with necrosis as a consequence of the black sigatoka attack showed significant differences between treatments on the sampling dates October 30th (p < 0.05), November 15th (p < 0.05), December 15th (p < 0.01) and December 30th (p < 0.05) (Fig. 9). The isolate RD_MAAMIA_05 (P. plecoglossicida) delayed the black sigatoka attack by between one and four leaves, depending on the sampling date, with such differences being statistically significant with respect to the control on the November 15th and December 15th sampling days (Fig. 9). Moreover, the results obtained with the isolate RD_DARAB_12 (P. tawanensis) did not significantly differ from the results with RD_MAAMIA_05, which also indicated an effect against black sigatoka. However the effect of RD_DARAB_12 was smaller because the difference with the control was only statistically significant in the sampling done on December 30th.

Neither plant height nor the diameter of the pseudostem at flowering time, nor the number of developed leaves at cropping time showed significant differences between treatments (Table 8).

Regarding yield components (Table 9), the treatments resulted in significant differences (p < 0.01) only in the average weight per fruit, but this also resulted in significant differences (p < 0.05) in the yield per plant. The average fruit weight, and the yield per plant (weight of the bunch) were higher in all of the treatments than in the control. However the differences with the control were only statistically significant for the isolate RD_MAAMIA_05 (P. plecoglossicida). In that treatment the average fruit weight was 30% greater and the average yield per plant 29% higher compared with the control. The treatment with RD_MAAAMIB_01 (P. tawanensis) produced a yield 14.3% higher than the control, but the difference was not statistically significant.

4. Discussion

Agriculture is concerned with the production of food in environmentally and socially sustainable ways (Godfray et al., 2010). The maximisation of the coadaptation between plants and rhizosphere microbial communities as an alternative to chemical agricultural inputs has become a subject of great interest with the aim of achieving sustainability and bio-safety in agriculture (Bhardwaj et al., 2014). Therefore, a better management of the soil micro-biota and a parallel reduction in the use of agro-chemicals has been proposed as the most adequate strategy for a more sustainable agriculture (Singh et al., 2011).

As organic farming manages without chemical inputs, organic crops are mostly dependent on the natural microflora of the soil (Sinha et al., 2010). As the long term goal of our work is to develop bacterial inoculants to be used as plant probiotics in banana in the Dominican Republic, we have paid great attention to knowledge about the cultivable microorganisms associated with the roots of organically managed banana crops in the Dominican Republic. This is because on the one hand development of inoculants based on microbes should be based on region-specific microbial strains (Deepa et al., 2010; Mulas et al., 2013; Majeed et al., 2013), and on the other hand, organic systems are naturally richer in soil biota than conventional ones (Mäder et al., 2002; Hole et al., 2005; Birkhofer et al., 2008; Henneron et al., 2015). Moreover there is substantial scientific evidence that plants, through root exudates, select the composition and total population of soil microflora (Chaparro et al., 2012), and therefore the most adequate bacterial species to be used as inoculants for a given crop (in this case banana) are those isolated from such a crop.

4.1. Strategy to identify the cultivable bacteria associated with the roots of banana trees

A comprehensive study of the soil bacteria associated with a given crop (i.e. community structure, population index and molecular phylogeny), should be accomplished by means of a metabolic analysis of certain genes, typically 16S rRNA (Iwai et al., 2011; Sueyaga, 2012; Keshri et al., 2013). However, when the goal of the work is to develop plant probiotics based on microorganisms, the efforts must focus on the cultivable microorganisms. In such a case a very high number of strains are frequently isolated, which must be identified from the taxonomic viewpoint.

Since the phylogenetic classification of the bacteria is currently

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**Table 7**

Average number of leaves per banana tree in the field trial during vegetative growth. Treatments correspond to inoculations with the indicated strains. Values followed by the same letter did not significantly differ (p < 0.05); ns, not significant; D.F., degrees of freedom.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>October 30th</th>
<th>November 15th</th>
<th>November 30th</th>
<th>December 15th</th>
<th>December 30th</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD_AZPV_05</td>
<td>10.5 a</td>
<td>10.8 a</td>
<td>6.8 a</td>
<td>8.8 a</td>
<td>10.2 a</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>10.3 a</td>
<td>10.8 a</td>
<td>6.6 a</td>
<td>8.7 a</td>
<td>9.8 a</td>
</tr>
<tr>
<td>RD_MAAMIA_05</td>
<td>10.5 a</td>
<td>10.8 a</td>
<td>6.8 a</td>
<td>8.8 a</td>
<td>10.2 a</td>
</tr>
<tr>
<td>RD_MOPEP_07</td>
<td>10.6 a</td>
<td>10.8 a</td>
<td>6.6 a</td>
<td>8.7 a</td>
<td>9.8 a</td>
</tr>
<tr>
<td>RD_DARAB_12</td>
<td>10.6 a</td>
<td>11.3 a</td>
<td>8.2 a</td>
<td>8.8 a</td>
<td>10.7 a</td>
</tr>
<tr>
<td>RD_MAAAMIB_01</td>
<td>10.9 a</td>
<td>11.2 a</td>
<td>8.7 a</td>
<td>9.4 a</td>
<td>10.7 a</td>
</tr>
</tbody>
</table>

ANOVA (F values and sign. level)  D.F.  Treatment: 5  Block: 2  Treatment * Block: 10  ANOVA (F values and sign. level)  D.F.

---

**Table 8**

Average number of leaves per banana tree in the field trial during vegetative growth. Treatments correspond to inoculations with the indicated strains. Values followed by the same letter did not significantly differ (p < 0.05); ns, not significant; D.F., degrees of freedom.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>October 30th</th>
<th>November 15th</th>
<th>November 30th</th>
<th>December 15th</th>
<th>December 30th</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD_AZPV_05</td>
<td>9.8 a</td>
<td>10.7 a</td>
<td>5.5 a</td>
<td>8.1 a</td>
<td>9.8 a</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>10.3 a</td>
<td>10.8 a</td>
<td>5.4 a</td>
<td>8.4 a</td>
<td>9.9 a</td>
</tr>
<tr>
<td>RD_MAAMIA_05</td>
<td>10.5 a</td>
<td>10.8 a</td>
<td>6.8 a</td>
<td>8.8 a</td>
<td>10.2 a</td>
</tr>
<tr>
<td>RD_MOPEP_07</td>
<td>10.6 a</td>
<td>10.8 a</td>
<td>6.6 a</td>
<td>8.7 a</td>
<td>9.8 a</td>
</tr>
<tr>
<td>RD_DARAB_12</td>
<td>10.6 a</td>
<td>11.3 a</td>
<td>8.2 a</td>
<td>8.8 a</td>
<td>10.7 a</td>
</tr>
<tr>
<td>RD_MAAAMIB_01</td>
<td>10.9 a</td>
<td>11.2 a</td>
<td>8.7 a</td>
<td>9.4 a</td>
<td>10.7 a</td>
</tr>
</tbody>
</table>

ANOVA (F values and sign. level)  D.F.  Treatment: 5  Block: 2  Treatment * Block: 10  ANOVA (F values and sign. level)  D.F.
Fig. 9. Average first necrotic leaf (counted from top to bottom) as a consequence of black sigatoka attack in the field trial, in the non-inoculated control and the different bacterial treatments. The bars followed by the same letter did not significantly differ ($p < 0.05$). The codes of the isolated strains give information about their origin according to Table 2. The prefix RD of the strain codes has been intentionally suppressed to save space. For more detailed information about each strain see Table S2.

Table 8

Average values for the different parameters in the banana trees corresponding to the field trial, at the stages of flowering and harvesting. Treatments correspond to inoculations with the indicated strains. Values followed by the same letter did not significantly differ ($p < 0.05$); ns, not significant; D.F., degrees of freedom.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flowering time (1st January 2013)</th>
<th>Harvest time (15th March 2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of leaves</td>
<td>Plant height (cm)</td>
</tr>
<tr>
<td>Control</td>
<td>13.2 a</td>
<td>162.7 a</td>
</tr>
<tr>
<td>RD_DARAB_12</td>
<td>15.2 a</td>
<td>206.0 a</td>
</tr>
<tr>
<td>RD_AZPVI_05</td>
<td>13.7 a</td>
<td>201.7 a</td>
</tr>
<tr>
<td>RD_MAAMIA_05</td>
<td>13.3 a</td>
<td>187.7 a</td>
</tr>
<tr>
<td>RD_MAAMIB_01</td>
<td>13.7 a</td>
<td>183.3 a</td>
</tr>
<tr>
<td>RD_MOPEP_07</td>
<td>12.2 a</td>
<td>184.2 a</td>
</tr>
<tr>
<td>ANOVA (F values and significance level)</td>
<td>D.F.</td>
<td>5</td>
</tr>
<tr>
<td>Treatment * Block</td>
<td>10</td>
<td>0.643 ns</td>
</tr>
</tbody>
</table>
based on the 16S rRNA gene (Woese, 2000), methods based on the analysis of this gene are the most suitable for the identification of bacterial isolates. Nevertheless, the complete sequencing of this gene, even with the reduction in the economic costs and efforts experienced during the last years, is not viable in large collections. For that reason several strategies for pre-grouping of a collection, prior to gene sequencing have been proposed. The ARDRA technique based on 16S rRNA-RFLP patterns was described in 1996 as a method to cluster bacterial strains corresponding well with known species (Moyer et al., 1996; Heyndrickx et al., 1996). More recently, Sklarz et al. (2009) re-evaluated the predictive power of this methodology concluding that it can be a suitable tool for genus differentiation of environmental isolates, and even for species identification, provided caution is taken in the type and number of restriction enzymes (RE) selected. This technique has previously been used for the characterization of PGPR collections (Martínez et al., 2003; Souza et al., 2013; Beneduzi et al., 2013; Arruda et al., 2013). Although the restriction analysis can be also applied to housekeeping genes (Martínez et al., 2003) and the nifH gene (Beneduzi et al., 2013; Souza et al., 2013), since the 16S rRNA gene is the only one amplified with universal primers and the only one able to allow the identification of bacterial isolates independent of the phylogenetic group to which they belong, this gene is the most appropriate. In our study, this analysis produced 40 RG with a different number of isolates within each group, ranging from one in several cases to 80 in the largest group.

Nevertheless, ARDRA has some limitations for analysis of bacterial biodiversity because bacterial species with identical 16S rRNA genes have been described. Therefore, it is necessary to use a secondary technique able to detect diversity within each ARDRA group in order to split each large group into several smaller ones. The selected technique was RAPD, which as well as ERIC, REP, etc. is strain dependent (Nocker et al., 2007). Several studies have used a 0/1 matrix obtained after the scoring of the RAPD bands patterns to cluster several strains using the UPGMA method, with the condition that the strains are phylogenetically close. In several cases, the dendrograms obtained maintained the taxonomic structure of the strains coherent with specific or subspecific levels (Mulas et al., 2011; Díaz-Alcántara et al., 2014). However in other cases different strains from the same species are located in different branches of the dendrogram, as for example in the works of De Goes et al. (2012), Bish et al. (2013) and in the present study (i.e. Fig. 5).

As on occasions we sequenced the 16S rRNA gene in several isolates per RG, it was possible to conclude that the RG obtained were in general terms genus-specific with some exceptions. For example three of these groups included different genera that were very close from the phylogenetic viewpoint: RG VI with Enterobacter and Pantoea; RG VII with Enterobacter and Leclercia and RG XXXI with Enterobacter and Luteimonas (Fig. 6). However, genera including phylogenetically very diverse species were distributed among different RG, each of them consisting of phylogenetically close species. For example, the genus Bacillus was distributed in eight different groups (Fig. 5), of which RG XVII included all of the isolates from the “Bacillus cereus group” and also B. marisflavi, both located on the same branch of the 16S rRNA phylogenetic tree (Fig. 5). RG XXIX included the “Bacillus pumilus group”, those RG were bi-specific (XXVI Bacillus subtilis and Bacillus siamensis), and finally several were mono-specific. Therefore, the ARDRA technique with the five RE used in the present study, proved to be valid for the preliminary identification of the isolates at the genus level, or even species level in some cases, yielding unambiguous results.

### 4.2. Species associated with the rhizosphere of banana trees in the Dominican Republic and their determinant factors

The plant drives the most determinant factors in the composition of the bacterial populations associated with a given crop. The plant species is the most important one (Andreote et al., 2010), although other factors such as the cultivar, the age of the plant, and other characteristics of the roots also play a role in the biodiversity and the predominant species associated with plant roots (Smalla et al., 2001; Macdonald et al., 2004). Root exudates determine the microorganisms selected by the plant (Chaparro et al., 2012; Prashar et al., 2014). Environmental factors such as the physical and chemical characteristics of the soil also influence the composition of bacterial populations associated with a given crop (Fang et al., 2005; Ibekwe et al., 2010), but in our research we observed that the soil features analysed had only a small influence on the biodiversity of the isolated bacteria. This was made explicit because the H index of diversity was the closest trait to the coordinates origin in the PCA bi-plot representing the H index and the soil factors in the different farms analysed. This indicates that the biodiversity index was the least variable trait between the farms, highlighting that it is not influenced by the soil parameters analysed. This indicates that the biodiversity index was the least variable trait between the farms, highlighting that it is not influenced by the soil parameters analysed, which showed high levels of variability. Other studies have analysed the influence of soil parameters on the biodiversity of the bacteria isolated in a given crop. Some of them arrived at the same conclusion as this study, with independence between the H index and the soil traits analysed (Ambrosini et al., 2012 in sunflower Helianthus annuus L.; De Souza et al., 2013 in rice Oryza sativa L.). However, other studies have observed that some soil parameters influenced the diversity of the bacteria associated with a given crop: Beneduzi et al. (2013) in sugarcane (Saccharum officinarum L.) related the pH and clay content with the H index; and Arruda et al. (2013) in corn (Zea mays L.) described a positive correlation between H index, clay and organic matter. There is a general opinion in the scientific community in favour of the decisive influence of pH on the composition and richness of the soil microorganisms (Fierer

### Table 9

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of hands per bunch</th>
<th>No. of fruits per bunch</th>
<th>Average weight per fruit (g)</th>
<th>Bunch weight (yield per plant) (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.2</td>
<td>100.50 a</td>
<td>151.29 a</td>
<td>15.22</td>
</tr>
<tr>
<td>RD_AZPV_05</td>
<td>7.0</td>
<td>96.00 a</td>
<td>169.28 ab</td>
<td>16.04 ab</td>
</tr>
<tr>
<td>RD_DARAB_12</td>
<td>7.3</td>
<td>101.20 a</td>
<td>160.28 a</td>
<td>16.18 ab</td>
</tr>
<tr>
<td>RD_MOPEP_07</td>
<td>7.3</td>
<td>109.20 a</td>
<td>152.39 a</td>
<td>16.70 ab</td>
</tr>
<tr>
<td>RD_MAMAB_01</td>
<td>7.3</td>
<td>103.70 a</td>
<td>167.34 ab</td>
<td>17.40 ab</td>
</tr>
<tr>
<td>RD_MAMAB_05</td>
<td>7.5</td>
<td>99.20 a</td>
<td>196.77 b</td>
<td>19.62 b</td>
</tr>
</tbody>
</table>

ANOVA (F values and signif. level) D.F.

Treatment 5 0.800 ns 1.771 ns 5.767** 3.324*
Block 2 0.125 ns 0.626 ns 0.114 ns 0.721 ns
Treatment * Block 10 1.025 ns 1.965 ns 0.489 ns 1.463 ns

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and Jackson, 2006), but in our case the variation of the pH across the farms was very small, as the pH was also plotted very near to the coordinates origin in the PCA biplot. Finally agronomic management factors like irrigation, tillage or fertilisation can also influence the composition of the bacterial populations (Fang et al., 2005; Ibekwe et al., 2010). However, in our research the agronomic management practices were fairly similar, as organic banana farmers usually follow the recommendations of processing and commercialisation companies.

The composition of the culture media used for the isolation of cultivable bacteria also has an unquestionable influence on the isolated taxa. For that reason caution must be employed when comparing between the results obtained in different studies if different culture media were used. The most numerous bacterial genera isolated in banana in our work were Bacillus (45%) followed by Pseudomonas-Luteimonas (20%), Enterobacter-Leclercia-Pantoeca (8%), and Stenotrophomonas (6%). Ambrosini et al. (2012) found that the most numerous genera in sunflower were Enterobacter, Burkholderia and Klebsiella; in rice De Souza et al. (2013) cited the predominant genera as Agrobacterium, Pseudomonas, Enterobacter and Burkholderia, and Mirzajan et al. (2013) Serratia, Pantoea, Ochrobactrum, Brevundimonas and Enterobacter. In oilseed rape (Brassica napus L.) Farina et al. (2012) isolated Agrobacterium, Burkholderia, Enterobacter, Klebsiella and Pseudomonas as the main genera. For comparison, we must ignore the genus Bacillus because the above-mentioned works used a culture media that excluded this genus. Enterobacter was a major genus in the five mentioned works; Pseudomonas was a major genus only in three. Burkholderia and Agrobacterium (Rhizobium) were major genera in other two works, and showed a minority representation in our work. However, the main differences in bacterial populations between crops were found in minority taxa. In total we identified 20 genera. Other researchers have isolated five other bacterial genera in banana tree roots that we did not isolate. In Brazil, De Souza et al. (2013) isolated bacteria from nine different genera, four of them in common with our work plus five different ones (Aneurinibacillus, Klebsiella, Micrococcus, Paenibacillus and Sporolactobacillus). Andrade et al. (2014), also in Brazil, isolated bacteria from six different genera all in common with the work of Souza et al. (2013). Martínez et al. (2003) isolated four genera of free N-fixing bacteria from Colima (Mexico), three in common with our work plus Klebsiella. Surprisingly, since they grow well in the media used, some genera such as Aneurinibacillus, Klebsiella, Micrococcus and Paenibacillus were not isolated in this work.

We reported for the first time the in vitro mechanisms of PGP from P. asaccharolyticum RD_AZPRC_25 and P. saccharolyticum RD_AZID_13, with a high IAA production; and for Ochrobactrum pseudogrognonense RD_MAELC_10, also with a significant production of IAA. On the other hand, other isolates that were not fully identified, but that were phylogenetically close to the following species, also showed a high expression of PGP traits, that had not been previously described: RD_MAAMIA_18 and RD_MAELC_05 (Bacillus sp.), phylogenetically close to B. aerophilus and B. subtilis subsp. Inaquosorum, respectively had a high production of side- rorphores; RD_MOLAP_05 (Bacillus sp.), phylogenetically close to B. gottheilii, and RD_MAAMIA_15 (Pseudoxanthomonas sp.), close to P. indica, were both able to grow in vitro in a N-free medium.

4.3. Development of inoculants for banana in the Dominican Republic

From the broad biodiversity of bacteria isolated in this work from banana tree roots, five strains were tested in the field, and the results are very promising for banana producers because most of them showed a tendency to increase the banana yield. This tendency was statistically significant in the case of RD_MAAMIA_05 (P. plecglossicida) compared with the uninoculated control, with a yield increase of 29%. The field trial with PGPR for banana trees of Kavino et al. (2010) observed a yield increase slightly higher than ours (between 36% and 39%) on inoculation with two chitinolytic Pseudomonas fluorescens strains, with the addition of chitin to improve bacterial survival. On the other hand, Selvaraj et al. (2014) observed an increase in fruit yield under field conditions from 36.5 to 45.6%, which was the consequence of the direct biocontrol of two soil-borne diseases (Fusarium oxysporum f. sp. cubense and the nematode Helicotylenchus multicinctus) with the strain Pf1 (P. fluorescens).

Unexpectedly in our case the yield increase seems to be at least partially the consequence of a reduction in the incidence of the leaf fungal disease black sigatoka, which is always present throughout the country, because the strain RD_MAAMIA_05 delayed the appearance of the first leaf with necrotic symptoms, thus increasing the number of fully functional leaves. To strengthen this hypothesis, the strain RD_AZPVI_05 had no effect on either the fruit yield or on the black sigatoka control. Tentatively the phenomenon of the disease control has been assigned to induced systemic resistance (ISR) mechanisms, although in the future it will be necessary to deepen the study of the production of the enzymes related to the enhancement of plant resistance. The main criteria for the selection of the bacteria in the field trial was siderophore production, and this trait has been frequently related with ISR (Garcia-Gutierrez et al., 2012; Dijavaheri et al., 2012). There are no references to the control of black sigatoka in banana attributed to ISR and triggered by rhizospheric bacteria, although there is one study concerning arbuscular mycorrhiza (Oye Anda et al., 2015). On the other hand Fisfal et al. (2010) mentioned positive preliminary results in the control of the banana disease caused by F. oxysporum by ISR processes triggered by one strain of Pseudomonas sp. and one of Burkholderia sp. Peenan et al. (2014) worked with Colletotrichum musae in banana trees and showed evidence of an increase in the activity of defence related enzymes after inoculation with the strain FP7 (P. fluorescens) in field conditions. Moreover Harish et al. (2008, 2009) controlled the Banana Bunch Top Virus (BBTV) with endophytic P. fluorescens and Bacillus spp, also attributed to ISR processes.

The finding that the biodiversity of the bacteria isolated in this work from banana trees roots is independent of the soil characteristics (and therefore showing small differences across the country) is important for the development of plant probiotics based on microorganisms for banana crops in the Dominican Republic. This could involve an adaptation range of the strains to the different environmental conditions across the island. The lack of differences in the average bacterial population diversity between provinces in the Dominican transect of about 150 km, contrasts with the large differences encountered by Marasco et al. (2013) in a thousand kilometre transect across the Mediterranean basin in vineyard crops, from northern Italy to northern Africa. The Island conditions of the Dominican Republic, which have reduced environmental differences due to the influence of the sea, could explain the lack of difference in bacterial diversity and work in favour of the smooth adaptation of a given autochthonous strain to the different productive regions on the island.

4.4. Conclusions

In conclusion, the biodiversity of the cultivable bacteria isolated in this study from roots of banana trees in organic farming systems in the Dominican Republic, contains genera most frequently associated with plant roots such as Bacillus, Pseudomonas and Enterobacter. However, we found that seven species not previously described as PGP bacteria showed high expression of PGP traits.
in vitro and consequently they are candidates to be tested in field conditions, for the eventual development of PGPR based inoculants for banana crops. It may be possible to describe new species among the large number of isolates that could not be fully identified based on comparison with the EzTaxon database and the subsequent phylogenetic reconstruction. In the field experiment the endophytic isolate RD_MAMIA_05 belonging to P. plegcologisida significantly improved the fruit yield and reduced the incidence of black sigatoka. The second best strain, RD_MAMIB_01 (P. taiwanensis) also showed PGPR effects in the field experiment, although with lower efficiency than the first one. The small variations in bacterial biodiversity among the banana producing regions in the Dominican Republic indicates the independence of bacterial biodiversity from the chemical and physical properties of the soil, and opens the door to the possible efficient adaptation of the selected strains to the whole region studied.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.04.013.

References


