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Effect of pesticides application on peanut (*Arachis hypogaea* L.) associated phosphate solubilizing soil bacteria

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ABSTRACT

In the peanut-growing area of Córdoba, Argentina, herbicides and fungicides utilization is a common practice. This study analyses the effect of pesticides applied at recommended rates on the number and diversity of phosphate solubilizing soil bacteria from this area and the frequency of pyrroquinoline quinone genes (*pqq*). Pesticide soil treatment did not affect the abundance of culturable phosphate solubilizing bacteria but increased their genetic diversity and altered the frequency of *pqqE* and *pqqC* genes. The presence of the *pqq*E and *pqq*C genes was observed in a high percentage of isolated Gram-negative phosphate solubilizing bacteria. The analysis of the diversity of 16S rDNA and *pqq*E genes of soil DNA samples indicated a higher diversity in pesticides treated soil samples compared to control soil samples. Results obtained indicated that pesticides application increases diversity of soil bacterial community and therefore phosphate solubilizing soil bacterial population probably as a result of an increase of bacterial populations that use pesticides applied as carbon and energy source. Analysis of *pqq* genes frequency and diversity suggested that they would be potential molecular marker of Gram-negative phosphate solubilizing soil bacteria.

1. Introduction

Peanut (*Arachis hypogaea* L.) is a widespread oilseed crop of great agricultural and economic significance. Argentina is one of the major producers and the principal peanut exporter in the world (Cámara Argentina del Maní, 2014) and about 92% of its production took place in the province of Córdoba (Fiant et al., 2013). However, due to the intensification of agricultural practices, peanut soils of Cordoba have decreased in their phosphorous (P) content (Sainz Rozas et al., 2012). P, next to nitrogen, is the second important macronutrient required for plant growth (Coyne, 2000). While the concentration of P in the soil is high, a low amount is in an available form for plants (Gulati et al., 2008). Its low availability is attributed to the reaction of soluble P with calcium, iron or aluminum ions causing its precipitation or fixation (Sashidar and Podile, 2010).

Peanut is a leguminous that establishes a symbiotic relationship with endophytic nitrogen fixing bacteria called rhizobia. For this process P content is critical since high requirement of ATP is involved (Dey et al., 2004).

Phosphate solubilizing bacteria can play an important role in plant nutrition increasing the available forms of P to plants (Rodriguez et al., 2006). The main mechanism of mineral phosphate-solubilization by microorganism is associated with the release of low molecular weight organic acids, mainly gluconate and 2-ketogluconate (Puente et al., 2004). These acids are produced in the periplasm of many Gram-negative bacteria through a non-phosphorylated direct oxidation pathway of glucose (DOPG–Direct Oxidation Pathway of Glucose) (Matsusshita et al., 2002). The bacterial enzyme glucose dehydrogenase (DGH), involved in the gluconic acid production, requires magnesium and the cofactor redox pyrroloquinoline quinone (PQQ), whose biosynthesis involves a PQQ operon which consists of at least 5–11 genes (Choi et al., 2008). PQQ is essential for the phosphate solubilizing phenotype in several bacteria (Shen et al., 2011). The cloning and expression of genes involved in PQQ synthesis has demonstrated the importance of the production of gluconic and 2-ketogluconic acid in phosphate solubilization phenotype (Han et al., 2008).

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Microorganisms are fundamental components of soil ecosystem as they degrade soil organic matter and sustain biogeochemical transformations of elements (Lin et al., 2007). Modern agriculture depends on the use of a wide variety of synthetic chemicals, including insecticides, fungicides, herbicides and others pesticides (López et al., 2002). Some microorganisms are able to degrade a great variety of chemical substances including agrochemicals to derive energy and other nutrients for their cellular metabolism (Debnath et al., 2002; Das et al., 2003). On the other hand, it has been demonstrated that agrochemicals can cause changes in microbial community structure, repressing or enhancing the microbial activity and growth, and changing or modifying their plant growth promoting activities (Girvan et al., 2004; Simonsen et al., 2008).

In a previous study we demonstrated that pesticides application caused a decrease on the number of cultivated nitrogen fixing population of peanut soils of Córdoba as well on the nitrogen fixing ability of these soils (Angelini et al., 2013). Considering the importance of phosphate solubilizing bacteria in P plant nutrition and nitrogen fixing process, the objectives of this research were (1) to evaluate the effect of pesticides applied at recommended rates on the number and diversity of phosphate solubilizing soil bacteria from peanut-growing area in Argentina and (2) to analyze the frequency of *pqq* genes in this bacterial population.

2. Materials and methods

2.1. Study sites and sampling

Assays were conducted at the experimental field of the National University of Rio Cuarto (33°07′ south latitude, 64°14′ west longitude of G), province of Córdoba, at an altitude of 421 m above the sea level. The experimental field was divided into two plots; one of them was designated as "control plot" in which no pesticides were applied (hand weeding was done) and the other as "treated plot" in which pesticides commonly used for peanut were applied (Table 1). Herbicides and insecticides were applied 2 days and fungicides 61 days after planting seeds (repeating this latter application every 15 days). Five rizospheric soil samples (each of them constituted by 25 subsamples) were taken at different times: 2 days (M1), 63 days (M2) and 158 days after application of herbicides and insecticides. Soil sub-samples were collected with an eyelet (cores of 2 cm diameter) at 10–15 cm deep from soil, pooled, sieved and stored in bags at 4 °C.

2.2. Determination of the number of culturable phosphate solubilizing soil bacteria

Five grams of each rhizospheric soil sample were transferred to Erlenmeyer flasks containing 45 ml of 0.1% sterile sodium pyrophosphate (NaPP). The flasks were shaken for 30 min at 180 rpm and 0.1 ml aliquots of serial dilutions (up to 10^{-8}) were streaked onto Petri dishes containing NBRIP–BPB medium (Mehta and Nautiyal, 2001) supplemented with dicloran to prevent fungal growth. The colony forming units (CFU) of phosphate solubilizing soil bacteria were counted after incubated at 28 °C for seven days. Ten g of soil samples was dried at 37 °C for a week in order to indicate the number of bacteria in dried weight.

2.3. Genotypic diversity of culturable phosphate solubilizing soil bacteria

Genotypic diversity analysis was performed by amplifying repetitive genomic regions (rep-fingerprint) of culturable phosphate solubilizing soil bacteria using ERIC (Enterobacterial Repetitive Intergenic Consensus, E1 (5′-ATGTAAGCTCCTGGGGATTCAC-3′)/E2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′) and BOX-AR1 (5′-CTACGGCAAGGCGACGCTGACG-3′) primers (Versalovic et al., 1994), widely used in studies of bacterial diversity. Total bacterial DNA was obtained using the procedure described by Walsh et al. (1991) and Meade et al. (1982). Amplifications were performed in a DNA thermalcycler (Mastercycler Eppendorf). The ERIC and BOX amplification products were separated according to molecular size by horizontal electrophoresis on 2.5% (w/v) agarose gels stained with SYBR Green II (Molecular Probes). Profiles were analyzed using FAMD 1.2 software (Schlüter and Harris, 2006)

2.4. available soil content

P available soil content was determinated by incubating 1 g soil of each sample in 15 ml NBRIP–BPB liquid medium at 28 °C for 15 days followed by estimation of soluble phosphorus (Fiske and Subbarow, 1925).

2.5. Amplification of pqq*E* and pqq*C* genes in culturable phosphate solubilizing soil bacteria

Total bacterial DNA was obtained as mentioned above. The amplifications of the ~700 bp fragment of *pqq*E gene and ~140 bp

Table 1
Pesticides used in the present study.

Pesticides		Chemical name	Recommended rates
Herbicides	S-metolaclor	2-chloro-6′-ethyl-*N*-(2-methoxy-1-methylethyl) acet-o-toluidide	1 l ha^{-1}
	Diclosulam	N-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro [1,2,4] triazolo [1,5c] pyrimidine-2-sulfonamide	20 g ha^{-1}
	Glyphosate	*N*-(phosphonomethyl) glycine	3.5 l ha^{-1}
	Imazethapyr	2-[4,5-dihydro-4-methyl-4- (1-methylethyl)-5-oxo-1H -imidazole-2-yl]-5-ethyl-3-pyridine carboxylic acid	1 l ha^{-1}
	Imazapic	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridine carboxylic acid	30 ml ha^{-1}
Insecticides	Gamma- cyhalothrin + Lambda-cyhalothrin	(S-α-cyano-3-phenoxybenzyl(1R,3R)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate) (S)-α-cyano-3-phenoxybenzyl (Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (R)-α-cyano-3-phenoxybenzyl (Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate	25 ml ha^{-1}
Fungicides	Azoxystrobin + Ciproconazol	(E)-2-{2-[6-(2-cyano-phenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate 3-chloro-4-[(2RS, 4RS, 2RS, 4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl] phenyl -4-chlorophenyl ether	1 l ha^{-1}

fragment of *pqq*C gene were developed using the primers F317 (5′TTYTAYACCAACCTGATCACSTC3′) and R1019 (5′TBAGCATRAASG-CCTGRCG3′) and primers pqqCF (5′GYGTSCGBTTYGCVGTBGA3′) and pqqCR (5′TARTGYTGSGGCCARCTGT3′), respectively (Anzuay et al., 2013). PCR products obtained were separated by horizontal electrophoresis on 1.5% (w/v) agarose gels stained with SYBR Green II (Molecular Probes) as described above.

2.6. Amplification reactions of pqqE and 16S rDNA genes of soil DNA and clone library construction

Soil DNA was extracted using the commercial kit Ultra Clean soil DNA extraction (MoBio) and purified as indicated by Petric et al. (2011) to remove PCR inhibitors. The *pqq*E gene was amplified as described above using F317 and R1019 primers and 16S rDNA gene using 27F (5′AGAGTTTGATCMTGGCTCAG3′) bacterial-specific primer (Lane, 1991) and 1492R (5′TACGGYTACCTTGTTACGACTT3′) Universal primer (Stackebrandt and Liesack, 1993). PCR products were ligated into the pGEM-T vector using commercial kit pGEM-T Easy (Promega) according to the manufacturer's instructions. Ligation products were transformed into *Escherichia coli* DH5α using the procedure described by Huff et al. (1990). Clones were picked and the presence of the expected size inserts was evaluated by colony PCR using T7 promoter (5′TAATACGACTCACTATAGGG3′) and T7 terminator (5′CTAGTTATTGCTCAGCGGTG3′) primers.

2.7. RFLP analysis of pqqE and 16S rDNA genes sequences on clone libraries

Sequence analysis of *pqq*E amplification products of clones was performed using *Cfo*I, *Msp*I and *Nde*II (PROMEGA) digestion enzymes. For 16S rDNA sequence analysis *Cfo*I, *Hinf*I and *Rsa*I (PROMEGA) enzymes were used. The RFLP products were separated by horizontal electrophoresis on 2% (w/v) agarose gels stained as described above.

2.8. Coverage analysis and diversity indexes

Analyses of the clone libraries were followed by calculation of coverage (C), where C is expressed by 1-$n1/N$, in which $n1$ is the number of clones that appeared only once, and (N) the total number of clones (Chelius and Triplett, 2001). Quantitative estimation of *pqq*E and 16S rDNA sequences diversity was analyzed by Shannon–Wiener (Margelef, 1958) and Simpson (1949) indexes.

2.9. Statistical analysis

Data analysis was carried out using the Infostat software. Data were subjected to repeated measures analysis ANOVA and differences among treatments were detected by Tukey test. For comparisons between two samples, t Student test ($p < 0.05$) was used.

3. Results and discussion

3.1. Effects of pesticides application on the number and genotypic diversity of culturable phosphate solubilizing soil bacteria and on the P available soil content

Abundance analysis at the three evaluated times indicated that addition of pesticides does not modifies the number of phosphate solubilizing bacteria (Fig. 1), in coincidence with others reports (Girvan et al., 2004). However, Das and Mukherjee (1994) and Das and Debnath (2006) observed an increase in the number of phosphate solubilizing bacteria after insecticides and herbicides addition. These authors attributed the increase observed to the utilization, by these microorganisms, of the applied herbicides and their degraded products to derive their energy, carbon and other nutrients for their cellular metabolism.

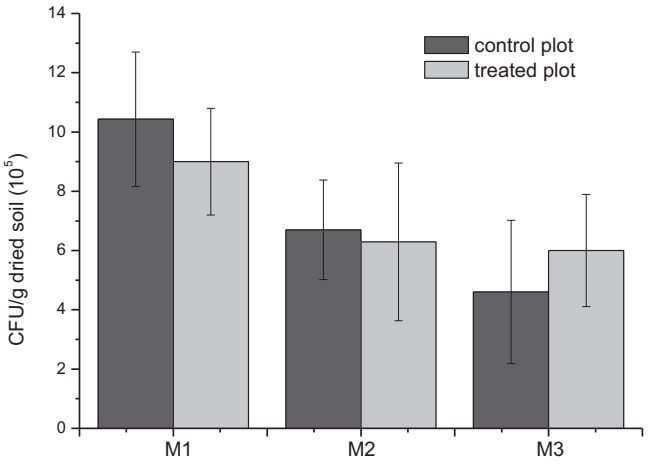

Fig. 1. Number of culturable phosphate solubilizing bacteria in peanut cultivated soils. Values are the mean ± SE of two determinations ($n = 6$).

To analyze the effect of pesticides application on the diversity of culturable phosphate solubilizing soil bacteria, genomic fingerprints of bacterial colonies selected from abundance analysis, were analyzed. BOX and ERIC primers were employed for Gram-positive and Gram-negative bacteria, respectively. A representative colony of each predominant profile was taxonomically identified by analysis of the partial sequence of the 16S rDNA gene. Approximately 30 colonies in NBRIP-BPB plates from each treatment were randomly selected. Comparison analysis indicated a higher number of genomic bacterial profiles in the samples from treated soils (Table 2).

The analysis by BOX-PCR of genomic DNA isolated from 44 bacteria showed 18 different profiles. Gram- positive bacteria were not obtained from M2 and M3 control soil samples. The dendrograms obtained for each sample grouped bacteria in 3–4 clusters (Fig. 2). Profiles 5 and 11 were the most predominant (31% and 18%, respectively). In addition, profile 5 was found both in M1 (control and treated soil) and M3 (treated soil) samples, suggesting that bacteria showing this profile were able to persist over time and to resist to the effect of the pesticides application. Sequence analysis of 16S rDNA genes from bacteria showing profile 5 revealed a high percentage of identity (99%) with bacteria belonging to the genus *Bacillus* (accession number JX675232).

ERIC–PCR fingerprinting analysis of genomic DNA isolated from 121 bacteria showed 62 different profiles. The dendrograms obtained for each sample grouped bacteria in 5–6 clusters (Fig. 3). In M1 soil samples the profiles number 27 and 21 were the most predominant (25 and 21%, respectively). Bacteria that showed profile 21 were isolated from control and treated soil samples and 16S rDNA sequence analysis indicated that this profile presented high percentage of identity (99%) with bacteria belonging to the genus *Enterobacter* (accession number JX675234). In control samples M2 and M3, profile 1 was the most abundant (38%) but it was not identified in treated samples. Taxonomic identification of one bacterium representative of this profile showed a high percentage of identity (99%) with bacteria from the genus *Enterobacter* (accession number JX675233). Profiles 18 and 39 corresponded to bacteria that were isolated from both control and treated soil samples in M2 and M3, respectively, which showed a high percentage of identity (99%) with bacteria belonging to the genera *Pseudomonas* (accession number

Table 2
Abundance of genomic profiles and Simpson and Shannon–Wiener indexes of culturable phosphate solubilizing bacteria and P available soil content in control (C) and treated (T) soil samples.

	M1 (2 days)[b]		M2 (63 days)[b]		M3 (158 days)[b]	
	C	*T*	*C*	*T*	*C*	*T*
Number of phosphate solubilizing isolates analyzed	23	32	30	28	24	28
Total number of genomic profiles	8	19	9	23	5	25
Simpson index	0.20 ± 0.04	0.10 ± 0.01	0.43 ± 0.10	0.04 ± 0.02	0.66 ± 0.02	0.010 ± 0.004[a]
Shannon–Wiener index	2.08 ± 0.48	2.97 ± 0.13	1.55 ± 0.20	3.68 ± 0.95	0.81 ± 0.19	3.69 ± 0.10[a]
Gram positive isolates	10	21	0	6	0	7
Number of BOX profiles	3	9	0	5	0	5
Simpson index	0.59 ± 0,09	0.21 ± 0.05	ND	0.05 ± 0.05	ND	0.09 ± 0.09
Shannon–Wiener index	0.77 ± 0.08	1.85 ± 0.40	ND	1.29 ± 0.29	ND	1.58 ± 0.05
Gram negative isolates	13	11	30	22	24	21
Number of ERIC profiles	5	10	9	18	5	20
Simpson index	0.26 ± 0.07	0.03 ± 0.03	0.43 ± 0.10	0.06 ± 0.04	0.66 ± 0.02	0.01 ± 0.01[a]
Shannon–Wiener index	1.57 ± 0.54	2.26 ± 0.25	1.55 ± 0.20	2.81 ± 0.84	0.81 ± 0.19	3.49 ± 0.01[a]
P available soil content (μg/ml)	10.9 ± 2.3	10.2 ± 3.6	10.3 ± 3.2	12.5 ± 4.8	11.4 ± 3.9	9.3 ± 3.6

Values are the mean ± SE of two determinations (n = 6–30).
[a] Statistically different from control soil samples ($p < 0.05$). C: control soil samples; T: treated soil samples. ND: not determinated.
[b] Days after pesticides application.

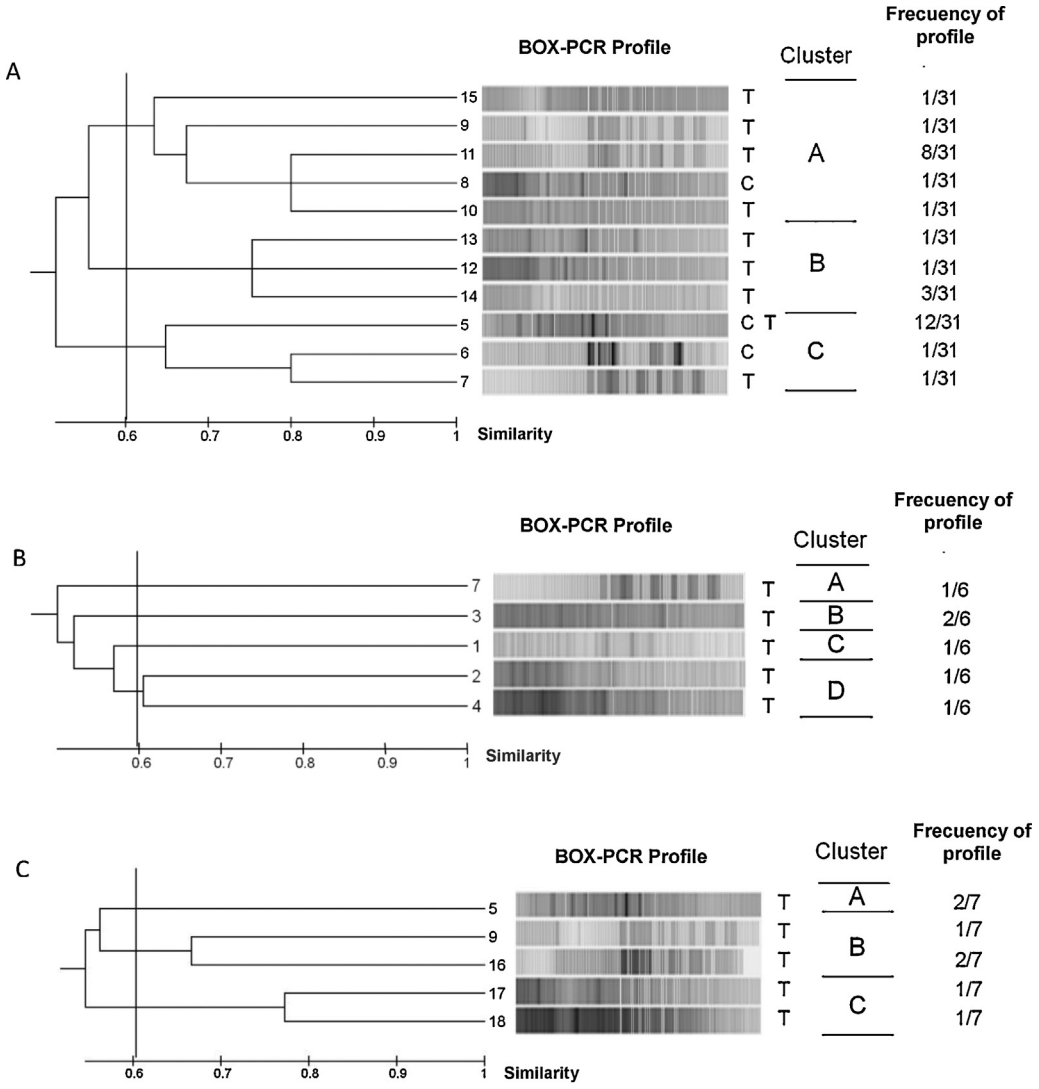

Fig. 2. UPGMA-dendrogram (Dice similarity coefficient) derived from BOX-PCR fingerprints of Gram-positive culturable phosphate solubilizing bacteria from control (C) and treated (T) soil samples from experiment on field. M1 (A), M2 (B) and (M3) samples. The BOX-PCR profiles obtained were identified with Latin numbers and the clusters are indicated with capital letters.

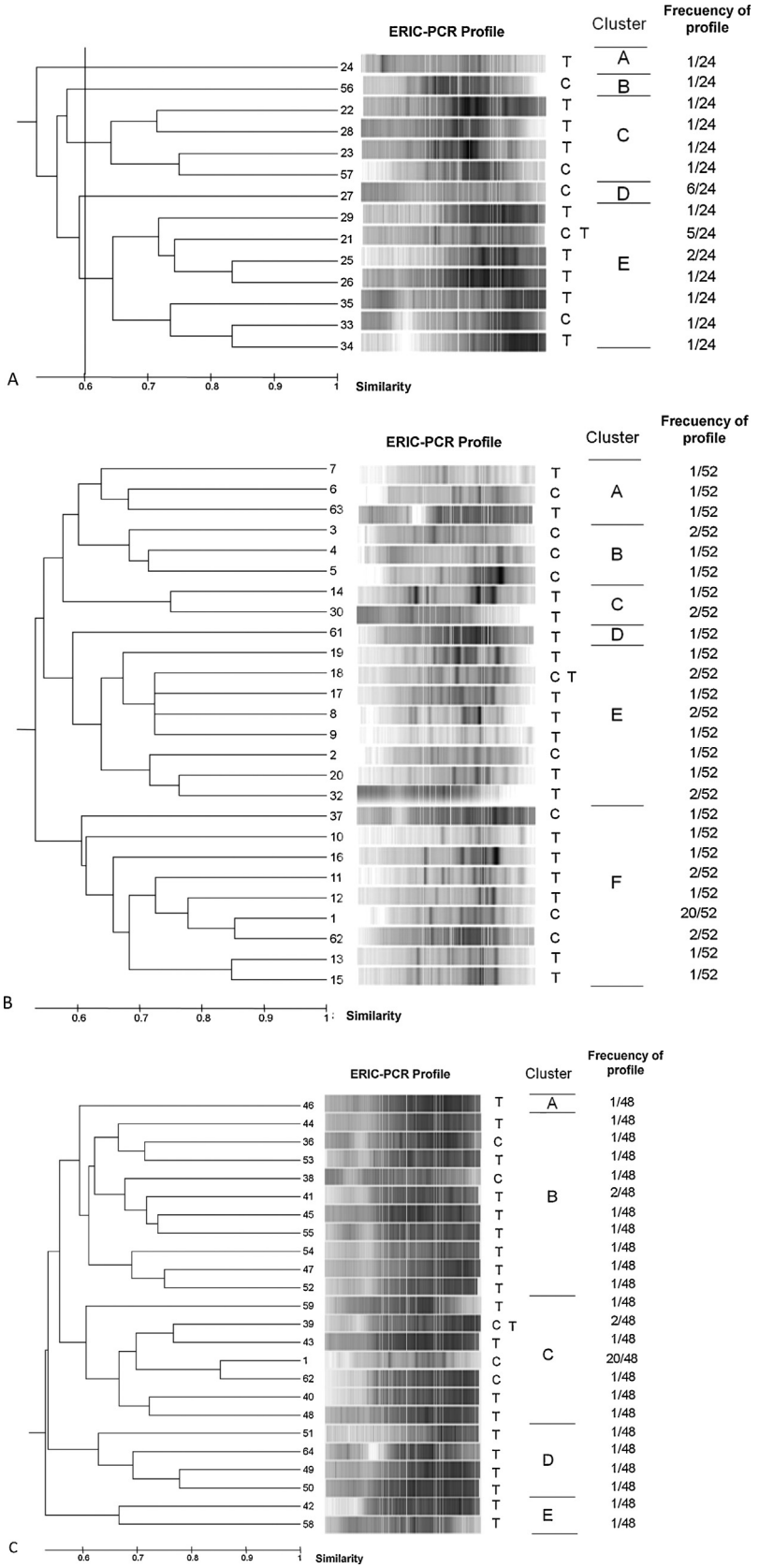

Fig. 3. UPGMA-dendrogram (dice similarity coefficient) derived from ERIC–PCR fingerprints of Gram-positive culturable phosphate solubilizing bacteria from control (C) and treated (T) soil samples from experiment on field. M1 (A), M2 (B) and (M3) samples. The ERIC–PCR profiles obtained were identified with Latin numbers and the clusters are indicated with capital letters.

JX675235) and *Enterobacter* (accession number JX675236), respectively.

In summary, in this study phosphate solubilizing bacteria able to persist over time (i.e. those showing the profile number 62 were found in control soil samples in M2 and M3) as well as to overcome the impact of the pesticides application were identified.

In order to quantitatively estimate the diversity of culturable phosphate solubilizing bacteria in the rhizospheric soil samples, the Simpson and Shannon–Wiener diversity indexes were estimated. The analysis of all phosphate solubilizing bacteria, regardless Gram staining, revealed that pesticides application increase the diversity of the phosphate solubilizing soil bacterial population (Table 2). In M3 treated soil sample a statistically higher diversity was observed with respect to control sample. In addition, in treated soil samples, diversity increased throughout time. Contrary to that, in control soil samples the diversity of this bacterial group decreased over the time, suggesting that the plant select a particular group of bacteria.

In order to estimate the effect of pesticides application on P solubilizing activity of soil samples, P soil content of treated and untreated soil samples was estimated. The results obtained indicated similar P available soil contents (μg/ml) in both control and treated soil samples in the three sampling times (Table 2). The analysis of these results together with those indicating that abundance of P solubilizing bacteria did not change when pesticides are applied, suggests that P solubilizing activity of these soils was not affected. Das and Debnath (2006) reported an increase in P soil content in rice rhizosphere treated with herbicides, and suggested that it was a consequence of an enhancement of P solubilizing activity.

3.2. Analysis of pqqE and pqqC genes in culturable phosphate solubilizing bacteria

In previous studies, presence of *pqq*C and *pqq*E genes in phosphate solubilizing bacteria belonging to phylogenetically distant genera has been reported (Anzuay et al., 2013). In order to investigate if these genes could be used as potential markers of bacteria belonging to this population, its frequency in the genome of 165 culturable phosphate solubilizing bacteria isolated from the field experiment was analyzed. A 700 bp fragment corresponding to *pqq*E gene was amplified from genomic DNA from the 25, 82 and 79% of bacteria isolated in M1, M2 and M3 soil samples, respectively. Amplification product corresponding to *pqq*C gene (~140 bp) was observed in 45, 81 and 86% of the bacteria isolated from M1, M2 and M3 soil samples, respectively. Bacterial isolates that showed both expected amplification products were all Gram-negative bacteria. The amplification products corresponding to *pqq*E and *pqq*C genes were observed in 84 and 86% of the Gram negative bacteria analysed, respectively. These results suggest that *pqq*E and *pqq*C genes could be used as potential molecular markers for the identification of Gram-negative phosphate solubilizing bacteria.

3.3. Effect of pesticides on the diversity of soil bacterial community and phosphate solubilizing bacteria population

Expected amplification product from soil DNA was obtained from M2 samples. A total of 72 clones (35 from M2 control samples and 37 from M2 treated samples) that showed the expected amplification product of *pqq*E gene were obtained from genomic soil DNA. RFLP–*pqq*E products showed 6 different profiles (A, B, C, D, E and F) using the enzyme *Cfo*I, 7 profiles (A′, B′, C′, D′, E′, F′ and G′) with the enzyme *Msp*I and 3 different profiles (A″, B″ and C″) using *Nde*II enzyme. The combination of fingerprint profiles obtained with the three enzymes used in both soil samples resulted in 16 different patterns. Five of them (2, 3, 4, 7 and 13) were observed in both control and treated soil samples. In control soil samples, 10 different patterns were obtained from the 35 clones analyzed, being 2 and 4 the predominant patterns (26 and 20%, respectively). Meanwhile, in the treated soil samples, 11 different patterns were obtained from the 37 clones analyzed and, as in the control sample, the predominant patterns were 2 and 4 (16 and 24%, respectively). In order to check whether the size of clone libraries analyzed was reflecting the real diversity, the coverage index was checked. Coverage values higher than 70% were obtained in both control and treated soil samples (Table 3). Values of Simpson and Shannon–Wiener indexes in the libraries of clones indicated an increase in *pqq*E gene diversity in treated soil samples.

Table 3
Number of RFLP-patterns, coverage and values of Simpson and Shannon–Wiener indexes of *pqq*E and 16S rDNA genes from control (C) and treated (T) samples from M2 soil samples.

	Clone library			
	*pqq*E gene		16S rDNA gene	
	C	*T*	*C*	*T*
Number of RFLP patterns	10 (35)[a]	11 (37)[a]	6 (27)[a]	8 (26)[a]
Coverage (%)	80%	73%	92%	96%
Values Simpson index	0.13	0.12	0.19	0.11
Values Shannon–Wiener index	2.75	3.10	2.29	2.59

C: control soil samples; T: treated soil samples. ND: not determinate
[a] Los valores entre paréntesis indican el número de clones analizados de cada tratamiento

The expected amplification product of 16S rDNA gene was obtained from 53 clones (27 from control samples and 26 from treated samples). Analysis of RFLP products of 16S r DNA gene showed 5 different profiles (A, B, C, D and E) using the enzyme *Cfo*I, 4 profiles (A′, B′, C′ and D′) with the enzime *Hinf*I and 3 different profiles (A″, B″ and C″) using *Rsa*I enzyme. The combination of genomic profiles obtained with the three enzymes used in both soil samples resulted in 9 different patterns. Five of these patterns (1, 3, 4, 6 and 7) were observed in both control and treated soil samples. In control soil samples, 6 different patterns were obtained from the 27 clones analyzed, being profile 5 the predominant one (18%). Meanwhile, in the treated soil samples, 8 different patterns were obtained from the 26 clones analyzed and pattern 1 was the predominant (19%). Coverage values higher than 90% were obtained in both control and treated soil samples (Table 3). Values of Simpson and Shannon-Wiener indexes in the libraries of clones indicated an increase in diversity of 16S rDNA gene in treated soil samples.

In studies of soil bacterial ecology the sequence of 16S rRNA gene is frequently analyzed (Lancaster et al., 2010; Singh et al., 2011). Other studies have analyzed diversity of *nif* genes encoding the bacterial enzyme nitrogenase (Ladha and Reddy, 2003; Mártensson et al., 2009). In this sense, in a previous study, Angelini et al. (2013) observed that pesticides application decrease the abundance and diversity of nitrogen-fixing bacteria from soil DNA extracts from peanut-growing area of Córdoba. The present study would be the first one that addresses the analysis of "representative" genes from a subpopulation of bacteria able to P solubilization. In addition, the high frequency of *pqq*E and *pqq*C genes in Gram-negative phosphate solubilizing soil bacteria encourages further studies to confirm them as potential molecular markers.

4. Conclusions

Application of pesticides commonly used in peanut production area of Córdoba produces a change in the structure of soil bacteria community and phosphate solubilizing population, increasing

their diversity. The presence of *pqq*C and *pqq*E genes in a high percentage of Gram-negative soil bacteria suggests them as potential molecular markers of this subpopulation to be used in soil bacteria ecological studies. Overall, our observations provide compelling evidence that, in soil from peanut-growing area, treatment with pesticides is enough to significantly disturb the composition of soil bacteria.

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