USE OF 16S rRNA GENE FOR CHARACTERIZATION OF PHOSPHATE-SOLUBILIZING BACTERIA ASSOCIATED WITH CORN

USO DEL GEN 16S rRNA PARA CARACTERIZACIÓN DE BACTERIAS SOLUBILIZADORAS DE FOSFATOS ASOCIADAS AL MAÍZ

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RESUMEN

Se caracterizaron fenotípica y genotípicamente 36 cepas de bacterias solubilizadoras de fosfato (BSP), aisladas de la rizosfera y rizoplano del maíz (*Zea mays* L.) en diferentes estados de México. Primeramente, se evaluó la capacidad de cada cepa para solubilizar fosfato, empleando fosfato tricálcico como fuente de fósforo en el medio de cultivo NBRIP-BPB. También, se evaluó su capacidad solubilizadora de fosfato pero adicionando al medio el amortiguador de pH MES (2-[Morfolino] ácido etanosulfónico). El análisis de patrones de restricción de ARN ribosomal amplificado (ARDRA) fue empleado para el estudio de la diversidad genética. A partir de la matriz generada, se construyó un dendrograma por el método UPGMA. El gen 16S rRNA de las cepas BUAP29, BUAP36 y CP08 fue amplificado, clonado y secuenciado para su clasificación taxonómicamente. Las 36 cepas bacterianas presentaron diversos niveles de actividad solubilizadora de fosfato tricálcico. Solamente las cepas BUAP33, BUAP17 y BUAP21 no presentaron el típico halo de solubilización cuando se adicionó al medio el amortiguador MES. El análisis de los patrones ARDRA así como el dendrograma mostraron una gran diversidad genética entre las 36 BSP analizadas, donde solamente las cepas BUAP36 y BUAP15 exhibieron 100% de similitud. El alineamiento de las secuencias del gen 16S rRNA de las cepas CP08, BUAP29 y BUAP36 presentó 99% de identidad con las secuencias de *Advenella incenata* cepa R-16599 (con número de acceso AY569458.1 en NCBI), *Burkholderia* sp (con número de acceso AY353696 en NCBI) y *Burkholderia gladioli* cepa 223-1 (con número de acceso DQ355168.1 en NCBI), respectivamente. En el presente estudio se reporta por primera vez a *A. incenata* como una BSP.

Palabras clave: ARDRA, bacterias solubilizadoras de fosfato, diversidad genética, 16S rRNA.

SUMMARY

Thirty-six strains of phosphate-solubilizing bacteria (PSB) isolated from the rhizosphere and rhizoplane of corn (*Zea mays* L.) crops in different states of México were subjected to phenotypic and genotypic characterization. The phosphate-solubilizing activity of each strain was first evaluated using tricalcium phosphate as the phosphorus source in the NBRIP-BPB culture medium. Phosphate-solubilizing capacity was also evaluated by adding the pH buffering agent MES (2-[Morpholine] ethanosulfonic acid) to the growth medium. Amplified ribosomal RNA restriction pattern analysis (ARDRA) was used to evaluate the genetic diversity. From the data matrix obtained, a dendrogram was built using the UPGMA method. The 16S rRNA gene of the BUAP29, BUAP36 and CP08 strains was amplified, cloned and sequenced for taxonomic identification. The 36 bacterial strains exhibited different levels of tricalcium phosphate solubilizing activity. Only BUAP33, BUAP17 and BUAP21 strains did not show the typical solubilization halo when the MES buffering agent was added to the growth medium. The analysis of ARDRA patterns as well as the dendrogram exhibited a large genetic diversity among the 36 PSB analyzed, with BUAP36 and BUAP15 strains showing 100 % similarity. The 16S rRNA gene sequence alignment of CP08, BUAP29 and BUAP36 strains showed 99 % identity with the sequences of *Advenella incenata* strain R-16599 (NCBI accession number AY569458.1), *Burkholderia* sp (NCBI accession number AY353696) and *Burkholderia gladioli* strain 223-1 (NCBI accession number DQ355168.1), respectively. In this study the *A. incenata* strain is reported as a PSB for the first time.

Index words: ARDRA, genetic diversity, phosphate-solubilizing bacteria, 16S rRNA.

INTRODUCTION

Phosphorus is one of the essential nutrients for plant growth and the absence of this element in the soil could limit plant development (Igual *et al*., 2001). It is well known that a large proportion of the inorganic phosphorus added to the soil as fertilizer is not available for plants because of its rapid immobilization (Mehta and Nautiyal, 2001).

The existence of soil microorganisms (bacteria, actinomycetes and some fungi) that solubilize soil-precipitated or soil-attached phosphate has been reported previously (Reyes *et al*., 2001). Most of the reported phosphate-solubilizing bacteria (PSB) belong to *Pseudomonas*, *Bacillus, Enterobacter*, *Rhizobium*, *Mesorhizobium, Burkholderia*, *Azotobacter, Azospirillum* and *Erwinia* genera, and the following are the most widely studied

Recibido: 8 de Octubre del 2007.
Aceptado: 7 de Octubre del 2008.

species: *Pseudomonas* sp. (Illmer and Shinner, 1992), *P. putida* (Kumar *et al.*, 2000), *P. aeruginosa*, *P. corrugata*, *P. stutzeri* (Vázquez *et al.*, 2000), *P. fluorescens* (Rodríguez and Fraga, 1999), *P. cepacia* (Bar-Yosef, 1996), *Bacillus firmus* (Banik and Dey, 1982), *B. amyloliquefaciens*, *B. atrophaeus* (Vázquez *et al.*, 2000), *Enterobacter agglomerans* (Laheurte and Berthenlin,1998), *E. taylorae, E. aerogenes, E. asburiae* (Vázquez *et al.*, 2000), *Rhizobium leguminosarum* (Halder *et al.*, 1990), *R. meliloti* (Halder and Chakrabartty, 1993), *Mesorhizobium mediterraneum* (Peix *et al.*, 2001), *Azospirillum halopraeferans* (Seshadri *et al.,* 2000), *Burkholderia cepacia* (Rodríguez and Fraga, 1999), *Erwinia herbicola* (Liu *et al.*, 1992), *Azotobacter chrococcum* (Kumar *et al.*, 2000) and *Pantoea agglomerans* (Son *et al.*, 2006).

The phosphate solubilizing activity of these microorganisms has been demonstrated through their growth on culture media supplemented with tricalcium phosphate, dicalcium phosphate, hydroxyapatite, phosphoric rock and other minerals containing insoluble P as sole phosphate source (Igual *et al.*, 2001). The phosphate-solubilizing activity is qualitatively assessed by the ability to form solubilization halos (light zones) around the microbial colonies (Mikánova and Nováková, 2002), when they grow on plates of distinct culture media such as Pikovskaya agar, NBRIP medium (Nautiyal, 1999) and NBRIP-BPB medium (Mehta and Nautiyal, 2001). These culture media contain bromophenol blue which is a pH change indicator due to the phosphate-solubilizing activity. The halo area is equivalent to the phosphate solubilizing activity measured by other techniques based on direct or indirect quantification of soluble phosphate, such as spectrophotometry and Olsen method (Rodriguez and Fraga, 1999). Several phosphate-solubilizing mechanisms have been described, including the production of 1) organic acids, H^+ and HCO_3^- ions, 2) polysaccharides (Goenadi *et al.*, 2000); and 3) phosphatase enzymes, mainly acid phosphatases (Rodriguez *et al.*, 2000).

The analysis of soil microbial diversity is relevant to define soil quality (Alkorta *et al.*, 2003). Polyphasic taxonomical studies, which include phenotypic, genetic and phylogenetic information, have been widely used in microbial diversity studies (Vandamme *et al.*, 1996). Regarding genetic and phylogenetic characterizations, molecular techniques such as gene sequencing have been used (Eisen, 1995). The sequence of the 16S rRNA gene has been widely used as phylogenetic marker in microbial ecology (Ludwig *et al.*, 1998), since the extent of divergence in the sequence of this gene provides an estimate of the phylogenetic distance existing between different species (Igual *et al*, 2001). Based on polyphasic taxonomical studies, new PSB species have been identified, such as *P. rhizospharae* (Peix *et al.*, 2003), *P. lutea* (Peix *et al.*, 2004) and *Microbacterium ulmi* (Rivas *et al.*, 2004). In the present work, 36 PSB strains isolated from corn (*Zea mays* L.) crops of different regions of México were phenotypically and genetically analyzed in order to know their tricalcium phosphate-solubilizing capacity, as well as the genetic diversity using the amplified DNA restriction analysis of the 16S rRNA gene (ARDRA). The strains with the greatest solubilizing capacity were classified taxonomically based on the 16S rRNA gene sequence.

MATERIALS AND METHODS

Biological material. From the 36 phosphate-solubilizing strains used, 19 belong to the collection of the Plant-Microorganism Molecular Interaction Laboratory of the Colegio de Postgraduados (labeled as CP) and 17 strains belong to the collection of the Soil Microbiology Laboratory of the Benemérita Universidad Autónoma de Puebla, (labeled as BUAP). All the strains were isolated from the rhizosphere and rhizoplane of maize plants during different seasons at different locations in México (Table 1). After isolation and purification, the strains were preserved in glycerol at -71 °C until needed.

Phosphate-solubilizing activity. Phosphate-solubilizing activity was determined on Pikovskaya (1948) culture plates. Each strain was streaked out in duplicate, and incubated at 27 °C for 72 h. The halo formation around the bacterial colony was considered as the indicator of phosphate-solubilizing activity (Seshadri *et al.*, 2000). Strains were spread on Pikovskaya plates for five consecutive times to confirm the phosphate-solubilizing activity (Igual *et al.*, 2001). Strain CP99, which lacks phosphate-solubilizing activity, was used as a negative control.

Phosphate-solubilizing efficiency. The *in vitro* phosphate-solubilizing capacity of each strain was determined on NBRIP-BPB medium containing bromphenol blue as a pH indicator (Mehta and Nautiyal, 2001). Two variants of the NBRIP-BPB medium were used: NBRIP with $Ca_3(PO_4)_2$ as sole P source (Medium 1), and NBRIP-BPB medium containing $Ca_3(PO_4)_2$ plus MES buffering agent (Medium 2). A 10 µL suspension of each strain containing 10^7 cells mL^{-1} was sown in triplicate, and incubated at 28 °C for 72 h. The solubilization efficiency (E) was determined by measuring the halo diameter (HD) and the colony diameter (CD), to obtain the relation of HD/CD x 100 (Seshadri *et al.*, 2000).

DNA isolation. Bacterial strains were cultivated in 10 mL of Luria-Bertani broth (LB) at 29 °C in agitation for18 h. One milliliter of the culture was placed in

microtubes and pelleted by centrifuging at 12 300 *x* g for 2 min. For total DNA extraction, the FastDNA kit (®Bio 101) was used following the manufacturer's instructions. The DNA extracted from each strain was visualized by electrophoresis in 1 % agarose gels using TAE 1X buffer containing ethidium bromide. Observations were carried out using the UV Eagle Eye ™ (Stratagene®) photo documentation system.

16S rRNA gene amplification. The region of the 16S ribosomal gene (rRNA) of the DNA extracted from each bacterial strain was amplified by the polymerase chain reaction (PCR). The reagent mixture was prepared with the fD1 and rD1 primers (Weisburg *et al.,* 1991), 1U of Taq polymerase enzyme and 1X reagent buffer (Promega®). The reagent mixture was incubated in a Hybaid PCR Express thermocycler (Thermohybaid, California, USA). The amplification conditions used for the PCR were as indicated by Weisburg *et al*. (1991). PCR products were observed by electrophoresis in agarose gels as described above.

Amplified ribosomal RNA pattern analyses (ARDRA). The amplified products of the 16S rRNA gene were subject to digestion with the following restriction enzymes: *DdeI, HaeIII, Hha1, HindI* and *MspI*. Five U of each restriction enzyme were used per 5 μL of amplified product and the mixture was incubated at 35 °C during 3 h. The restriction fragments obtained were subjected to electrophoresis on a 3 % agarose gel in TAE 1X buffer containing ethidium bromide and observed as described above.

Table 1. Phosphate-solubilizing efficiency of the phosphate-solubilizing bacteria.

Strain	Origin	Biochemical identification	Medium 1	Medium 2
BUAP06	Iquimita, Puebla	*Burkholderia gladioli*	166.67 ijk	141.33 st
BUAP15	San Antonio Virreyes, Puebla	*Burkholderia cepacia*	220 cd	196.67 jklmn
BUAP16	San Antonio Virreyes, Puebla	*Burkholderia cepacia*	160 jklm	158.33 opqr
BUAP17	San Antonio Virreyes, Puebla	*Burkholderia cepacia*	198.33 fgh	nt
BUAP19	Acajete, Puebla	*Burkholderia gladioli*	256.67 bc	176.67 mnopq
BUAP21	Acajete, Puebla	*Burkholderia gladioli*	213.33 ef	nt
BUAP29	Huasca, Hidalgo	*Burkholderia gladioli*	326.67 a	258.33 bcd
BUAP31	Huasca, Hidalgo	*Burkholderia cepacia*	140 lmn	120 tuv
BUAP33	Huasca, Hidalgo	*Burkholderia gladioli*	180 hij	nt.
BUAP36	Huasca, Hidalgo	*Burkholderia cepacia*	213.33 ef	266.7 hijkl
BUAP02	Tenextepec, Puebla	*Enterobacter cloacae*	330 a	300 a
BUAP22	San José Teacalco, Puebla	*Enterobacter cloacae*	280 b	280 ab
BUAP30	Ixmiquilan, Hidalgo	*Enterobacter cloacae*	260 bc	250 cde
BUAP32	Huasca, Hidalgo	*Enterobacter cloacae*	186.67 ghi	176.67 mnopq
BUAP37	Mixquiahuala, Puebla	*Enterobacter cloacae*	253.33 c	216.67 ghij
BUAP45	Atotonilco, Tlaxcala	*Enterobacter cloacae*	250 c	208.33 hijk
BUAP46	Progreso, Hidalgo	*Enterobacter cloacae*	240 cd	240 defg
CP01	Pabellón de Arteaga, Aguascalientes	*Pseudomonas fluorescens*	260 bc	248.33 def
CP02	Pabellón de Arteaga, Aguascalientes	*Pseudomonas fluorescens*	200 fgh	185 klmn
CP13	Pabellón de Arteaga, Aguascalientes	*Pseudomonas fluorescens*	260 bc	243.3 def
CP07	Villadiego, Guanajuato	*Pseudomonas fluorescens*	180 hij	176.6 mnopq
CP04	Pabellón de Arteaga, Aguascalientes	*Pasteurella multocida*	150 klmn	141.67 rstu
CP16	Villadiego, Guanajuato	*Panteoa agglomer*	14l mn	110 uv
CP24	Villadiego, Guanajuato	*Pantoea agglomer*	130 n	110 v
CP15	Villadiego, Guanajuato	*Panteoa agglomer*	126.67 n	118.33 tuv
CP34	Pabellón de Arteaga, Aguascalientes	No identified	245 c	228 ef
CP25	Santa Isabel de Ajuno, Michoacán	No identified	132 n	115 v
CP5	Villadiego, Guanajuato	No identified	260 bc	225 efgh
CP19	Pabellón de Arteaga, Aguascalientes	No identified	180 hij	158.33 opqr
CP10	Pabellón de Arteaga, Aguascalientes	No identified	170 ijk	146.67 rs
CP11	Villadiego, Guanajuato	No identified	136.67 mn	110 v
CP12	Villadiego, Guanajuato	No identified	160 jklm	128.33 stuv
CP14	Villadiego, Guanajuato	No identified	250 c	228.33 efgh
CP17	Santa Isabel de Ajuno, Michoacán	No identified	250 c	220 fghij
CP18	Santa Isabel de Ajuno, Michoacán	No identified	180 hij	175 nopq
CP08	Villadiego, Guanajuato	No identified	310 a	270 bc

Mean values of each column with the same letter are not statistically different (Tukey, 0.05). Growth medium 1 = NBRIP-BPB with $Ca_3(PO_4)_2$; Growth medium 2= NBRIP-BPB with $Ca_3(PO_4)_2$ plus MES buffering agent. Identified by Apilab® plus API 20N and/or 20NE; nt = Not tested.

Dendrogram construction. The band patterns obtained (genomic profile) were used to generate a data matrix using the Jaccard similarity coefficient. A dendrogram was then constructed through the unweighted pair group method with arithmetic averages (UPGMA) (Sneath and Sokal, 1973), using the NTSYS-pc V1.8 program (Rohlf, 1993) to determine the genetic diversity of PSB groups.

16S rRNA gene partial sequencing. Sequencing was only carried out for the BUAP29, BUAP36 and CP08 strains. A TA Cloning® Kit (Roche®) and the pCR®2.1 ligation vector were used to clone PCR products of approximately 1.5 kb of the 16S rDNA gene of each of the three strains. Sequencing was carried out using the universal and reverse m13 pUC oligonucleotides by automated DNA fluorescent sequencing. The sequence was obtained from the Synthesis Unit of the Biotechnology Institute of the Universidad National Autónoma de México (UNAM).

16S rRNA gene partial sequence analysis. The partial sequences of the 16S rRNA genes of the BUAP29, BUAP36 and CP08 strains were compared to sequences reported in the NCBI (National Center for Biotechnology Information) databases using the nucleotide-nucleotide BLASTn program (Altschul *et al*., 1997).

RESULTS AND DISCUSSION

Phenotypic characterization of phosphate-solubilizing bacterial strains

Solubilization efficiency. All the strains maintained their phosphate-solubilizing activity after being re-sown five consecutive times in Pikovskaya medium (Igual *et al*., 2001). Table 1 shows the strain solubilization efficiency assessed with different P sources.

All bacteria analyzed presented phosphate-solubilizing activity although to different levels on the NBRIP-BPB medium carrying $Ca_3(PO_4)_2$ (Medium 1) as sole P source, suggesting that these strains may be used to generate soluble P in soils containing tricalcium phosphates in their minerals (Goenadi *et al*., 2000).

It has been demonstrated that there are at least two P solubilization mechanisms: production of organic acids, such as the citric, malic, oxalic, glutamic acids (Dirk and Lesas, 2000), lactic, succinic, isovaleric, isobutyric, acetic acid (Rodríguez and Fraga, 1999), and gluconic acid (Babu-Khan *et al*., 1995), among others, and the production of phosphatase enzymes (Yang *et al*., 1997), especially acid phosphatase (Rodríguez *et al*., 2000).

Most strains showed a lower phosphate-solubilizing efficiency when the medium was supplemented with MES buffering agent. The BUAP33, BUAP17 and BUAP21 strains did not show the typical solubilization halo in the culture medium supplemented with the MES buffering agent. The MES buffering agent prevents pH modification of the medium surrounding the bacterial colony; therefore, the phosphate-solubilizing activity could be due to a mechanism different to organic acid production (Mehta and Nautiyal, 2001). Gyaneshwar *et al*. (1998) isolated from alkaline vertisol two PSB strains using conventional screening media could not release phosphorus from alkaline Indian vertisol soils supplemented with carbon and nitrogen sources. They found that the two PSBs could solubilize both rock phosphate and di-calcium phosphate inunbuffered media but failed to solubilize rock phosphate in buffered media. The organic acids secreted by these PSBs were 20-50 times less than that required to solubilize phosphorus from alkaline soil.

Thakuria *et al*. (2004) have demonstrated that there is no statistically significant relation between the pH increase in the Pikovskaya broth and its soluble P content, which indicates that the solubilizing activity may occur of because of factors other than the production of organic acids. In another study, Johri *et al*. (1999) reported an increase in the solubilizing activity by the NBR14 and NBR17 strains cultured in NBRIP medium when NaCl was replaced by $CaCl_2$ or KCl and incubated at 30 °C. Also they found an increase in the P solubilizing activity when bacterial strains where incubated at 37 °C rather than at 30 °C. These data clearly indicate the influence of the salt source and the incubation temperature on the phosphate-solubilizing activity on the NBRIP medium. Several studies have shown that the composition of the medium affects the phosphate-solubilizing activity (Gibson and Mitchell, 2004) as well as the type of soil (Nautiyal *et al*., 2000), and the location from where the bacteria were isolated (Coenye and Vyame, 2003).

The BUAP29, BUAP36 and CP08 strains showed high P solubilizing activity in both media (Table 1), and were therefore selected for genetic taxonomic characterization up to the species level by 16S rRNA gene sequencing.

Genetic diversity of PSB strains

The restriction patterns of the amplified fragments of the 16S rRNA gene of the 34 strains were different from each other (Figure 1), except BUAP36 and BUAP15 which were identical. Digestion carried out using the enzyme *DdeI*, showed 14 different patterns, while the enzymes HhaI and *Himf* exhibited 15; the enzymes *HaeIII* 10 and *MspI* gave nine patterns. The presence of different

patterns when using the same restriction enzymes indicates that there are differences within the sequence of the 16S rRNA gene and therefore, there exists a large genetic diversity in the studied population. Analysis of variation of the ribosomal 16S gene allows the inference of the phylogenetic relationships among taxonomically relatively close and distant organisms (Eisen, 1995; Ludwig *et al.*, 1998). The dendrogram (Figure 2) indicates the existence of a great genetic diversity of PSB. There were no bacterial isolates with the same genetic pattern, except for BUAP36 and BUAP15 strains, which showed the same pattern in the profiles obtained with the five restriction enzymes and had 100 % similarity, thus suggesting that these two strains may belong to the same taxon. Amos and Harwoud (1998) mention that there could be many factors related to the genetic diversity in a population, for instance, the site of origin of the strains as well as the associated crop. It seems that such factors may have influenced the PSB population in the present study.

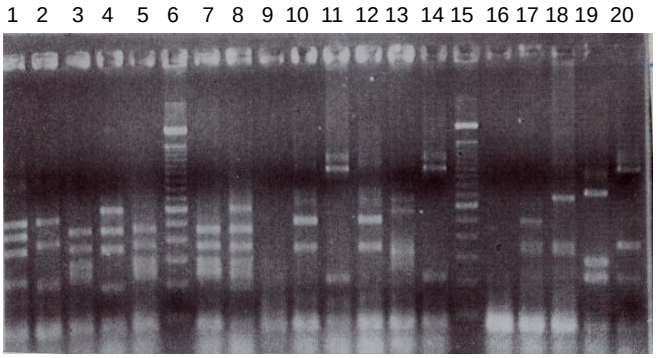

Figure 1. Electrophoretic profile of the 16S rRNA gene restriction analysis (ARDRA) obtained from the restriction of the amplified gene16S rRNA with Dde enzyme. Strains analyzed per lane: 1, CP14; 2 CP 17;3 BUAP 01; 4, CP08; 5, BUAP 32; 6, 1kb plus Marker; 7, CP12; 8 CP10; 9 BUAP 07; 10, CP15; 11, BUAP 19; 12, BUAP 21; 13, BUAP 16; 14, CP06; 15 1Kb Marker; 16, BUA9 31; 17, CP 17; 18, CP18; 19, BUAP 21; 20, CP 21.

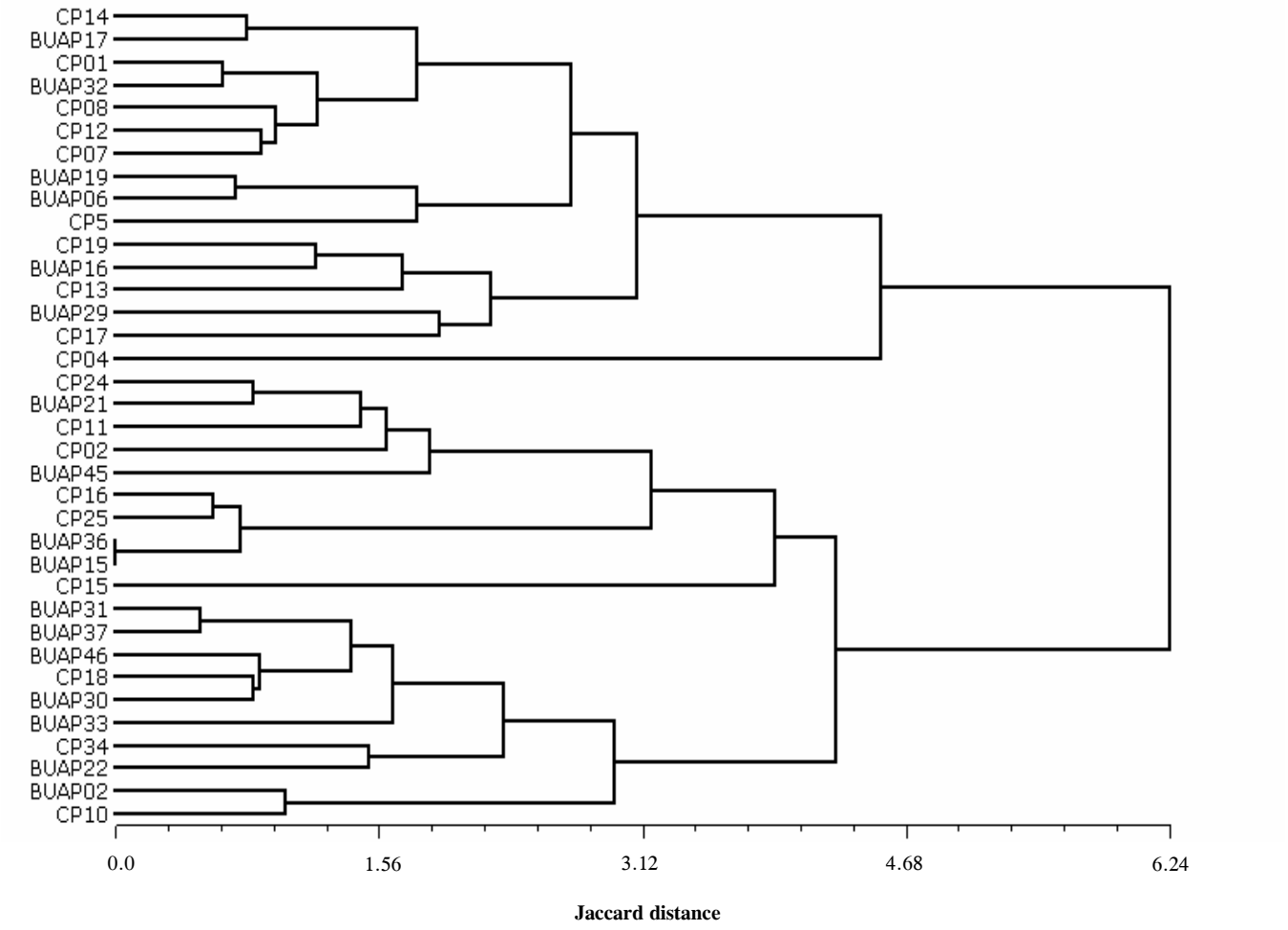

Figure 2. Dendrogram of phosphate solubilizing bacteria strains, based on the partially PCR-amplified 16S rRNA gene restriction patterns analysis.

Taxonomic classification. The sequences of the 16S rRNA gene of the BUAP29 and BUAP36 strains showed 99 % identity with the sequence of the 16S rRNA gene reported in the Genbank for *Burkholderia sp* (accession number AY353696 in NCBI), *Burkholderia gladioli* strain 223 gr-1 (NCBI: DQ355168.1) and the CP08 strain showed 99 % identity to *Advenella incenata* strain R-1659 (NCBI: AY569458.1). These results differ from the classification obtained with biochemical testing using the API 20NE and API 20N system; BUAP 29 was identified as *Burkholderia gladioli* and BUAP 36 as *Burkholderia cepacia*. The CP08 strain has not been identified based on biochemical testing with API 20NE, and therefore may be classified as *Advenella incenata*, a poorly studied species. Coenye *et al*. (2005) suggested it as a new species, belonging to the family of the *Alcaligenacea*, subclass-β of *Proteobacteria*. These results suggest the need to carry out cloning and sequencing of the 16S rRDN gene of the remaining strains in order to achieve a correct taxonomic classification (Pace, 1997), and emphasize the importance of carrying out polyphasic taxonomy studies. Igual *et al*. (2001) mentioned some useful molecular techniques for isolation and efficient identification of PSBs (16S rRNA sequencing, LMW RNA profiles, TP-RADP and rep-PCR fingerprinting), and Peace (1996) described a method to obtain strain-specific DNA probes.

In the present study *Advenella incenata* is reported for the first time as a phosphate-solubilizing microorganism due to its capacity to solubilize tricalcium phosphate in NBRIP-BPB culture medium.

CONCLUSIONS

Thirty six strains analyzed solubilized tricalcium phosphate when used as the sole P source in the NBRIP-BPB culture medium. Most of the strains showed a decrease in their phosphate-solubilizing efficiency when MES buffering agent was added to the NBRIP-BP medium.

BUAP33, BUAP17 and BUAP21 strains did not show the typical solubilization halo in the culture medium supplemented with the MES buffering agent, because the buffering agent prevented the pH modification of the medium surrounding the bacterial colony. Therefore, the phosphate-solubilizing efficiency could be due to a different mechanism for organic acid production.

The ARDRA analysis indicates the existence of a large genetic diversity in the studied population, and the dendrogram only grouped the BUAP36 and BUAP 15 strains with 100 % similarity. No correlation was found between ARDRA patterns and biochemical taxonomic classification.

The analysis of the 16S rDNA gene partial sequences at Genbank showed 99 % similarity, classifying BUAP29 strain as *Burkholderia sp*, BUAP36 strain as *Burkholderia gladioli*, and CP08 strain as *Advenella incenata*.

This study reports for the first time the species *Advenella incenata* as phosphate-solubilizing bacteria.

ACKNOWLEDGEMENTS

To M.Sc. Moisés Carcaño for the donation of BUAP strains, Q.F.B. Rosalba Palma Sánchez and Dr. Teresa Terrazas for their support in the construction of the dendrograma, as well as to Dr. Jesús Caballero Mellado and Lourdes Martinez Aguilar for their support in the ARDRA assay.

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