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Bacterial diversity with plant growth-promoting potential isolated from *Agave americana* L., rhizosphere

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ABSTRACT

Objective: Study the diversity of cultivable rhizospheric bacteria associated to *Agave americana* L. and select native strains with potential as plant growth-promoting bacteria (PGPB).

Design/methodology/approach: The isolated bacteria were phenotypically characterized. The genetic diversity and identity of the strains were revealed by genomic fingerprints and by sequencing of 16S rRNA gene. Plant growth promoting ability and plant inoculation assays were evaluated to know the potential as PGPB.

Results: A total of 235 strains were isolated from *A. americana* rhizosphere and were classified within of 10 different bacterial genera. *Rhizobium*, *Pseudomonas*, *Acinetobacter* had high potential as PGPB.

Study limitations/implications: Cultivable approach was used to study rhizobacteria. A metagenomic study could expand the knowledge about the structure and diversity of bacterial community associated to *A. americana*.

Findings/conclusions: Rhizosphere bacteria have potential use as biofertilizer for the cultivation and propagation of *A. americana* and other agave species.

Keywords: Agave, plant growth promoters, biofertilizer.

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INTRODUCTION

The soil firmly adhering to the roots, designated as rhizosphere soil is constituted by groups of microorganisms including bacteria, fungi, nematodes, protozoa, algae and micro-arthropods (Prashar *et al.*, 2014). The interactions between the roots of plants and soil microorganisms are essential for the function and stability of ecosystems, mainly for the growth and development of plants (Bhattacharyya *et al.*, 2016). Bacteria colonize the root, the rhizosphere or both, and promote the growth of plants and increase the absorption and availability of soil nutrients. Research related to microbial symbiosis is growing significantly, mainly with focus on the structure, function, and health of plants (Chandra *et al.*, 2018). Plant Growth Promoting Bacteria (PGPB) have the capability to improve growth through different direct and indirect mechanisms, such as phosphate solubilization, siderophore production, biological nitrogen fixation, phytohormone

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production, antifungal activity, induction of systemic resistance, promotion of beneficial plant-microbe symbioses, among other functions. Different bacterial species grouped within the genera *Azospirillum*, *Bacillus*, *Enterobacter* and *Pseudomonas* have been used as PGPB. On the other hand, Mexico is the site of origin, evolution, and diversification of Agave genus (Figueredo *et al.*, 2015). Approximately 163 species are found in Mexico, out of which 123 are endemic. *Agave americana* L. is a species from the Chiapas (Mexico) highlands. It has an economic, social, and cultural importance, as it is a source of natural fiber, medicine, and fructans. Notably it is used to produce traditional alcoholic beverages. Local farmers have established plantations of *A. americana* to obtain sufficient raw materials for agroindustrial use. However, the water and soil nutrient scarcity, and some diseases caused by fungi, primarily *Fusarium* species (Ramírez-Ramírez *et al.*, 2017), limit plant growth, consequently, plants reach maturity only after 5 to 7 years instead of instead of a few years. An alternative for obtaining mature plants for industrial use is the application of plant growth-promoting bacteria, but it is necessary to assess the possible effects of PGPB on *A. americana* to increase the survival and growth of plantlets. Thus, the objective of this study was to determine the phenotypic and genotypic characteristics of native bacterial strains isolated from *A. americana* rhizosphere, and to evaluate their potential as PGPB.

MATERIALS AND METHODS

Experimental site and rhizosphere soil sampling

The samples were obtained from suckers (young plants) of *A. americana* that grow in experimental plots "Tulaito", located at 16º 16´ N and 92º 10´ W, with an altitude of 1832 masl, in the municipality of Comitán de Domínguez, Chiapas (Mexico). The plants with rhizosphere were obtained from five sampling points into the plot. The soil attached to the roots was obtained carefully as indicated by Schafer *et al.* (2014).

Phenotypic characterization of strains

The cell morphologies of the strains were examined by light microscopy. The Gram reaction it was done using a kit (Merck®), according to the manufacturer's procedure, and colony morphology was determined. Bacteriological characterizations of the isolates were performed in distinct nutrient solid medium. The salinity tolerance, ability to grow at different pH levels and the capacity to produce exopolysaccharides (EPS) was determined with the methodology described by Paulo *et al.* (2012).

Genotypic characterization of strains

The isolates were grown in 2.0 ml of nutrient medium overnight. Genomic DNA was extracted using a DNA Isolation Kit (Roche®) and then ERIC genomic fingerprints were analyzed (Versalovic *et al.*, 1994). The richness (d) and diversity (H) index (Shannon-Weaver index) were calculated based on ERIC genetic profiles of the strains. PCR was performed with the universal 16S rRNA primers for bacteria 27-F and 1492-R. The amplification products were digested with a restriction endonuclease Alu I (Thermo Scientific®). The amplified ribosomal rDNA restriction analysis (ARDRA) profiles obtained were used to calculate the diversity of the bacterial species (Rincón-Rosales

et al., 2013). PCR products were purified and sequenced. The taxonomically related sequences obtained from the National Center for Biotechnology Information (NCBI) were aligned by the CLUSTAL X (2.0) software with default settings. Phylogenetic analysis was performed with MEGA v5.2 (Tamura *et al.*, 2011) and phylogenetic tree was constructed by Neighbour-Joining. The 16S rRNA gene sequences of strains were deposited in the GenBank database (Table 1).

Measurement of PGPB efficiency

Indole acetic acid (IAA) production. The isolates were streaked on LB agar amended with 5 mmol L^{-1} L-tryptophan. When the bacterial strain grew, it was covered with Whatman filter paper and incubated at 28 °C for 72 h. The paper was removed and treated with Salkowski's reagent. The production of IAA-like substances was identified by the formation of a red halo on the paper surrounding the colony.

Inorganic phosphate solubilization. Isolates were individually grown in YM broth medium and then inoculated in NBRIP medium containing insoluble tricalcium phosphate, pH was adjusted to 7.0. Phosphate solubilizing bacteria were recognized by clear halos after 5 days of incubation at 30 °C and then the phosphate solubilization index (PSI) was calculated.

Acetylene reduction assay (ARA). Acetylene reduction assay was performed with a single colony of bacteria grown in N-free minimal semisolid medium. Cultures were incubated for 72 h at 28 °C, and then with acetylene at final concentrations of 1% and 10%. The ARA was measured with a Varian 3300 gas chromatograph with a flame ionization detector.

ACC deaminase. The isolates was inoculated in culture medium containing: 0.25 g K_2HPO_4; 0.05 g $MgSO_4.7H_2O$; 0.025 g $FeSO_4.7H_2O$; 0.25 g $CaCO_3$; 0.05 g NaCl; 0.0012 g $NaMoO_4.2H_2O$; 2.5 g glucose; 3.75 g agar; 240 ml distilled water, and 0.03% of ACC as the sole source of nitrogen. The strains were incubated at 30 °C for 4 days. Colonies were subcultured in fresh medium containing ACC and incubated under the same conditions to confirm growth. Development of bacterial colonies indicates production of ACC deaminase.

Production of siderophore. Bacterial isolates were grown in CAS-agar medium [chromeazurol-S (CAS), iron (III) and hexadecyl trimethyl ammonium bromide (HDTMA)] at 28-30 °C for 5 days until there was a color change from blue to a fluorescent orange surrounding the bacterial colonies indicating the production of siderophore (Amaresan *et al.*, 2013).

Plant inoculation assay

Representative strain of each of the bacterial genus identified in this study were selected for the inoculation test. *A. americana* plantlets obtained by micropropagation were used as test plants. After 60 days after transplantation, the plants were transferred to pots containing peat moistened with free N Fahraeus medium as a nutrient source. The plants were inoculated with 2 mL of a suspension of each strain at a concentration of 10^6 UFC mL^{-1}. Uninoculated plants and others treated with 30 mg of KNO_3-N per plant served

as controls. Four replicates were used per treatment, and the plants were arranged in a completely randomized design. The agave plants were grown under greenhouse conditions for 90 days. The variables studied were the dry weight, diameter of the stem, number of leaves, and the length of the roots. Measurements were made on the plants during the transplantation phase and after 90 days. Data were analyzed by variance analysis and means compared by Tukey test with the Statgraphic software.

RESULTS AND DISCUSSION

A total of 235 bacterial strains were isolated from the *A. americana* rhizosphere. Bacteria isolated were generally Gram negative, aerobic, small bacilli, cocci and coccobacilli. Bacterial cells formed colonies with various sizes and colors. Approximately 85% of isolates had capacity to form pigments and abundant exopolysaccharides (EPS). The ACO-13A, ACO-17B, ACO-34A and ACO-31B strains were characterized by their ability to produce abundant exopolysaccharides (EPS). The EPS forms a protective layer for the bacteria, which allows tolerance to abiotic stress and contributes to the colonization of root surface (Sandhya & Ali, 2015). Most of the isolates have capacity to grow in the range from pH 5.0 to 9.0. For tolerance to NaCl, rhizospheric strains have to ability to grow in the range from 0.5 to 3.0%, except Rhizobium strains ACO-5A, ACO-143A and ACO-34A. This result is important due to salinity because it is one of the most severe soil problems affecting the crop yield. The strains were grouped by ERIC-PCR into 25 genomic fingerprints. This technique is widely used to discriminate at the level of strains (Versalovic *et al.*, 1994). According to the Shannon-Weaver index, a high diversity (H=3.01) and abundance (d=4.79) of strains associated with this agave species were determined. The phylogenetic analysis of the 16S rDNA gene sequences showed that the bacterial community isolated from *A. americana* plant included three major phylogenetic groups (α, β and γproteobacteria) and that the rhizospheric strains belonged to genera *Achromobacter*, *Acinetobacter*, *Comamonas*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Pseudochrobactrum*, *Novosphingobium*, *Rhizobium* and *Stenotrophomonas* (Table 1). On the other hand, 19 different ARDRA genomic profiles were obtained. The Shannon-Weaver index showed a higher diversity (H=2.82) and abundance (d=4.82) of bacterial species isolated from the rhizospheric soil of *A. americana*. The study of genomic fingerprints, as well as phylogenetic analysis allowed to determine that there is a wide diversity and abundance of bacterial species associated with this agave. Several of these bacteria could be considered as plant growth-promoting bacteria (PGPB).

The potential of rhizospheric isolates as PGPB was evaluated base on the multifunctional biochemical features. All bacterial strains with the exception of isolates *Achromobacter marplatensis* ACO-4A and *Novosphingobium resinovorum* ACO-14A and *Stenotrophomonas maltophilia* ACO-31B synthesized indole acetic acid (IAA). For phosphate solubilization, most of the strains showed clear zones (solubilization halos) around the colonies and the phosphate solubilization index ranged from 2.15 to 3.44 (Table 2). Only six strains (ACO-13A, ACO-17-B, ACO-54, ACO-5A, and ACO-143 had nitrogenase activity (ARA). The *Rhizobium daejeonense* ACO-34A strain was the one that registered the highest ARA activity (712 nmol C_2H_4 per culture h^{-1}). The ACC deaminase activity was present in six isolates,

Table 1. Phylogenetic affiliation of bacterial strains isolated from the *Agave americana* rhizosphere.

Representative strain (T)	Closest NCBI match / Similarity (%)	Accession number
ACO-4A^{T}	*Achromobacter marplatensis* B2^{T} / 99	MH393463
ACO-8	*Achromobacter marplatensis* B2^{T} / 99	MH393466
ACO-216^{T}	*Achromobacter xylosoxidans* DSM 10346^{T} / 86	MH454099
ACO-13A^{T}	*Acinetobacter johnsonii* ATCC 17909^{T} / 99	MH393467
ACO-41	*Acinetobacter pittii* LMG 1035^{T} / 98	MH393476
ACO-67^{T}	*Acinetobacter pittii* LMG 1035^{T} / 98	MH393480
ACO-109	*Acinetobacter johnsonii* ATCC 17909^{T} / 99	MH393485
ACO-145	*Acinetobacter pittii* LMG 1035^{T} / 98	MH393489
ACO-183	*Acinetobacter johnsonii* ATCC 17909^{T} / 99	MH454098
ACO-107^{T}	*Comamonas thiooxydans* S23^{T} / 97	MH393484
ACO-98^{T}	*Enterobacter asburiae* JCM6051^{T} / 98	MH393481
ACO-99^{T}	*Klebsiella michiganensis* LH-2^{T} / 98	MH393482
ACO-17B^{T}	*Pseudomonas taiwanensis* BCRC 17751^{T} / 97	MH393470
ACO-21A	*Pseudomonas taiwanensis* BCRC 17751^{T} / 97	MH393472
ACO-21B	*Pseudomonas taiwanensis* BCRC 17751^{T} / 97	MH393473
ACO-53^{T}	*Pseudomonas fuscovaginae* ICMP 5940^{T} / 96	MH393477
ACO-54^{T}	*Pseudomonas soli* F-279208^{T} / 99	MH393478
ACO-106	*Pseudomonas taiwanensis* BCRC 17751^{T} / 97	MH393483
ACO-116^{T}	*Pseudomonas hibiscicola* JQZST-1 / 97	MH393486
ACO-138^{T}	*Pseudomonas stutzeri* ATCC 17588 / 92	MH393487
ACO-210	*Pseudomonas taiwanensis* BCRC 17751^{T}/ 97	MH393491
ACO-141	*Pseudomonas soli* F-279208^{T} / 99	MH454097
ACO-42B^{T}	*Pseudochrobactrum saccharolyticum* CCG46 / 99	MH454100
ACO-14A^{T}	*Novosphingobium resinovorum* NCIMB 8767^{T} / 97	MH393468
ACO-14B	*Novosphingobium resinovorum* NCIMB 8767^{T}/ 97	MH393469
ACO-5A^{T}	*Rhizobium nepotum* 39/7 /97	MH393464
ACO-6	*Rhizobium pusense* NRCPB10^{T} / 96	MH393465
ACO-19	*Rhizobium pusense* NRCPB10^{T} / 98	MH393471
ACO-26	*Rhizobium pusense* NRCPB10^{T} / 98	MH393474
ACO-27B^{T}	*Rhizobium pusense* NRCPB10^{T} / 96	MH393475
ACO-62	*Rhizobium pusense* NRCPB10^{T} / 97	MH393479
ACO-143^{T}	*Rhizobium radiobacter* IAM12040^{T} /98	MH393488
ACO-34A^{T}	*Rhizobium daejeonense* L61^{T} / 96	KM349967
ACO-157	*Stenotrophomonas maltophilia* IAM12423^{T} / 98	MH393490
ACO-31B^{T}	*Stenotrophomonas maltophilia* ATCC 19861^{T} / 94	MH454096

except in ACO-4A, ACO-67, ACO-14A and ACO-31B. Recently, endophytic bacteria belonging to the genera *Acinetobacter*, *Bacillus* and *Pseudomonas* with a capacity for nitrogen fixation, auxin production and phosphate solubilization were isolated from blue agave plants (*Agave tequilana*) from Nayarit, Mexico (Martínez-Rodríguez *et al.*, 2014).

Table 2. Plant growth promotion activities in bacterial strains isolated from the *Agave americana* rhizosphere.

Bacterial strain	IAA production	P solubilization Index	ARA¥	ACC deaminase	Siderophore
Achromobacter marplatensis ACO-4A^T	−	2.15 ± (0.25) *	0.0	−	−
Acinetobacter johnsonii ACO-13A^T	+	3.12 ± (0.15)	253 ± (1.3)	+	+
Acinetobacter pittii ACO-67^T	+	2.95 ± (0.22)	0.0	−	+
Pseudomonas taiwanensis ACO-17B^T	+	3.44 ± (0.11)	358 ± (1.4)	+	+
Pseudomonas soli ACO-54^T	+	3.24 ± (0.21)	275 ± (1.2)	+	+
Novosphingobium resinovorum ACO-14A^T	−	2.73 ± (0.10)	0.0	−	+
Rhizobium nepotum ACO-5A^T	+	3.12 ± (0.15)	328 ± (0. 8)	+	+
Rhizobium radiobacter ACO-143^T	+	2.94 ± (0.26)	476 ± (0.9)	+	+
Rhizobium daejeonense ACO-34A^T	+	3.36 ± (0.17)	712 ± (1.1)	+	+
Stenotrophomonas maltophilia ACO-31B^T	−	2.08 ± (0.24)	0.0	−	+

+: positive activity; − : negative activity.
* Mean values of three replicates. The values in parenthesis are standard deviation.
¥ ARA = acetylene reduction assay (nmol C_2H_4 per culture h^{-1}).

In the case of siderophores, only nine strains had the capacity to produce this metabolite. Siderophores contribute to the protection of the bacteria against rhizospheric pathogens that compete for iron ions (Sandhya & Ali, 2015). Inoculation of plants with PGPB enhances the assimilation of essential nutrients and plant-associated biological nitrogen fixation (Calvo *et al.*, 2014). These results are important due that the nitrogen and phosphorus are key elements for the growth and metabolism of agave plants. With respect to inoculation of selected isolates, we observe a positive effect on the growth of *A. americana* plants (Table 3). Strain *Rhizobium daejeonense* ACO-34A had the higher positive effect on the plant dry weight (4.01 g) and on the stem diameter (2.99 cm) compared to non-inoculated control plants and to those with added KNO_3. Plants inoculated with the strain *Pseudomonas soli* ACO-54 or *Rhizobium nepotum* ACO-5A showed significantly ($P<0.05$) higher number of leaves. The inoculated plants with the strain *Rhizobium nepotum* ACO-5A and *R. daejeonense* ACO-34A recorded a greater length of the main root. Similar results concerning the occurrence and diversity of diazotrophic bacteria in rhizosphere soil and in root and leaf tissues of *Agave sisalana* plants have been reported by Santos *et al.* (2014) as well as a test of their potential for plant growth promotion. Therefore, PGPB strains investigated in this study could be alternative *A. americana* inoculants that would improve its growth and development.

CONCLUSIONS

We isolated and characterized rhizospheric bacteria associated with *A. americana*. The strains were characterized by their ability to produce auxins (IAA), solubilize phosphate, synthesize siderophores, ACC deaminase and nitrogenase and showed a positive effect on the growth of plants. The diversity of bacteria associated to this agave had multifunctional qualities as PGPB that may contribute to their adaptation, healthy proliferation and improve its growth in soils of low fertility.

Table 3. Plant growth promotion activities in bacterial strains isolated from the *Agave americana* rhizosphere.

Bacterial strain	Plant dry weight (g)	Stem diameter (cm)	Number of leaves	Root length (cm)
Achromobacter marplatensis ACO-4A^T	2.50 cde [y]	1.66 de	3.0 cd	13.32 d
Acinetobacter johnsonii ACO-13A^T	3.02 abcd	1.80 cde	3.75 abc	17.15 bcd
Acinetobacter pittii ACO-67^T	2.38 de	1.51 e	3.0 cd	15.77 cd
Pseudomonas taiwanensis ACO-17B^T	3.41 abc	2.28 bc	4.5 ab	22.07 ab
Pseudomonas soli ACO-54^T	3.55 ab	2.26 bc	4.75 a	20.87 abc
Novosphingobium resinovorum ACO-14A^T	2.11 de	1.36 e	2.75 cd	14.52 d
Rhizobium nepotum ACO-5A^T	3.91 a	2.21 bcd	4.75 a	25.82 a
Rhizobium radiobacter ACO-143^T	3.50 abc	2.8 ab	3.75 abc	22.77 ab
Rhizobium daejeonense ACO-34A^T	4.01 a	2.99 a	4.0 abc	24.25 a
Stenotrophomonas maltophilia ACO-31B^T	1.98 e	1.37 e	2.25 d	13.2 d
KNO_3-N	2.72 bcde	2.18 cd	3.25 bcd	17.4 bcd
Uninoculated	1.79 e	1.57 e	2.25 d	12.27 d
HSD ($P<0.05$)	1.0105	0.5865	1.3643	6.1795

[y]Mean values of four replicates. Means followed by the same letter are non-significant (Tukey test, $P<0.05$).
HSD: Honest Significant Difference.

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REFERENCES

Amaresan, N., Kumar, K., Sureshbabu, K., Madhuri, K. (2013). Plant growth promoting potential of bacteria isolated from active volcano sites of Barren Island. India. *Lett. Appl. Microbiol.* 58 (2), 130–137. Doi: 10.1111/lam.12165

Bhattacharyya, P. N., Goswami, M. P., Bhattacharyya, L. H. (2016). Perspective of beneficial microbes in agriculture under changing climatic scenario: a review. *Journal of Phytology.* 8(2), 26-41. Doi: 10.19071/jp.2016.v8.3022

Calvo, P., Nelson, L., Kloepper, J. W. (2014). Agricultural uses of plant biostimulants. *Plant Soil.* 383, 3–41. Doi: 10.1007/s11104-014-2131-8

Chandra, D., Barh, A., Sharma, I. P. (2018). Plant growth promoting bacteria: a gateway to sustainable agriculture. Microbial Biotechnology in Environmental Monitoring and Cleanup. IGI Global. pp. 318–338. DOI: 10.4018 / 978-1-5225-3126-5.ch020

Figueredo, C. J., Casas, A., González-Rodríguez, A., Nassar, J. M., Colunga-GarcíaMarín, P., Rocha-Ramírez, V. (2015). Genetic structure of coexisting wild and managed agave populations: implications for the evolution of plants under domestication. *AoB Plants.* 7 (14),114. Doi: 10.1093/aobpla/plv114

Paulo, E. M., Vasconcelos, M. P., Oliveira, I. S., Affe, H. M. J., Nascimento, R., Melo, I. S., Roque, M. R. A., Assis, S. A. (2012). An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with rapid confirmation. *Food Sci. Technol. Int.* 32(4), 710–714. Doi: 10.1590/S0101-20612012005000094

Martínez-Rodríguez, J. C., De la Mora-Amutio, M., Plascencia-Correa, L. A., Audelo-Regalado, E., Guardado, F. R., Hernández-Sánchez, E., Peña-Ramírez, Y. J., Escalante, A., Beltrán-García, M. J., Ogura, T. (2014). Cultivable endophytic bacteria from leaf bases of *Agave tequilana* and their role as plant growth promoters. *Braz. J. Microbiol.* 45(4). 1333-1339. Doi: 10.1590/s1517-83822014000400025

Prashar, P., Kapoor, N., Sachdeva, S. (2014). Rhizosphere: its structure, bacterial diversity and significance. *Rev. Environ. Sci. Biotechnol.* 13 (1), 63–77.

Ramírez-Ramírez, M. d. J., Mancilla-Magalli, N.A., Meza-Álvarez, L., Turincio-Tadeo, R., Guzmán-de Pena, D., Avila-Miranda, M.E. (2017). Epidemiology of Fusarium agave wilt in *Agave tequilana* Weber var. azul. *Plant Protect. Sci.* 53, 144-152. Doi: 10.17221/142/2016-PPS

Rincón-Rosales, R., Villalobos-Escobedo, J. M., Rogel, M. A., Martínez, J., Ormeño-Orrillo, E., Martínez-Romero, E. (2013). *Rhizobium calliandrae* sp. nov., *Rhizobium mayense* sp. nov. and *Rhizobium jaguaris* sp. nov., rhizobial species nodulating the medicinal legume *Calliandra grandiflora*. *Int. J. Syst. Evol. Microbiol.* 63(9), 3423–3429. Doi: 10.1099/ijs.0.048249-0

Sandhya, V., & Ali, S.Z. (2015). The production of exopolysaccharide by *Pseudomonas putida* GAP-P45 under various abiotic stress conditions and its role in soil aggregation. *Microbiology*. 84, 512–519. Doi: 10.1134/S0026261715040153

Santos, A.F.D., Martins, C.Y. S, Santos, P. O. (2014) Diazotrophic bacteria associated with sisal (*Agave sisalana* Perrine exEngelm): potential for plant growth promotion. *Plant Soil*. 385, 37–48. Doi: 10.1007/s11104-014-2202-x

Schafer, J., Hallett, S., Johnson, W. (2014). Rhizosphere Microbial Community Dynamics in Glyphosate-Treated Susceptible and Resistant Biotypes of Giant Ragweed (*Ambrosia trifida*). *Weed Science*, 62(2), 370-381. Doi:10.1614/WS-D-13-00164.1

Versalovic, J., Schneider, M., Brujin, J. F., Lupski, J. R. (1994). Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods Mol. Cell Biol.* 5 (1), 25–40.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei M., Kumar, S. (2011). MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28 (10), 2731–2739.