DIVERSITY OF CULTURABLE BACTERIAL MICROBIOTA OF THE *Eisenia foetida* DIGESTIVE TRACT

DIVERSIDAD DE LA MICROBIOTA BACTERIANA CULTIVABLE DEL TRACTO DIGESTIVO DE *Esenia foetida*

J. Abraham Pérez-Pérez[1], David Espinosa-Victoria[1]*, Hilda V. Silva-Rojas[1] and Lucía López-Reyes[2]

[1]Postgrado en Edafología, Colegio de Posgraduados. km 36.5 Carretera México-Texcoco. 56230, Montecillo, Texcoco, Estado de México, México. [2]Benemérita Universidad Autónoma de Puebla. 4 Sur #104. 72000 Col. Centro, Puebla de Zaragoza, Puebla, México.

*Corresponding author (despinos@colpos.mx)

SUMMARY

Bacteria are an unavoidable component of the natural earthworm diet; thus, bacterial diversity in the earthworm gut is directly linked to decomposition of organic matter and development of the surrounding plants. The aim of this research was to isolate and to identify biochemically and molecularly the culturable bacterial microbiota of the digestive tract of *Eisenia foetida*. Earthworms were sourced from Instituto de Reconversión Productiva y Bioenergética (IRBIO) and Colegio de Postgraduados (COLPOS), México. Bacterial isolation was carried out on plates of Brain Heart Infusion (BHI) culture medium. Fifty six and 44 bacterial isolates were obtained from IRBIO and COLPOS, respectively. The population was composed of 44 Gram-negative and 56 Gram-positive isolates. Over 50 % of the bacterial isolates were rod-shaped cells. The 16S rRNA gene was sequenced and nine genera were identified in worms from IRBIO (*Bacillus, Paenibacillus, Solibacillus, Staphylococcus, Arthrobacter, Pantoea, Stenotrophomonas, Acinetobacter* and *Aeromonas*) and six in worms from COLPOS (*Bacillus, Paenibacillus, Stenotrophomonas, Staphylococcus, Acinetobacter* and *Aeromonas*). Bacillus was the predominant genus, with eight and six species in the oligochaetes from IRBIO and COLPOS, respectively. The most represented bacteria in the worms from both sites were *Bacillus* sp. and *B. subtilis*. The predominance of *Bacillus* was probably due to spore formation, a reproductive strategy that ensures survival and dispersion in the soil and oligochaetes digestive tract. The gut of *E. foetida* not only harbored bacterial species of agronomic importance but also species potentially pathogenic for humans (*Staphylococcus warneri, Pantoea agglomerans* and *Stentrophomonas* sp.). The larger bacterial diversity in worms from IRBIO could be due to their feeding on cattle manure, which is a rich source of bacteria.

Index words: *Eisenia foetida, Bacillus*, bacterial diversity, gut, 16S rRNA gene.

RESUMEN

Las bacterias son parte inevitable de la dieta natural de las lombrices de tierra; de esta manera, la diversidad bacteriana en el intestino de la lombriz de tierra está directamente relacionada con la descomposición de la materia orgánica y con el desarrollo de las plantas circundantes. El objetivo de esta investigación fue aislar e identificar bioquímica y molecularmente la microbiota bacteriana cultivable del tracto digestivo de *Eisenia foetida*. Las lombrices de tierra provinieron del Instituto de Reconversión Productiva y Bioenergética (IRBIO) y del Colegio de Postgraduados (COLPOS), México. El aislamiento bacteriano se realizó en placas de medio de cultivo Agar Infusión Cerebro Corazón (AICC). Se obtuvieron 56 y 44 aislamientos bacterianos de IRBIO y COLPOS, respectivamente. La población estuvo compuesta por 44 aislamientos Gram-negativos y 56 Gram-positivos. Más del 50 % de los aislamientos bacterianos presentaron células en forma *de bacilo*. Se secuenció el gen 16S rRNA y se identificaron nueve géneros en los vermes de IRBIO (*Bacillus, Paenibacillus, Solibacillus, Staphylococcus, Arthrobacter, Pantoea, Stenotrophomonas, Acinetobacter* y *Aeromonas*) y seis en los de COLPOS (*Bacillus, Paenibacillus, Stenotrophomonas, Staphylococcus, Acinetobacter* y *Aeromonas*). El género predominante fue *Bacillus*, con ocho y seis especies en los oligoquetos de IRBIO y del COLPOS, respectivamente. *Bacillus* sp. y *B. subtilis* fueron las bacterias mayormente representadas en los vermes de ambos sitios. La predominancia de *Bacillus* esté relacionada probablemente con la formación de esporas, estrategia de reproducción que asegura su supervivencia y dispersión en el suelo y en el tracto digestivo de los oligoquetos. El intestino de *E. foetida* no sólo albergó especies bacterianas de importancia agronómica, sino también especies potencialmente patógenas para humanos (*Staphylococcus warneri, Pantoea agglomerans* y *Stentrophomonas* sp.). La mayor diversidad bacteriana en los vermes de IRBIO pudo deberse a la alimentación con estiércol de bovino, que es una fuente rica en bacterias.

Palabras clave: *Eisenia foetida, Bacillus*, diversidad bacteriana, intestino, gen 16S rRNA

INTRODUCTION

Earthworms are indicators of soil health (Bhadauria and Saxena, 2010). Earthworms play a crucial role in the soil structure modification, water infiltration, acceleration of organic matter decomposition, nutrient recycling and bio-remediation (Brown and Doube, 2004; Domínguez *et al.*, 2009).

The soil inhabited by these worms contains huge amounts of microorganisms. It is known that a gram of soil contains at least one million of microorganism (Torsvik and Øvreås, 2002), being the bacteria the most abundant and diverse group (Venter *et al.*, 2004).

Thus, bacteria are inevitable part of the natural diet of earthworms. Therefore, the bacterial diversity in the gut of

Recibido: 14 de agosto de 2017
Aceptado: 11 de abril de 2018

the earthworm will reflect the bacterial composition of the ingested soil or plant debris (Jayasinghe and Parkinson 2009; Knapp *et al*., 2008). Brito-Vega and Espinosa-Victoria (2009) reported that bacterial diversity in the gut of the earthworm *Pontoscolex coretrurus* is associated to the habitat and type of food.

Kim *et al*. (2004) isolated 91 bacterial colonies from the digestive tract of *E. foetida* inhabitant of a contaminated soil. They sequenced the 16S rDNA gene and identified 12 groups: *Aeromonas* 6 %, *Agromyces* 3 %, *Bacillus* 31 %, *Bosea* 1 %, *Gordonia* 6 %, *Klebsiella* 6 %, *Microbacterium* 7 %, *Nocardia* 2 %, *Pseudomonas* 10 %, *Rhodococcus* 19 %, *Tsukamurella* and *Streptomyces* 7 %. Valle-Molinares *et al.* (2007) identified seven typical soil *Bacillus* species (*B. insolitus, B. megaterium, B. brevis, B. pasteurii, B. sphaericus, B. thuringiensis* and *B. pabuli*) from the gut of *Onychochaeta boricana*. On the other hand, Brito *et al.* (2010 Pers. Com.[1]) reported eleven (*Bacillus subtilis* subsp. *subtilis, Bacillus mycoides, Bacillus cereus, Bacillus* sp., *Bacterium* sp., *Pseudomonas aeruginosa, Pseudomonas* sp., *Massilia timonae, Acinetobacter* sp., *Aeromonas* sp. and *Citrococcus*) and six (*Bacillus megaterium, Bacillus horikoshii, Aeromonas punctata, Bacillus* sp., *Bacterium* sp., *Terribacillus*) bacterial species in the digestive tract of individuals of *Pontoscolex corethrurus* inhabitants of a livestock area and an ecological reserve, respectively.

Méndez *et al.* (2003) indicated that bacteria establish a mutualistic symbiosis during their passage through the worm gut. It is known that earthworms accelerate the rate of decomposition of organic matter (Aira and Domínguez, 2008; Aira *et al*., 2006); however, strictly speaking, the microorganisms that inhabit the gastrointestinal tract have the enzymatic machinery to perform this activity. It is therefore necessary to know first the bacterial species that reside in the digestive tract of these worms, and then proceed to determine the functional group to which they belong. Therefore, the aim of this investigation was to isolate and identify biochemically and molecularly the resident bacteria in the digestive tract of the composting worm *E. foetida*.

MATERIALS AND METHODS

Origin and fixation of the individuals of *Eisenia foetida*

The individuals of *Eisenia foetida* used in this study came from both Instituto de Reconversión Productiva y Bioenergética (IRBIO), state of Chiapas and Colegio de Postgraduados (COLPOS), State of Mexico, Mexico. The worms fed on pasture and cattle manure in the first case and organic waste (fruit peels, vegetable residue and eggshell) in the second case. Ten individuals of each locality were used. The specimens were washed superficially with distilled water until they were free of soil; then, they were immersed in 70 % ethanol three times for 30 s; subsequently, they were rinsed in sterile distilled-water and fixed with distilled water at 50 ºC for 10 s (Kim *et al*., 2004). The earthworms were longitudinally dissected with a sterile scalpel to reach the intestine. The oligochaete digestive tract was divided into three sections: a) including the segments 1 to 45, b) segments 46 to 90, and c) segments 91 to 135.

Bacterial isolation from the digestive tract of *Eisenia foetida*

A portion of the intestinal content of each gut section was inoculated on plates of Brain Heart Infusion (BHI) (DIFCO®) culture medium. Plates were incubated at 30 ºC for 24 h. Isolation and purification of the bacterial colonies occurred by repeated streaking of a single colony on fresh BHI plates (Valle-Molinares *et al*., 2007).

Colonial and cellular morphologies of bacterial isolates

Colonial morphology was analyzed according to Gómez *et al.* (2006), with the aid of a stereomicroscope SMZ140/143 (Motic® Instruments Inc., Richmond, Canada). The Gram stain and purity of isolates was confirmed using an optical microscope MOTICBA-200 (Motic® Instruments Inc., Richmond, Canada).

Bacterial DNA extraction, amplification and sequencing of the 16S rRNA gene

DNA extraction was performed using 2 % CTAB protocol (Tris-HCl 100mM pH 8.0; EDTA 20 mM; CTAB 2 %; NaCl 1.4 M) (Doyle and Doyle, 1990).

The amplification of the 16S rRNA gene was performed using primers 8F (5´AGAGTTTGATCCTGGCTCAG3´) and 1492R (5´GGTTACCTTGTTACGACTT3´). The PCR was performed in a C1000 Touch™ thermal cycler (BIO-RAD, Hercules, CA, USA) with the following conditions: initial denaturation temperature 95 ºC for 2 min; 30 cycles of denaturation at 95 ºC for 1 min, annealing temperature 50 ºC for 30 s and extension at 72 ºC for 2 min, and a final extension at 72 ºC for 10 min (Silva *et al*., 2009; Vickerman *et al*., 2007). An agarose gel stained with 1.5 % GelRed® (Biotium, Fremont, CA, USA) exhibited the amplified fragment. The sequencing was carried out using the BigDye™ Terminator version 3.0 kit (Applied Biosystems, Foster City, CA, USA).

[1]Brito V. H., D. Espinosa V., I. Barois, P. Lavelle and A. Gómez-Vásquez (2010) Genetic identification of bacteria isolated from the digestive tract of the earthworm *Pontoscolex corethurus*. *In*: The 9th International Symposium on Earthworm Ecology. 5th - 10th September 2010. Xalapa, Veracruz, México. p:172.

Finally, the sequences were compared to the GenBank of the NCBI employing the option Blast_nucleotide 2.2.29 (Benson *et al*., 2009). The phylogenetic analysis was performed using the MEGA 6.0 software (Tamura *et al*., 2013). The evolutionary history of the sequences of the both sites was inferred using Maximum Parsimony method. The Maximum Parsimony trees were obtained using the algorithm Tree-Bisection-Regrafting (Lewis, 2001). The reliability of the formed trees associated in groups of taxa was conducted with the bootstrap test with 1000 replicates.

Biochemical analysis of bacterial isolates

Biochemical characterization required BioMerieux kits: API® 20NE identification system for non-fastidious and non-enteric Gram-negative rods, and API® 50 CHB for the species of the genus *Bacillus*. Each API gallery, inoculated according to the manufacturer recommendations, was incubated at 30 ºC and read at 24 and 48 h. The biochemical profile obtained in the database was compared using the program ApiwebTM (Logan and Berkeley, 1984). Catalase and oxidase activities were assayed using hydrogen peroxide (3 % v/v) and tetramethyl-p-phenylenediamine dihydrochloride 1 % (Sigma Co.), respectively.

RESULTS AND DISCUSSION

Colonial and cellular morphology of bacterial isolates

One hundred bacterial isolates from *E. foetida* digestive tract were obtained, 56 and 44 from IRBIO and COLPOS earthworms, respectively. The colonial morphology of the bacteria isolated from earthworms at both sites was similar. There were yellow and white colonies, with convex or flat elevation and wavy or whole edges; however, only rhizoid-shaped colonies were observed in digestive tract of IRBIO earthworms (Figure 1). Microscopic analysis revealed that the bacterial population consisted of 44 Gram-negative and 56 Gram-positive isolates. Over 50 % of the bacterial isolations corresponded to shaped-bacilli cells.

Molecular identification of bacterial isolates

The amplification of the 16S rRNA gene of the strains isolated from the *E. foetida* digestive tract exhibited a band of 1500 base pairs (bp) (Figure 2).

Nine bacterial genera were identified in the worms of IRBIO (Table 1). *Bacillus* was the predominant genus with eight species (*Bacillus* sp., *B. subtilis, B. cereus, B. megaterium, B. safensis, B. pumilus, B. simplex* and *B. flexus*). Six genera were identified in the COLPOS oligochaetes. Similarly, *Bacillus* was the predominant genus with six species (*B. subtilis, B. cereus, B. megaterium, B. safensis, B. aryabhattai* and *B. stratosphericus*). Ten different *Bacillus* species of *E. foetida* that were present in some intestine sections (A, B and C) were found in both worm populations, (Tables 1 and 2). As in the present experiment, Valle-Molinares *et al.* (2007) and Kim *et al.* (2004) reported the predominance of the genus *Bacillus* in the digestive tract of worms *Onychochaeta boricana* and *E. foetida*, respectively.

Seven bacterial species were present only in the worms of IRBIO: *Bacillus pumilus, B. simplex, B. flexus, Solibacillus* sp., *Paenibacillus* sp., *Arthrobacter* sp. and *Pantoea agglomerans*, while just three bacterial species were found in the oligochaetes of COLPOS: *Bacillus aryabhattai, B. stratosphericus* and *Staphylococcus warneri* (Table 1). Probably, the highest bacterial diversity observed in the digestive tract of worms of IRBIO was due to pasture and the cattle manure ingested. Particularly, bovine manure is a rich source of bacterial species, including some species potentially pathogenic to humans. Brito *et al.* (2010 Pers. Com[1]) reported larger bacterial diversity in the gut of *Pontoscolex coretrurus*, inhabitant of a livestock area.

Bacillus is a typical inhabitant of the soil. It is a Gram-positive spore-forming bacterium. Spore formation is a reproductive strategy ensuring its survival and spread not only in the soil but also in the digestive tract of the Oligochaeta (Kim *et al*., 2004; Valle-Molinares *et al*., 2007).

The mostly represented bacterial species in the intestine of worms of both sites were *Bacillus subtilis* and *Bacillus* sp., with 16 and 9 isolates (Table 1), respectively. *Staphylococcus warneri* was another species with significant representation in the *E. foetida* intestine, with nine isolates. This species is a Gram-positive coccus, coagulase negative, commonly found on the skin microbiota of humans and animals, which can cause infection in humans with weakened immune system (Predari, 2007); however, it is necessary to corroborate in this species the existence of human pathogenicity genes.

A single isolation of *Pantoea agglomerans* was detected in the worms of IRBIO. *P. agglomerans* is a Gram-negative bacillus, inhabitant of soil, plant pathogen, but also reported as a pathogen of humans. It has been associated with bacteremia in blood, soft tissues and joints in children (Cruz *et al*., 2007). As in the case of *S. warneri*, it is necessary to determine the presence genes for human pathogenicity in this isolate.

The bacterial species were not uniformly distributed throughout the digestive tract, except for *Bacillus* sp., which was found in sections A, B and C of the worms of both IRBIO and COLPOS, with a total of 16 isolates (Table 2). Some bacterial species were detected in the

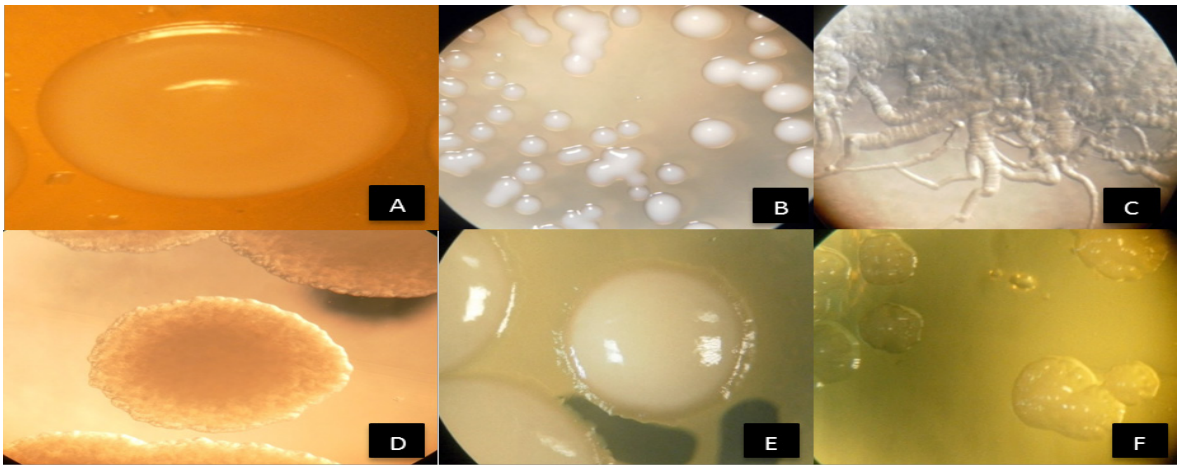

Figure 1. Colonial morphology of the bacterial strains isolated from *Eisenia foetida* digestive tract. A) yellow colony, mucoid, with whole edge and convex; B) white colony, circular, whole edge and conve; C) rhizoid-shaped colony, filamentous, high and umbilicated; D) whitish colony, flat, irregularly shaped, lobed edge with dry appearance, like a fried egg; E) whitish colony like wax, convex, fusiform and circular, with rounded edge; F) irregular-shaped colony, umbilicated with wavy edge.

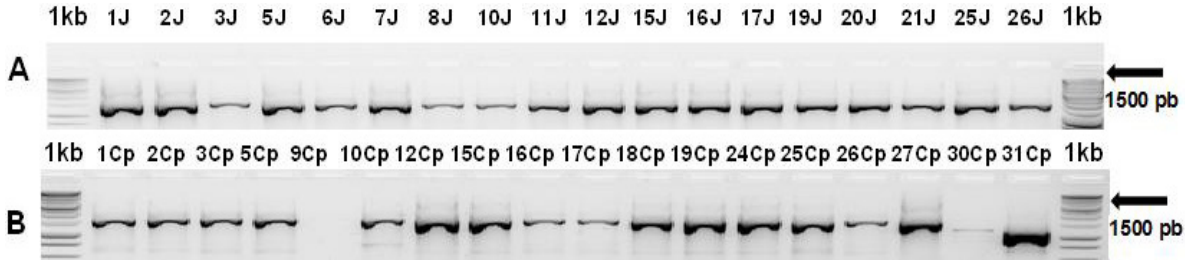

Figure 2. Amplification products of the 16S rRNA gene of bacteria isolated from the intestinal content of *Eisenia foetida,* using primers 8F and 1492R. J and Cp refer to the bacterial DNA of worms from A) Instituto de Reconversión Productiva y Bioenergética and B) Colegio de Postgraduados. Lines 1kb correspond to the molecular weight markers.

three intestinal sections of either worms, as is the case of *Bacillus simplex, Solibacillus* sp. and *Arthrobacter* sp., present in sections A, B and C of the digestive tract of IRBIO worms, with frequencies of 5, 5 and 4, respectively. *Staphylococcus warneri* was present in the three sections of the worms of COLPOS, with frequencies of 3, 4 and 2, respectively (Table 2).

There is no experimental evidence to explain the distribution of bacterial species in the digestive tract of the studied oligochaetes. The different bacterial species found in the three intestinal sections of *E. faetida*, as well as their metabolic variability, could explain the role of the earthworm in the modification of microbial populations in the composted materials (Pathma and Sakthivel, 2012); however, similar bacterial communities are reported in vermicomposts of different organic wastes (Fernández-Gómez *et al.*, 2012).

Phylogenetic analysis of bacterial populations

Figures 3 and 4 show the phylogenetic trees of the bacterial strains isolated from intestine of worms of IRBIO and COLPOS, respectively. The terminal nodes of the tree correspond to the studied organisms, while internal nodes represent the common ancestors that share two or more taxa (Gregory, 2008).

Figure 3 shows that some species of the genus *Bacillus* are within the same clade (*B. subtilis, B. safensis* and *B. pumilis*); however, other species of the same genus, such as *B. flexus, B. megaterium* and *B. simplex*, share *Staphylococcus* sp. as a common ancestor. That means that the conservative gene for these three bacilli species arose from some *Staphylococcus*. Moreover, as the bacillary morphology groups different species, four species of *Solibacillus* sp. present in the worms of IRBIO denote proximity to the genus *Bacillus*, which arose from *Paenibacillus* sp. Figure 4 presents an overview of the major taxonomic

Table 1. Bacterial species identified in the digestive tract of *Eisenia foetida* individuals from IRBIO and COLPOS using 16S rRNA gene sequencing.

IRBIO		COLPOS		
Species	Number of strains	Species	Number of strains	Total
Bacillus sp.	9	*Bacillus* sp.	7	16
Bacillus subtilis	4	*Bacillus subtilis*	5	9
Bacillus cereus	1	*Bacillus cereus*	2	3
Bacillus megaterium	1	*Bacillus megaterium*	2	3
Bacillus safensis	2	*Bacillus safensis*	4	6
Bacillus pumilus	3	-	-	3
Bacillus simplex	5	-	-	5
-	-	*Bacillus aryabhattai*	2	2
-	-	*Bacillus stratosphericus*	2	2
Bacillus flexus	4	-	-	4
Paenibacillus sp.	2	*Paenibacillus* sp.	1	3
Solibacillus sp.	5	-	-	5
Staphylococcus sp.	5	-	-	5
-	-	*Staphylococcus warneri*	9	9
Arthrobacter sp.	4	-	-	4
Pantoea agglomerans	1	-	-	1
Acinetoacter lwoffii	6	*Acinetoacter lwoffii*	1	7
Stenotrophomonas sp.	1	*Stenotrophomonas* sp.	6	7
Aeromonas media	3	*Aeromonas media*	3	6

IRBIO: Instituto de Reconversión Productiva y Bioenergética, State of Chiapas, Mexico; COLPOS: Colegio de Postgraduados, Campus Montecillo, State of Mexico, Mexico.

groups. It exhibits the sequences of 45 bacterial isolates of the intestine of *E. foetida* from COLPOS. It is noted that some *Bacillus* species lie within the same clade (*Bacillus* sp. and *B. subtilis*).

Biochemical analysis of bacterial populations

Table 3 shows the enzyme activity and use of C source by Gram-negative bacteria isolated from the gut of *E. foetida*. *Stentrophomonas* showed catalase, β-glucosidase, protease, and β-galactosidase activities. *Aeromonas* and *Arthrobacter* only showed β-glucosidase and catalase activities, respectively, while *Acinetobacter* presented protease and β-galactosidase activities. This is evidence of the biochemical potential of bacterial communities along the different sections of the *E. foetida* intestine. The transformation of organic matter passing through the intestine is closely related to the biochemical versatility of the bacterial species of composting worms (Pathma and Sakthivel, 2012).

Stentrophomonas was the most versatile genus in the use of C source because it assimilated glucose, arabinose, mannose and mannitol. *Arthrobacter* and *Acinetobacter* shared with *Stentrophomonas* the use of mannitol; however, these two genera, along with *Aeromonas*, used caprate and malate as C source. *Acinetobacter* was the only positive citrate genus. Probably, the biochemical versatility of *Stentrophomonas* enables it to be present in the digestive tract of worms of IRBIO and COLPOS (Table 2). Hong *et al.* (2012) reported the same biochemical versatility, but in different bacterial species associated o the gut of the red Californian worm.

Table 4 shows the capacity of *Bacillus* species of the digestive tract of *E. foetida* to use different C sources. *B. safensis* hydrolyzed 11 of the 20 tested carbon sources, while *Bacillus* sp., *B. cereus* and *B. flexus* used 10 of those. *Paenibacillus* was the less versatile species since it only used three carbon sources: esculin, salicin and sucrose. All *Bacillus* species used esculin as C source, except *B. pumilus*. Seven of the 10 species of *Bacillus* used salicin and sucrose. Ribose was used only by *B. cereus*, whereas inulin and starch were hydrolyzed only by *Bacillus* sp. In addition to the spore-formation strategy, that ensures the survival and spread, the versatility of *Bacillus* to use different C

Table 2. Frequency of bacterial species in sections A, B and C of the digestive tract of *Eisenia foetida* individuals from IRBIO and COLPOS.

Bacterial species	Section A		Section B		Section C		Total
	IRBIO	COLPOS	IRBIO	COLPOS	IRBIO	COLPOS	
Bacillus sp.	2	3	4	2	3	2	16
Bacillus subtilis	-	-	3	3	1	2	9
Bacillus cereus	-	-	-	1	1	1	3
Bacillus megaterium	-	-	1	2	-		3
Bacillus safensis	-	2	2	-	-	2	6
Bacillus pumilus	-	-	-	-	3	-	3
Bacillus simplex	1	-	2	-	2	-	5
Bacillus aryabhattai	-	1	-	-	-	1	2
Bacillus stratosphericus	-	2	-	-	-	-	2
Bacillus flexus	1	-	1	-	2	-	4
Paenibacillus sp.	1	-	1	1	-	-	3
Solibacillus sp.	2	-	2	-	1	-	5
Staphylococcus sp.	2	-	3	-	-	-	5
Staphylococcus warneri	-	3	-	4	-	2	9
Arthrobacter sp.	1	-	1	-	2	-	4
Pantoea agglomerans	1	-	-	-	-	-	1
Acinetoacter Iwoffii	4	-	1	-	1	1	7
Stenotrophomonas sp.	-	2	-	2	1	2	7
Aeromonas media	2	-	-	2	1	1	6
Number of species by section	10	6	11	8	11	8	
Total	17	13	21	17	18	14	100

IRBIO: Instituto de Reconversión Productiva y Bioenergética, State of Chiapas, Mexico; COLPOS: Colegio de Postgraduados, Campus Montecillo, State of Mexico, Mexico.

sources contributes to the exploration and permanence in niches such as the digestive tract of *E. foetida*.

This research represents the basis for future studies in order to know the bacterial diversity of the gut of *E. foetida*. The ultimate goal is to improve vermicompost biotechnology. There is the possibility of modifying the bacterial flora of the worm, favoring the presence of most efficient bacterial species for transformation of organic matter or detoxification of xenobiotic compounds.

CONCLUSIONS

Detection of nine and six bacterial genera in the gut of *E. foetida* from IRBIO and COLPOS, respectively, is an evidence of intestine microbial diversity. Bacterial species were not uniformly distributed along the digestive tract, except for the case of *Bacillus* sp., which was isolated in sections A, B and C of the worms of IRBIO and COLPOS, with 16 isolates. The type of food contributed to the extent of bacterial diversity in the digestive tract of IRBIO oligochaetes, because cattle manure is a rich source of bacterial species. *Bacillus* was the predominant genus with eight and six species in the intestine of worms of IRBIO and COLPOS, respectively. Ten different *Bacillus* species were identified in both populations of worms. These species were present in some of the sections of the intestine of *E. foetida*. *Bacillus* sp. and *Bacillus subtilis* were the mos widely represented strains in the worms of IRBIO and COLPOS, with 16 and 9 isolates, respectively. The digestive tract of *E. foetida* housed not only typical bacterial species of soil and water, but also species reported as potentially pathogenic for humans (*Staphylococcus warneri, Pantoea agglomerans* and *Stentrophomonas* sp.).

ACKNOWLEDGEMENTS

To Consejo Nacional de Ciencia y Tecnología (CONACYT) for the financial support to carry out this research.

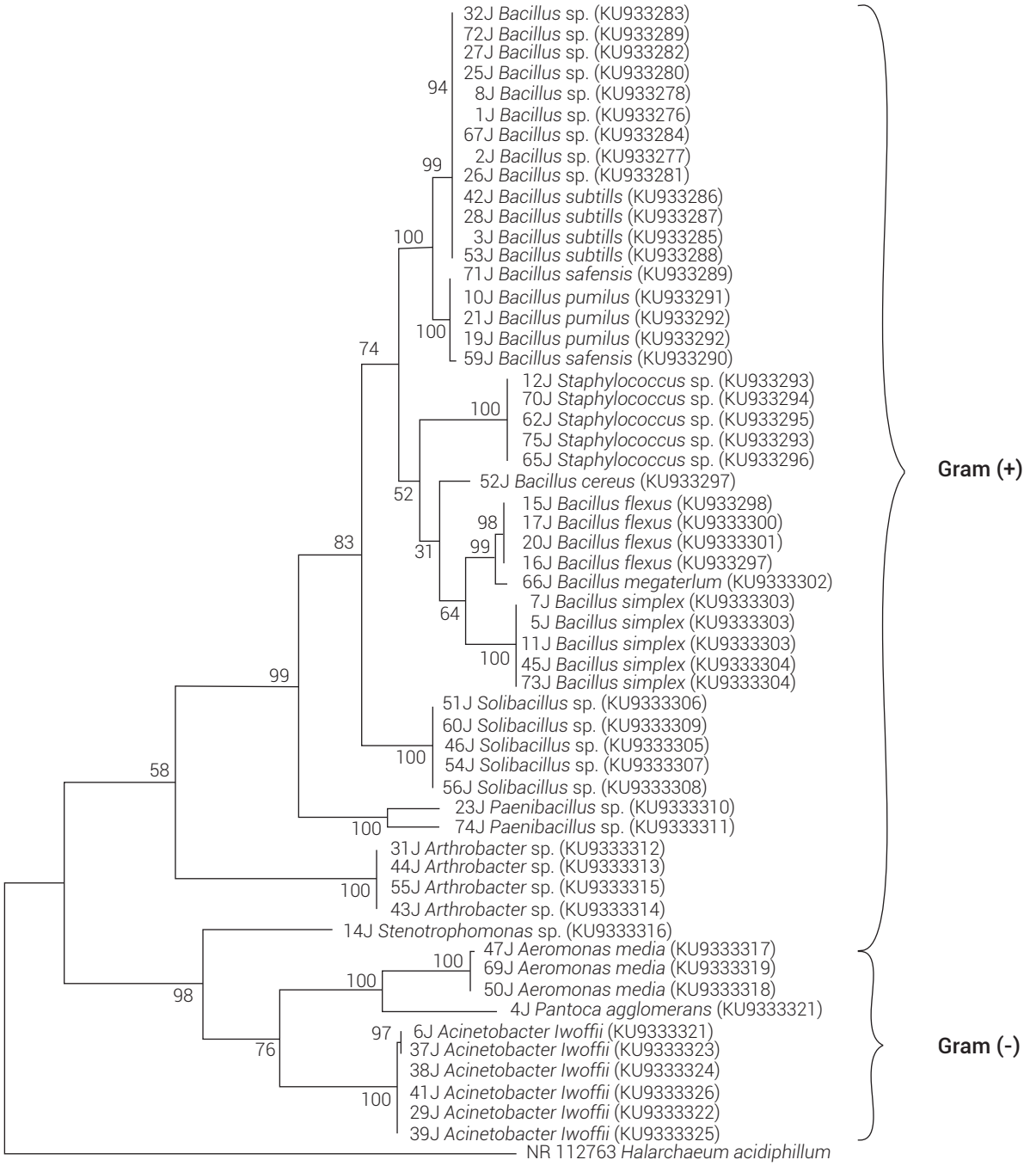

Figure 3. Phylogenetic tree constructed using partial 16S rRNA gene sequences obtained with Maximum Parsimony method. The bacteria were isolated from the intestine of *Eisenia foetida* from IRBIO, State of Chiapas, Mexico. The analysis included 57 nucleotide sequences. The consistency index was 0.607890, the retention index was 0.901973 and the composite index was 0.598406 for all sites. Numbers in parenthesis correspond to the access numbers in the GenBank.

BIBLIOGRAPHY

Aira M. and J. Domínguez (2008) Optimizing vermicomposting of animal wastes: effects of rate of manure application on carbon loss and microbial stabilization. *Journal of Environmental Management* 88:1525-1529, https://doi.org/10.1016/j.jenvman.2007.07.030

Aira M., F. Monroy and J. Domínguez (2006) Changes in microbial biomass and microbial activity of pig slurry after the transit through the gut of the earthworm *Eudrilus eugeniae* (Kinberg, 1867). *Biology and Fertility of Soils* 42:371-376, doi: 10.1007/s00374-005-0047-4

Benson D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell and E. W. Sayers (2009) GenBank. *Nucleic Acids Research* 37:D26-D31, https://

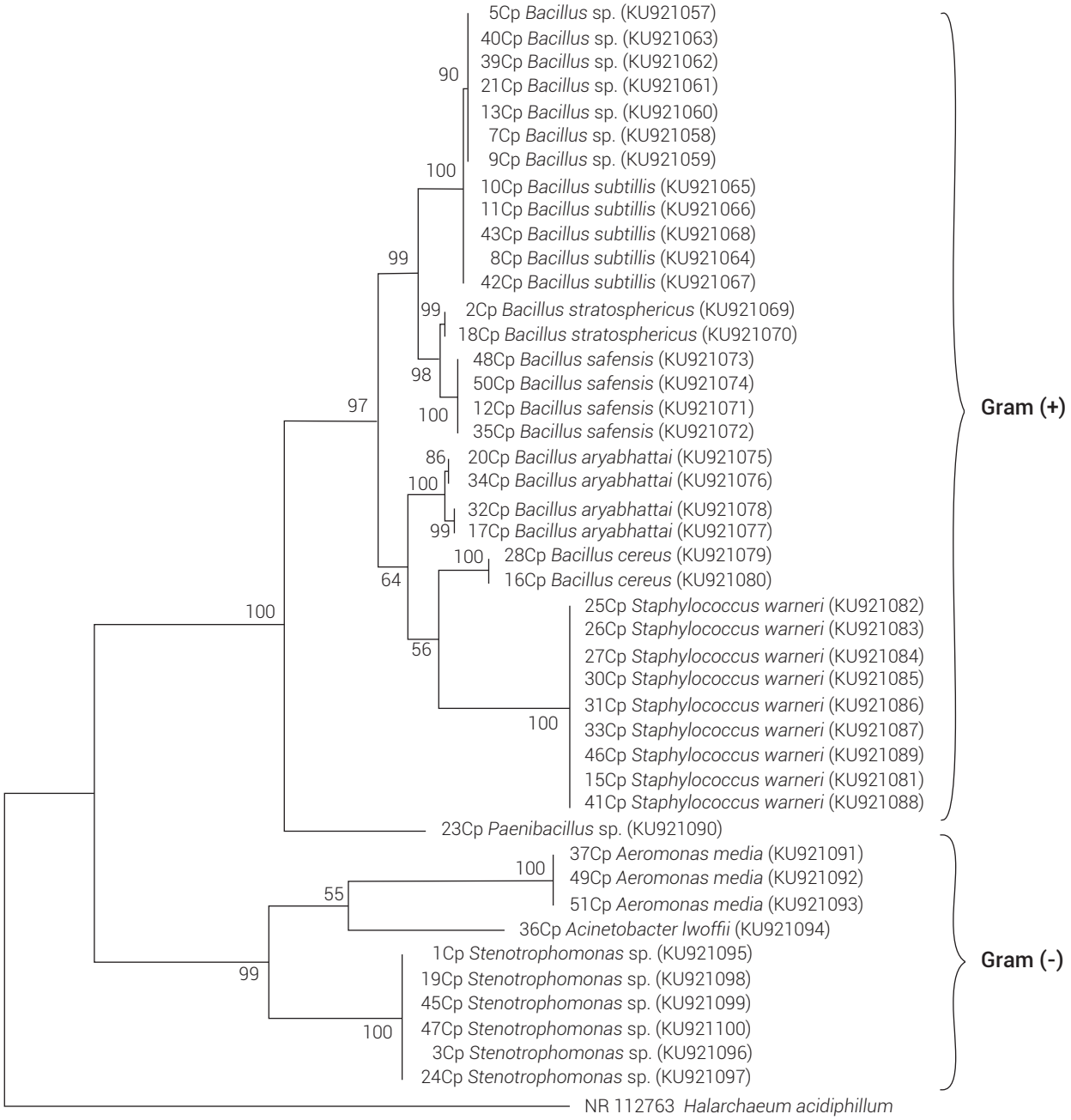

Figure 4. Phylogenetic tree constructed using partial 16S rRNA gene sequences obtained with Maximum Parsimony method. The bacteria were isolated from the intestine of *Eisenia foetida* from COLPOS, State of Mexico, Mexico. The analysis included 45 nucleotide sequences. The consistency index was 0.697309, the retention index was 0.927575 and the composite index was 0.709227 for all sites. Numbers in parenthesis correspond to the access numbers in the GenBank.

doi.org/10.1093/nar/gkn723

Bhadauria T. and K. G. Saxena (2010) Role of earthworms in soil fertility maintenance through the production of biogenic structures. *Applied and Environmental Soil Science* 2010:816073, http://dx.doi.org/10.1155/2010/816073

Brito-Vega H. and D. Espinosa-Victoria (2009) Bacterial diversity in the digestive tract of earthworms (Oligochaeta). *Journal of Biological Sciences* 9:192-199, doi: 10.3923/jbs.2009.192.199

Brown G. G. and B. M. Doube (2004) Functional interactions between earthworms, microorganisms, organic matter, and plants. *In*: Earthworm Ecology. 2nd edition. C. A. Edwards (ed.). CRC Press. Boca Raton, FL, USA. pp:213-239, https://doi.org/10.1201/9781420039719.pt6

Cruz A. T., A. C. Cazacu and C. H. Allen (2007) *Pantoea agglomerans*, a plant pathogen causing human disease. *Journal of Clinical Microbiology* 45:1898-1992, https://doi.org/10.1128/JCM.00632-07

Domínguez J., M. Aira y M. Gómez-Brandón (2009) El papel de las lombrices de tierra en la descomposición de la materia orgánica y el

Table 3. Enzymatic activity and use of C sources by Gram-negative bacterial genera isolated from the digestive tract of *Eisenia foetida*.

Bacterial genus	CAT	ESC	GEL	PNPG	GLU	ARA	MNE	MAN	CAP	MLT	CIT
Arthrobacter	-	+	-	-	-	-	-	+	+	+	-
Stenotrophomonas	+	+	+	+	+	+	+	+	-	-	-
Aeromonas	+	-	-	-	-	+	-	-	+	+	-
Acinetobacter	-	-	+	+	-	-	-	+	+	+	+

CAT: catalase activity, ESC: esculin hydrolysis by β-glucosidase, GEL: hydrolysis by proteases, PNPG: β-galactosidase activity, GLU: glucose assimilation, ARA: arabinose assimilation, MNE: mannose assimilation, MAN: mannitol assimilation, CAP. caprate assimilation, MLT: malate assimilation, CIT: trisodium citrate assimilation, (+): positive rection, (-): negative reaction.

Table 4. Use of different C sources by the species of the genus *Bacillus* isolated from the digestive tract of *Eisenia foetida*.

Bacillus species	GLI	RIB	GLU	FRU	MaN	MAN	NAG	AMY	ARB	ESC	SAL	CEL	MAL	SUC	TRE	INU	RAF	STA	GLIG	TAG
Bacillus sp.	-	-	+	-	-	-	+	-	+	+	+	-	+	-	+	+	-	+	+	-
Bacillus subtilis	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	+	-
Bacillus cereus	+	+	+	+	-	-	-	-	-	+	-	-	+	+	+	-	+	-	+	-
Bacillus megaterium	-	-	-	-	-	-	-	+	-	+	+	+	-	+	+	-	-	-	-	+
Bacillus safensis	+	-	+	-	+	+	+	+	-	+	+	+	-	+	-	-	-	-	-	+
Bacillus pumilus	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+
Bacillus simplex	-	-	-	-	+	+	-	+	-	+	+	-	+	-	-	-	+	-	+	-
Bacillus aryabhattai	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+	-	-	-	-	-
Bacillus flexus	-	-	+	+	+	-	-	+	+	+	+	-	-	-	+	-	+	-	+	-
Paenibacillus sp.	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-

GLI: glycerol; RIB: ribose; GLU: glucose; FRU: fructose; MaN: mannose; MAN: mannitol; NAG: N-acetyl-glucosamine; AMY: amygdaline; ARB: arabinose; ESC: esculin; SAL: salicin; CEL: celobiose; MAL: maltose; SUC: sucrose; TRE: trehalose; INU: inulin; RAF: Rafinose; STA: starch; GLIG: glycogen; TAG: tagatose; Positive (+) and Negative (-) reactions.

ciclo de nutrientes. *Ecosistemas* 18:20-31.

Doyle J. J. and J. L. Doyle (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.

Fernández-Gómez M. J., R. Nogales, H. Insam, E. Romero and M. Goberna (2012) Use of DGGE and COMPOCHIP for investigating bacterial communities of various vermicomposts produced from different wastes under dissimilar conditions. *Science of the Total Environment* 414:664-671, https://doi.org/10.1016/j.scitotenv.2011.11.045.

Gómez G. M. L., L. J. Vivas A., R. A. Ruiz R., V. R. Reyes D. y C. A. Hurtado (2006) Bacterias Marinas Nativas Degradadoras de Compuestos Orgánicos Persistentes en Colombia. Instituto de Investigaciones Marinas y Costeras, INVEMAR. Santa Marta, Colombia. 32 p.

Gregory T. R. (2008) Understanding evolutionary trees. *Evolution: Education and Outreach* 1:121-137, https://doi.org/10.1007/s12052-008-0035-x

Hong S. W., J. M. Park, S. J. Kim and K. S. Chung (2012) *Bacillus eiseniae* sp. nov., a swarming, moderately halotolerant bacterium isolated from the intestinal tract of an earthworm (*Eisenia foetida* L.). *International Journal of Systematic and Evolutionary Microbiology*. 62:2077-2083, https://doi.org/10.1099/ijs.0.034892-0

Jayasinghe B. A. T. D. and D. Parkinson (2009) Earthworms as the vectors of actinomycetes antagonistic to litter decomposer fungi. *Applied Soil Ecology* 43:1-10, https://doi.org/10.1016/j.apsoil.2009.06.001

Kim H. J., K. H. Shin, C. J. Cha and H. G. Hur (2004) Analysis of aerobic and culturable bacterial community structures in earthworm (*Eisenia foetida*) intestine. *Agricultural Chemistry and Biotechnology* 47:137-142.

Knapp B. A., J. Seeber, S. M. Podmirseg, E. Meyer and H. Insam (2008) Application of denaturing gradient gel electrophoresis for analysing the gut microflora of *Lumbricus rubellus* Hoffmeister under different feeding conditions. *Bulletin of Entomological Research* 98:271-279, https://doi.org/10.1017/S0007485308006056

Lewis P. O. (2001) Phylogenetic systematics turns over a new leaf. *Trends in Ecology and Evolution* 16:30-37, https://doi.org/10.1016/S0169-5347(00)02025-5

Logan N. A. and R. C. W Berkeley (1984) Identification of *Bacillus* strains using the API system. *Journal of General Microbiology* 130:1871-1882.

Méndez R., S. Borges and C. Betancourt (2003) A microscopical view of the intestine of *Onychochaeta borincana* (Oligochaeta: Glossoscolecidae). *Pedobiologia* 47:900-903. https://doi.org/10.1078/0031-4056-00278

Pathma J. and N. Sakthivel (2012) Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. *SpringerPlus* 1:26, https://doi.org/10.1186/2193-1801-1-26

Predari S. C. (2007) Estafilococos coagulasa negativos: el enemigo silente. *Revista Argentina de Microbiología* 39:1-3.

Silva R., R. Negri and V. Lutz (2009) Summer succession of ultraphytoplankton at the EPEA coastal station (Northern Argentina). *Journal of Plankton Research* 31:447-458, https://doi.org/10.1093/plankt/fbn128

Tamura K., G. Stecher, D. Peterson, A. Filipski and S. Kumar (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725-2729, https://doi.org/10.1093/molbev/mst197

Torsvik V. and L. Øvreås (2002) Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology* 5:240-245, doi: 10.1016/S1369-5274(02)00324-7

Valle-Molinares R., S. Borges and C. Ríos-Velázquez (2007) Characterization of possible symbionts in *Onychochaeta borincana* (Annelida: Glossoscolecidae). *European Journal of Soil Biology* 43(Suppl. 1):S14-S18, https://doi.org/10.1016/j.ejsobi.2007.08.057

Venter J. C., K. Remington, J. F. Heildelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y. H. Rogers and H. O. Smith (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304:66-74, https://doi.org/10.1126/science.1093857

Vickerman M. M., K. A. Brossard, D. B. Funk, A. M. Jesionowski and S. R. Gill (2007) Phylogenetic analysis of bacterial and archaeal species in symptomatic and asymptomatic endodontic infections. *Journal of Medical Microbiology* 56:110-118, https://doi.org/10.1099/jmm.0.46835-0