The bacterial diversity in *Zea mays* L: A critical review

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Abstract

Maize is a plant of great value to humanity, so the bacterial diversity in association with the cultivation is equally important. Various protocols have been developed for studied bacterial diversity associated with maize from the cultivable bacterial diversity to nonculturable bacterial diversity. In this article the importance of maize, microbial diversity associated with the plant and bacterial diversity associated with the rhizosphere and inside of the plant is review. The methods that have been used for information in the past 20 years and a reflection on the prospects of the current knowledge of the diversity of bacteria associated in *Zea mays* L

Keywords: bacterial diversity, functional diversity, genetic diversity

Introduction

Maize is one relevant plant world so knowledge about bacterial diversity association is essential. Studies with maize have been approached by various authors, from which provide general information, to which include studies with specific bacteria. The development of different methods for bacterial diversity associated with maize includes from general and fundamental studies, from the assessment of the detection of cultivable bacterial diversity to the detection of non-cultivable bacterial diversity. This article covers basic specific aspects since the importance of maize the microbial diversity associated with the plant and bacterial diversity associated with the rhizosphere and inside the plant. With emphasis on the methods that have been used for their information in the past 20 years for bacterial diversity and a reflection on the perspectives of the present state of knowledge of the diversity of bacteria associated with *Zea mays* L

The maize importance

Maize (Zea mays L), also called corn is the most studied plant for its high genetic diversity and the ample variety of uses. It is currently the most widely grown crop in the world, and is used not only for food/feed but also to produce ethanol, industrial starches and oils. (Pohl et al, 2007; Strable and Scanton, 2009). It is the most important crop in Mexico because it is where probably originated with apparent dispersion in the continent, from North America to southern Argentina (Pohl et al, 2007). The world maize production ranges up to 558 million t year^{-1}, the main producers are United States, China, Brazil, India and Mexico (Naqvi et al, 2011; UNDP, 2010). Different studies in maize have been in relation to the development of strategies to improve grain yield and in relation to their interactions with abiotic and biotic agents of interest resulting knowledge of the organism associated with the crop. Maize interacts with several types of organism bacteria whiting them, since beneficial to harmful (Marschner et al, 2006).

Bacterial diversity associated with Zea mays L

Maize interacts with several kinds of microorganisms, the studies of bacterial diversity in Zea mays L have been strongly focused on determination of the taxonomic group (Figure 1), from the point of view of the classics microbial ecology to the environmental perspective. Numerous restrictions are associated with the bacterial culture that can influence in the real diversity of the microbial community: i.e. techni-

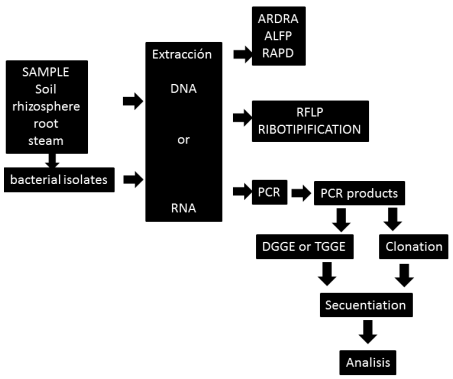

Figure 1. The different steps that were followed to study the bacterial diversity associated with maize plants

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Table 1 - Proteobacteria associated with maize plants

Class	Gender species	Level of association	Reference
Alpha	*Acetobacter*	Rh	Kozdrój, 2008
	Agrobacterium	E, Rh	Kozdrój, 2008; McInroy and Kloepper, 1995
	Radiobacter		
	Azorhizobium	Rh	Roesch *et al.,* 2008
	Azospirillum	Rh, R, T	Caballero-Mellado, 1999; Espinosa-Victoria *et al.,* 2006; Roesch *et al*., 2008; Estrada de los Santos *et al.,* 2002.
	brasilense		
	lipoferum		
	Beijerinckia	E, Rh	Roesch *et al*., 2008
	Bradyrhizobium	Rh, R, T	Roesch *et al*., 2008
	Gluconacetobacter	Rh	Roesch *et al*., 2008
	Mesorhizobium	Rh	Roesch *et al*., 2008
	Methylobacterium	E, T	McInroy and Kloepper, 1995; Roesc *et al*., 2008
	Methylocella	Rh	Roesch *et al*., 2008
	Methylocystis	Rh, R	Roesch *et al*., 2008
	Methylosinus	Rh, T	Roesch *et al*., 2008
	Phyllobacterium	E	McInroy and Kloepper, 1995
	Rhizobium	E, Rh, T	Chelius and Triplet, 2001; Guitierrez-Zamora y Martínez-Romero 2001; McInroy and Kloepper, 1995; Rosenblueth and Martinez-Romero, 2004; Roesch *et al.,* 2008
	etli		
	japonicum		
	Rhodoblastus	Rh, T	Roesch *et a*l., 2008
	Rhodovulum	Rh	Roesch *et al*., 2008
	Sinorhizobium	Rh, R, T	Roesch *et al.,* 2008
	Sphingomonas	E	McInroy and Kloepper, 1995
	paucimobilis		
	Xanthobacter	Rh, R, T	Kozdrój, 2008; Roesch *et al.,* 2008
Beta	*Achromobacter*	E	Pereira *et al*., 2011
	Xylosoxidans		
	Advenella	E	Espinosa-Victoria *et al*., 2009
	incenata		
	Alcaligenes	Rh, T	Kozdrój, 2008; Roesch *et al*., 2008
	Azoarcus	Rh, R, T	Roesch *et al.,* 2008
	Azohydromonas	R	Roesch *et al*., 2008
	Azonexus	R	Roesch *et al*., 2008
	Burkholderia	E, Rh, R	Chelius and Triplett, 2001; Di Cello *et al.,* 1997; Espinosa-Victoria *et al.,* 2009; Estrada de los Santos *et al*., 2002. Estrada de los Santos *et al*., 2001; Hebbar *et al.,* 1994; Kozdrój, 2008; McInroy and Kloepper, 1995; Perin *et al*., 2006. Roesch *et al.,* 2008, Dalmastri *et al.,* 1999.
	cepacia		
	gladioli		
	graminis		
	pickettii		
	solanacearum		
	silvatlantica		
	vietnamiensis		
	Dechloromonas	Rh, R, T	Roesch *et al*., 2008

Table 1 - continued

	Derxia	Rh, R	Roesch *et al*., 2008
	Delftia	R	Roesch *et al*., 2008
	Duganella zoogloides	E	Chelius and Triplet, 2001
	Herbaspirillum Seropedicae	E, Rh, R, T	Chelius and Triplet, 2001; Olivares *et al.,* 1996, Roesch *et al*., 2008
	Hydrogenophaga	E	McInroy and Kloepper, 1995
	Ideonella	Rh, R, T,	Roesch *et al.,* 2008
	Pelomonas	R	Roesch *et al.,* 2008
	Variovorax paradoxuss	E	McInroy and Kloepper, 1995
Gamma	*Azotobacter*	Rh, Rp, ERh, R, T	Cavaglieri *et al*., 2005; Roesch *et a*l., 2008
	Citrobacter koseri	E	Palus *et al.,* 1996, McInroy and Kloepper, 1995
	Enterobacter asburiae cloacae	E	Espinosa-Victoria *et a*l., 2009; Hinton *et al*., 1995; McInroy and Kloepper, 1995
	Enterobacterias	Rh	Chelius and Triplet, 2001
	Erwinia pyrifoliae	E, Rh	Kozdrój, 2008; Palus *et al.,* 1996; Pereira *et al.,* 2011
	Escherichia	E	McInroy and Kloepper, 1995
	Klebsiella oxytoca pneumoniae terrigena variicola	E, Rh	Berge *et al.,* 1991; Chelius and Triplet, 2001; McInroy and Kloepper, 1995; Palus *et al.,* 1996 ; Pereira *et al*., 2011; Rosenblueth *et al.*, 2004, Roesch *et al*, 2008
	Kluyvera	E	McInroy and Kloepper, 1995
	Pantoea	E	McInroy and Kloepper ,1995; Espinosa-Victoria et al., 2009
	Pasteurella multocida	E	Espinosa-Victoria *et al.,* 2009
	Pseudomonas chlororaphis fluorescens putida saccharaphila talassii	E, Rh, Rp, ERh, T	Cavaglieri *et al*., 2005; Espinosa-Victoria *et al.,* 2009; Kozdrój, 2008; McInroy and Kloepper, 1995; Pereira *et al.,* 2011; Roesch *et al*., 2008
	Rahnella aquatilis	Rh	Berge *et al*., 1991
	Raoultella	Rh; R, T	Roesch *et al.,* 2008
	Serratia	E	McInroy and Kloepper, 1995; Palus *et al.,* 1996
	Stenotrophomonas maltophilia	E	McInroy and Kloepper, 1995; Pereira *et al*., 2011
	Xanthomonas campestris	E	Kozdrój, 2008; McInroy and Kloepper, 1995
	Yersinia frederiksenii	E	McInroy and Kloepper, 1995
Delta	*Chondromyces lanuginosus*	Rh	Chelius and Triplet, 2001
	Flavimonas oryzihabitans	E	McInroy and Kloepper, 1995
	Geobacter	Rh	Roesch *et al.,* 2008

E=Endophyte, Rh=Rhizospheric, Rp=Rizoplane, ERh=Internal tissue of rhizosphere, S=Soil, R=Raize, T=Stem

cal difficulties in liberate bacteria from soil particles, appropriate selection of culture medium, the negative interaction between microorganisms and the presence of organisms with dissimilar growth rate or the different growth conditions such as temperature, light and pH (Fakruddin and Khanjada, 2013; Kirt et al, 2004). The bacterial diversity associated with Zea mays L rhizosphere is the best studied, this is the slice of soil adhering to the plant root, root is an interface that harbors numerous microorganisms both beneficial and harmful (Berg and Zachow, 2011; Brusetti et al, 2004; Gomes et al, 2001; Remenant et al, 2009); in this place, secretion of a mixture of substances of radical origin that is selectively elicited by the growth of fungi and bacteria populations, is released to soil in the vicinity of the root (Yang and Crowley, 2000; Gomes et al, 2001). The rhizosphere is a microhabitat with competition both root-root and root-microorganisms by nutrients such as carbon and nitrogen among others (Dalmastri et al, 1999; Da Mota et al, 2008; Xu et al, 2008), in this place the diversity of organisms is more evident by their effects on the growth and health of plants (Berg and Zachou, 2011). The rhizosphere effect on maize has been most pronounced in young roots compared with mature roots, particularly in the bacterial population as demonstrated in Arthrobacter species of bacteria that decrease with increasing age of the plant (Gomes et al, 2001). Biotic and abiotic factors such as soil type and plant lineage have been detected that affect the size and composition of the microbiotic population in the rhizosphere of maize in natural communities (Dalmastri et al, 1999; Da Mota et al, 2008). The taxonomic affiliation of the bacteria associated with maize plants reveals a high dominance of Actinobacteria and Proteobacteria mainly α-proteobacteria and β-proteobacteria (Chelius and Triplett, 2001; Roesch et al, 2008). The bacterial community associated with maize harbors multiple orders: Actinomycetales, Burkholderiales, Clostridiales, Rizobiales, Rubrobacteriales and Xanthomonadales (Da Mota et al, 2008) and the bacterial genera commonly associated with maize have been identified as Enterobacter, Klebsiella, Pseudomonas, Bacillus and Azospirillum (Palus, 1996) . Most plant-associated bacteria soil migrate toward the rhizosphere and rhizoplane posteriomente then the inside of the roots. Microbial populations associated with plants, rise in areas where biochemical interactions between microorganisms and the plant are more pronounced as the rhizosphere (Compant et al, 2010). In this regard, have been studied 52 genera represented by 71 species associated with maize plants in the rhizospheric soil, root surface soil or rhizoplane, inside the root (endorhizosphere) and internal stem tissues (Table 1 and Table 2). Of them, 31 bacterial genera associated in the internal tissues of the stem and root (endophytes) of sweet maize (McInroy and Kloepper, 1995), with various metabolic activities with needed to meet widely. The endophytic bacteria associated with plants possess multiple functions in the environment as some endophytic bacterial genus promote plant growth, suppress pathogens, help to remove contaminants, solubilize phosphate or contribute assimilation nitrogen to plants (Rosenblueth and Martínez-Romero, 2006). Some bacteria associated with the rhizosphere of maize have been used in the inoculation of seeds, resulting in positive effects on plants by significant increases in dry matter of roots and stems (Sangeetha et al, 2013). Futhermore maize plants from different regions of Mexico have been associated with phosphate solubilizing activity coming from bacterial communities that have been characterized to phenotypic and genotypic level (Espinosa-Victoria et al, 2009). The symbiotic nitrogen-fixing bacteria *R. etli* hosts Phaseolus sp has been found as a natural endophyte of maize plants in traditional farmland where maize and bean grown so associated (Gutiérrez-Zamora and Martinez-Romero, 2001). Members of the Azospirillum, Herbaspirillum, Burkholderia genus and other free-living bacteria are diazotrophs associated with maize (Baldani and Baldani, 2005; Roesch et al, 2008). In addition, there are bacteria with activities different to nitrogen fixation as Pseudomonas species with antagonistic activity towards fungi (Pandey et al, 2001) and towards bacteria as Azotobacter and Arthrobacter (Cavaglieri et al, 2005). Many studies have been conducted to determine the biodiversity of natural microbial communities in the rhizosphere of maize, only a few studies have been conducted to understand the diversity among species. On the other hand, comparisons between populations of Burkholderia cepacia have brought to the genetic diversity is influenced by the type of soil, and cultivating maize root compartments (Dalmastri et al, 1999). Their influence of root metabolites play an important role in the establishment of microbial diversity with respect to the age of the plant and the site of isolation as demonstrated with *Paenibacillus azotofixans* (Seldin et al, 1998). Finally have been found variability in microbial diazotrophic population in two maize cultivars in relation to plant age (Roesch et al, 2006), while on bacteria that oxidize ammonia no differences were found between wheat and maize (Song et al, 2007).

Bacterial count in maize

Maize supports a bacterial diversity which is partially represented by the population developed in culture medium. The methodology for calculating the bacterial diversity in maize by counting cultivable organisms has been to take a portion of stem (Rosemblueth and Martínez-Romero, 2004), soil (Belay et al, 2002), rhizosphere (Chiarini et al, 1998; Reyes and Valery, 2007) or rhizoplane (Reyes and Valery, 2007). The subsequent preparation of sample in suspension with dilutions and plating specific nutritious agars, with further growth and separation of bacteria based on the morphology of the grown colonies. Trosvick et

Table 2 - No proteobacteria in association with *Zea mays* L.

Phylum/Class	Bacteria	Level of association	Reference
Actinobacteria	*Actinomadura*	Rh	Kozdrój, 2008
Actinobacteria	*Arthrobacter* *globiformis* *nicotinoborans* *polychromogenes*	E, Rh, Rp, ERh	Cavaglieri *et al*., 2005; Chelius and Triplett 2000; Gomes *et al.,* 2001; Kozdrój, 2008; McInroy and Kloeper, 1995
Actinobacteria	*Clavibacter* *michiganensis*	E	McInroy and Kloepper, 1995
Actinobacteria	*Corynebacterium*	Rk	Kozdrój, 2008
Actinobacteria	*Curtobacterium*	E	McInroy and Kloepper, 1995
Actinobacteria	*Microbacterium* *testaceum*	E	McInroy and Kloepper, 1995; Zinniel *et al*., 2002
Actinobacteria	*Micrococcus*	E, Rp, ERh	Cavaglieri *et al*., 2005; McInroy and Kloepper, 1995
Actinobacterias	*Agromyces*	Rp, ERh	Cavaglieri *et al*., 2005
Bacteroidetes,	*Flavobacterium.*	E, Rh	Kozdrój, 2008; McInroy and Kloepper, 1995
Bacteroidetes	*Cytophaga (Cytophagales)*	Rh	Chelius and Triplet, 2001; Kozdrój, 2008; Olson y Person, 1999
Firmicutes	*Bacillus* *amyloliquefaciens* *drentensis* *megaterium* *pumilu* *subtilis* *thuringiensis*	E, Rh, Rp, ERh	Berge *et al.,* 1991; Cavaglieri *et al*., 2005; Kozdrój, 2008 ; McInroy y Kloepper, 1995; Pereira *et al*., 2011; Roesch *et al*., 2008
Firmicutes	*Clostridium*	Rh	Kozdrój, 2008
Firmicutes	*Enterococcus*	Rh	Kozdrój, 2008
Firmicutes	*Listeria*	Rp, ERh	Cavaglieri *et al*., 2005
Firmicutes	*Paenibacillus* *azotofixans*	Rh, Rp, S, T	Seldin *et al.,* 1998; Rosado *et al* ., 1998
Firmicutes	*Staphylococcus*	E	McInroy and Kloepper, 1995
Nitrospirae	*Nitrospira* *moscoviensis*	Rh	Chelius and Triplet, 2001

al (2002) and Wintzingerode et al (1997) consider that only grown in culture media in the laboratory represents a small fraction of diversity of microorganisms of an ecosystem. The technique of microbial plate count is widely used where the soil samples from the rhizosphere, roots or stems are washed and suspended in saline phosphate in a ratio of 1:10. After 45 min stirring at 200 rpm, serial dilutions are made in sterile saline and subsequent seeding Trypticase Soy Agar (TSA) or Nutrient Agar (NA). The colony forming units (cfu) performed at 48h of incubation at average temperatures of 29°C are counted (Dalmastri et al, 1999; Gomes et al, 2001; Pereira et al, 2011; Di Cello et al, 1997). Bacteria growing in culture are subjected to biochemical, physiological and genetic identification. Table 3 shows the estimated values in maize by bacterial plate counts. One gram of maize soil rhizosphere contain more than eight billion bacteria (ufc) (Gomes et al, 2001). Root samples have shown 6.2-7.45 x 10^1 ufc g^{-1} (Dalmastri et al, 1999; Buyer and Kaufman, 1996) and 2.2 x 10^3 ufc g^{-1} in maize tissue (Palus et al, 1996). The huge bacterial variability has been described in samples based on the availability and amount and type of nutrients, moisture, aeration, temperature, pH, microbial interactions, the presence of roots and farming practices. The maize plant development affects bacterial diversity in connection with the instability of the atmosphere surrounding the plant (Seldin et al, 1998). Compatibility between Rhizobium etli isolated from maize stem and in the rhizosphere, roots and nodules of other crops such as beans has been demonstrated (Rosenblueth and Martínez-Romero, 2004).

Community level physiological profiles (CLPPs)

The functional diversity is measured under various aspects based on the ability of microbial communities to metabolize a range of organic substrates as: carbohydrates, polymers, phenolic compounds, carboxylic acids, amino acids, and amines (Carvalho et al, 2011; Khan et al, 2014; Ros et al, 2008; Sharma et al, 1998; Mulder et al, 2006). The community in the rhizosphere of gramineous plants established

a reduction of functional diversity in soils under the impact of heavy metals using a single carbon source, functional diversity is calculated based on the number, type, activities and usage rates of carbon sources used by the bacterial community associated with maize (Sharma et al, 1998). It It has been possible to to compare the temporal variation in soil bacteria due to the activity that reflects the influence of the toxin CryAb synthetized by transgenic maize (Mulder et al, 2006). Furthermore with different level of nitrogen application during intercropping of maize and peanut showed the soil rhizosphere increasing bacterial community by 6 different kinds of carbon source utilization (Khan et al, 2014).

Fatty acid methyl ester (FAME) profiles as measure of bacterial diversity in maize

FAMEs profiles are obtained by the composition of methyl esters of fatty acids from bacteria are purified and saponified with NaOH or KOH (Marschner et al, 2006; Rai et al, 2006). The method is based in the esterification of the extracted lipids from the samples, followed by the separation and identification of compounds by gas chromatography (Kozdrój, 2008). FAMEs are identified by comparison of their retention times against chemical patterns on a chromatographic column. The quantification of each compound is made based on the determination of the areas formed by the corresponding gas chromatogram (Kirk et al, 2004, Pérez et al, 2009). It is a technique that is interpreted by the software Microbial Identification System software (MIDI Inc) (Kozdrój, 2008; Rai et al, 2006). In maize rhizosphere has been detected between 45-63 bacterial FAMEs diversity and it has been shown that young maize plants inoculated with *Pseudomonas chlororaphis* and *Pseudomonas putida* altered microbial community. The lipid profiles uninoculated samples register 43 FAMEs, while the presence of *P. chlororaphis* and *P. putida* detected 47 and 42 FAMEs respectively (Kozdrój, 2008). It has been identified *Bacillus pumilus*, *B. subtilis*, *Pseudomonas aeruginosa* and *P. fluorescens* as the relatively more predominant group of bacterial species residing in maize stem (Rai et al, 2006).

Extraction and isolation of total bacterial DNA

Bacterial DNA analysis is useful for the studies of bacterial diversity in different environments. Maize plants bacteria are searched in subsamples as rhizosphere soil, plant tissue, isolated bacteria and bacteria extract grown in broth (Marrschner et al, 2006; Chelius and Triplet, 2001; Gomes et al, 2001). Protocols involve different technical aspecs between these are enzymatic or chemical treatments, for example lysozyme and phenol cleaning. The DNA is cleaned up using CsCl and subsequent precipitation with sodium acetate for purification (Smalla et al, 1993). Methods using a commercial kit according to the manufacturer's protocol are also useful (Song et al, 2007). DNA concentration is determined with a spectrophotometer and visualized on agarose gels at 0.8% weight-volume for integrity and purity of the sample (Da Mota et al, 2008). The collected DNA is purified using a commercial system like the Promega Wizard (Chelius and Triplett, 2001). The extracted DNA is used as template to amplify target sequences by PCR. Amplified DNA compared by electrophoresis and also used to construct phylograms. Thus, the

Table 3 - Bacterial diversity associated with maize by plate counts.

Sample	Bacterial count (ufc g^{-1})	Reference
Root	6.68 - 7.45 x 10^1 (dry weight)	Buyer and Kaufman, 1996
Root	10^6-10^8 (fresh weight)	Di Cello *et al*., 1997
Root	6.20 ± 0.24 log (fresh weight)	Dalmastri *et al*., 1999
Root	8.55-9.01 log cfu	Chiarini *et al*., 1997
Root (inside)	3.4 log (fresh weight)	García-Reyna, *et al*.,
Root (washed)	5.56 log (fresh weight)	Picard *et al* 2000
Root (tissue)	10^5-10^9	Cavaglieri *et al*., 2005
Rhizoplane	10^9-10^{10}	Cavaglieri *et al*., 2005
Rhizosphere	6.12 log (dry weight)	Picard *et al* 2000
Rhizosphere	9 x10^7- 4x10^8	Gomes *et al*., 2001
Rhizosphere (Bt y no Bt)	8 x 10^3-3 x 10^4 (sporulating)	Brusetti *et al*., 2004
Rhizosphere (Bt y no Bt)	3 x 10^6-3 x 10^7 (fresh weight)	Brusetti *et al*., 2004
Soil	6 x 10^7- 5x10^8	Gomes *et al*., 2001
Tissue	2.2 x 10^3 N_2 fixing	Palus *et al*., 1996
Tissue	10^4 (fresh weight)	Palus *et al*., 1996
Root	9.5 x10^6 a 3.1 x 10^7	Pereira *et al*., 2011
Tissue root (inside)	5 x 10^4 a 2.8 x 10^6; 10^5	Pereira *et al*., 2011; Rai *et al*., 2006
Rhizosphere	4 x 10^6 N_2 fixing	Estrada de los Santos *et al*., 2001
Soil	4 x 10^5 N_2 fixing	Estrada de los Santos *et al*., 2001
Soil	10^3 *Azospirillum brasilense*	Espinosa-Victoria *et al*., 2006

ufc (units forming of colony)

bacterial diversity in some ecosystems has been determined and compared between samples belonging to different environments.

DNA polymorphism amplified arbitrarily (RAPDs)

Is performed by restriction analysis of amplified 16S rDNA using the fingerprints as Burkholderia cepacia has to show a high variability expressed 145 (80%) haplotypes in 180 isolated from maize and Euclidean distance calculation using the method of AMOVA (Dalmastri et al, 1999). Furthermore, soil type and climatic conditions have not changed the genetic structure of *Azospirillum brasilense* associated with maize in traditional systems and conservation tillage (Espinosa-Victoria et al, 2006).

Analysis of patterns of bacterial diversity by amplified ribosomal gen 16S rRNA (ARDRA)

Studies of molecular fingerprints as the 16S rRNA prove to be an alternative in the knowledge of soil bacterial communities (Gomes et al, 2001). The region of 16S ribosomal DNA obtained from each DNA bacterial strain amplified by the Polymerase Chain Reaction or (PCR), based on the mixture of reactants with initiators as fD1 and rD1 (Pereira et al, 2011) or T7 and SP6 (Chelius and Triplett, 2001). 1U Taq polymerase is added and 1x buffer solution with $MgCl_2$ and dNTP. Incubate in a thermo cycler with a suitable programming previously standardized and PCR products are observed on agarose gels after electrophoresis. Is digested with various restriction enzymes ie: DdeI, HaeIII, HhaI, HindI and MspI, AluI with 5 U of each restriction enzyme for 5 µl of amplified product and the mixture is incubated at 35°C for 3h. The restriction fragments are subjected to electrophoresis on an agarose gel 2.75-3% in 1X TAE buffer containing ethidium bromide and observed on Gel Imaging Systems (Espinosa-Victoria et al, 2009). For bacteria from maize and by PCR was enriched α-and β-proteobacteria or Gram-positive bacteria with high G + C followed by gel electrophoresis studies with temperature gradient (TGGE-PCR) and eubacterial primers (F984GC; r1378) (Gomes et al, 2001. The compared the restriction patterns, 86 ARDRAS have been found with restriction analysis in the rhizosphere and 14 in maize roots washed (Picard et al, 2000). The number of selected clones is insufficient to reveal all kinds of sequences in the library with coverage of only 70% (Chelius and Triplett, 2001). Furthermore, characterized by amplifying 16S rDNA with the enzyme AluI showing the same ARDRA to B. cepacia isolated from three cultivars of maize in fields Italians (Dalmastri et al, 1999). The genotype and age of maize plants have marked influence on the profile of the associated bacteria (Pereira et al, 2011).

Analysis of amplified ribosomal DNA restriction-ARDRA

Is performed to the PCR products, using as primers universal from bacteria like 5'AAGGAGGTGATCCAGCCGCA3 'and 5' AGAGTTTGATCCTGGCTCAG3 ', the first 799f and 1492r *Escherichia coli* among others (Chelius and Triplet, 2001; Di Cello et al, 1997). Subsequently restriction analysis with different enzymes are performed within them has been used for Hae III, HhaI, and AluI (Chelius and Triplett, 2001) The separation is performed on agarose gels at concentrations of 2.5% weight to volume to 80 V for 5h and comparing restriction patterns estimating the distance (Chelius and Triplett, 2001; Di Cello et al, 1997). The number of clones recognized for Pioneer 3751 maize has been between 1 and 70 ARDRA patterns (Chelius and Triplet 2001). Affiliation has been found between maize bacterial container clones of α-proteobacteria, β-proteobacteria, δ- and γ-proteobacteria, Gram positive bacteria and actinobacteria (Chelius and Triplet, 2001). There is a report of the amplification of 1450 bp and nine ARDRA patterns with the enzyme AluI coinciding with Burkholderia cepacia and Pseudomonas putida LMG 11351 PaW340 plus Burkholderias isolated from maize in Mexico (Estrada de los Santos et al, 2002; Reis et al, 2004)

Electrophoresis in denaturing gradient gel (DGGE)

Amplification of 16S rDNA is performed by PCR with 968f-GC and 140R initiators. The reaction is made with the DNA template of interest in Tris-HCl pH 8.3, $MgCl_2$, dNTPs and Taq polymerase with display products in 1.4% agarose with subsequent ethidium bromide revealed. DGGE is performed in a system where the PCR products are amplified directly in gels with 6% polyacrylamide in buffer solution TAE at pH 7.4, 40 mM Tris acetate, 20 mM sodium acetate, 1 mM EDTA containing urea as denaturing formamide gradient and varying from 45 to 65% and from 25% to 40% of 16S rDNA. The gels run for 15 h at 60°C and 16 h 100 V and 60 °C and 150 V. Following electrophoresis the gels are revealed by 30 min with SYBR Green I (Invitrogen-Molecular probes, SP, Brazil) and photographed under UV light. A matrix of data indicating the presence or absence of bands is constructed and examined by multivariate statistical analysis (Da Mota et al, 2008, Kandeler et al, 2002). DGGE profiles were used to construct a hierarchical dendrogram using cluster analysis (HCA). The technique has been revealed that the bacterial communities of the rhizosphere of maize are more affected by the stress of aluminum in the soil by the plant line (Al-sensitive and Al-tolerant). Rhizobial and Actinomycetales are the most abundant ribotypes in maize by DGGE. Furthermore rhizosphere populations are determined by soil conditions and not by the line or

maize variety. The data demonstrates that the plant influences the structure of the microbial community in the rhizosphere and is related to the following effect in the case of maize varieties tolerant to Al that increase exudation of organic acids such as citrate and malate, when they are grown in presence of Al (Da Mota et al, 2008). Besides using DGGE have shown high similitude between bacterial communities of transgenic with non-transgenic maize (Fang et al, 2005). 75% to 29 characterized by DGGE bands are shared ground maize rhizosphere and shows that about 10% of broad bands represent bacterial specificity in the rhizosphere (Kandeler et al, 2002). In addition, the community composition of ammonia oxidizing bacteria was found to be similar in the rhizosphere of wheat, beans and maize (Song et al, 2007).

Temperature gradient gel electrophoresis (TGGE)

Is a PCR with amplification of 16S DNA including DNA template. The reaction components (10 mM KCl, 10 mM Tris.HCl, pH 3), 0.2 mM dNTPs, 3.75 mM $MgCl_2$, 4% (w/v) acetamide, 100 nM of the primers and 2 U of Taq DNA polymerase in 25 µL according to manufacturer's instructions (Heuer and Smalla, 1997; Gomes et al, 2001) and is conducted with a gradient of 38-53°C for 16 h at 110 V. Silver is applied subsequently in acid medium, for the detection of DNA. The technique has been used to compare different maize varieties as the Nitrodent Nitroflint generated in Brazil and used as a model plant for exploring the resolving power of the gel electrophoresis technique with temperature gradient (TGGE), in analysis of fragments amplified 16S rDNA PCR. The enrichment of some bacterial species in the root of maize has been reported, predominantly α-proteobacteria in the rhizosphere of young plants also no differences were found in populations of rhizosphere of plants 20 and 40 days of growth. The authors demonstrated that the genus Arthrobacter genus decreases with age of the maize plant and showed the predominant TGGE-fingerprints were both soil at rhizosphere although for this one last with greater intensity (Gomes et al, 2001). The diversity and dynamics of bacterial populations in the rhizosphere of two cultivars of maize grown in a tropical soil has been determined by traditional culture techniques and molecular analysis based on DNA directly extracted from soil and rhizosphere 16S rRNA genes (Heuer and Smalla, 1997). The data provided are of use to assessing the potential effects of transgenic maize in the rhizosphere microbial communities (Gomes et al, 2001).

Mulilocus electrophoresis enzymes (MLEE)

MLEE data and plasmids patterns of *Rhizobium etli* from inside maize stem with a clear differentiation present in the rhizosphere, root or nodules of Phaseolus vulgaris (Rosenblueth and Martínez-Romero, 2004). In addition, Estrada de los Santos et al (2002), used as MLEE technique to determine the identity of the bacteria isolated from maize plants.

Conclusion

The future strategies in the study of the maize diversity include methods proposed by various researchers in the study of phenotypic diversity spend up to one genotype represented a major contribution to environmental microbiology associated with *Zea mays* L. Both methods can evaluate a large number of microorganisms present in the rhizosphere and other anatomic sites of maize plant. Some methods study culturable microbial organisms or non-cultivable in laboratory media, the according to some authors, representing 1% and 99% respectively of the total microbiota. Now the road is observed in this research should focus on elucidating the interactions that occur in ecosystems and our partners to plant in order to bring this knowledge to conservation and productivity improvement without impairment expense environmental. Future work may involve clonal analysis of bacterial communities associated with maize plants, including comparisons between single seed of endophyte origin, the rhizoplane, rhizosphere and soil,because these projects are interesting subjects of new knowledge.

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