Article

Diversity and Potential Function of the Bacterial Rhizobiome Associated to *Physalis ixocarpa* Broth. in A Milpa System, in Michoacan, Mexico

Ariza-Mejía Daniella[1†], Oyoque-Salcedo Guadalupe[1†], Angóa-Pérez Valentina[1*], Mena-Violante Hortencia[1], Álvarez-Bernal Dioselina[1] and Torres-García Jesús R.[1,2*]

[1] Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR) del Instituto Politécnico Nacional, Unidad Michoacán, Jiquilpan de Juárez 59510, México 1; darizam1900@alumno.ipn.mx (A.M.D.); goyoque@ipn.mx (O.S.G.); vangoa@ipn.mx (A.P.V.); hmena@ipn.mx (M.V.H.); dalvarezb@ipn.mx (A.B.D.); jrtorresg@ipn.mx (T.-G.J.R.)

[2] Investigadores por México, CONACyT.

† both author contributed equally to this publication

* Correspondence: vangoa@ipn.mx, jrtorresg@ipn.mx,

Abstract: Michoacan state has a long history in plant domestication's. *Physalis ixocarpa* is a native plant that growth associated to corn crops from this region. Such plants have similar fruits to *Physalis philadelphica* (husk tomate). Due to the domestication process includes the adaptation to environmental factors, we ask if 1) Does *P. ixocarpa* has the capacity of association with bacterial communities of the zone where it was domesticated?, and 2) Does the rhizobiome of this plant can increase the potential functions in the soil?. An experiment was established in a traditional milpa system. Samples of rhizobiome from corn, *P. ixocarpa, P. philadelphica,* and soil were sequenced using Next Generation Sequencing in the region 16S. The potential function, metabolic pathway reconstruction and participation of each bacteria genus was inferred using iVikodak platform. A total of 34 Phyla and 795 genera were identified. Purine metabolism's was the principal function, where all rhizobiomes showed similar metabolic pathways. However, the difference among plant species is the participation of the distinct genera in the purine metabolism. We conclude that rhizobiome of *P. ixocarpa* shows complementarity for the soil functions, and their utilization can be helpful in zones where the agricultural practices have degraded microbiological soil conditions.

Keywords: iVikodak; Balsas Basin; tomate milpero; native plants

1. Introduction

The Michoacan state in Mexico is inside to Balsas Basin, which has been recognized as one floristic region with a high number of endemic species [1]. Besides, the evidence indicates that such region is an important center of domestication of many important species as maize (*Zea mays* L.), bean (*Phaseolus vulgaris* L.), squash (*Cucurbita spp.*), husk tomatoes (*Physalis spp.*)[2], among others [3]. Moreover, the region presents a high diversity of climates, orographic conditions, and the presence of human groups for many centuries, conditions ideals for the domestication process [4].

In this zone, agriculture is one of the principal economic activities. However, the agricultural practices of the zone show marked differences in the technification level. For example, the strawberries and berries crops are equipped with greenhouses, irrigation systems, and many pesticides to eliminate insects, fungi, and weeds. On the other hand, in rural regions, the cultivation of maize for auto consumption based on the polyculture (maize intercropped with beans, squash, tomatoes) is already frequent; this type of agricultural system is knowledge as "milpa system" [5]. In such systems, the principal characteristics are utilizing remanent seed of the previous cycle, organic fertilization, and manual control weeds [3,5]. This type of practice drives the selection of genotypes adapted

to growth conditions such as temperature, humidity, precipitation, radiation (domestication process) [4].

Besides physiological, genetic, and morphological adaptations, it is probably that plants under the domestication process also is adapted to microbiota associated with roots. Plants and their respective microbiome maintain intricate cooperation to maintain the essential functions of the plants [6]. This microbiome can exert diverse functions; these include the degradation of toxic compounds, detoxification by enzymes, or participation in essential functions such as nutrient uptake, solubilization of minerals, and hormone synthesis [6]. Soil microbiomes show functional redundancy whereby multiple species play similar roles in the soil ecosystem [7]. The redundancy of functions is an important property of microbiomes; in the absence of a determinate bacterial genus, another genus can make such function [8]. This is important for zones where the application of herbicides, fungicides, or another type of xenobiotic has changed the structure of microbial communities [7].

An alternative for the restoration of soils where the application of agrochemicals has modified the structure of the bacterial communities of the soil is the introduction of native plants that are adapted to environmental conditions and conserve plant-microbe-soil interactions. For example, in the region of Balsas Basin, some species of the *Physalis* genus are present and probably had their center of origin and domestication [9].

In particular, *Physalis ixocarpa* Brot. ex Hornem is a species highly appreciated by producing edible fruits similar to commercial husk tomato (*Physalis philadelphica* L.). The differences between species are the size of fruits (*P. ixocarpa* fruits are smaller than *P. philadelphica*), and the organoleptic characteristics that confer to P. ixocarpa a sweet flavor that is preferred by local people [10]. In this sense, the market price of *P. ixocarpa* fruits is 5 to 6 folds the cost of commercial husk tomatoes. This plant grows inside the maize crops and is tolerated by the farmers that avoid eliminating such plants during the manual weeding; for this reason, the local people call them "tomate milpero". These plants cover the soil surface and reduce the light caption of another weed. In the region exist documented studies that describe the genetic diversity and show evidence of human selection [10].

Metagenomics methods based on the 16s sequencing allow the identification of genera presents in a sample, know the abundance, richness, and diversity of bacterial communities [11]. Moreover, some methods based on identifying the potential function of microbiomes have been developed; these tools can be helpful to identify the participation of the different plant species in soil bacterial diversity in a milpa system [12].

Due to *P. ixocarpa* being a native species of the region, we ask if 1) Does *P. ixocarpa* maintain the capacity of association with bacterial communities of the zone where it was domesticated? 2) the presence of *P. ixocarpa* in the milpa system can increase the potential functions in the soil? 3) Exist differences among *P. ixocarpa* and the domesticated husk tomatoes (*P. phliladelphica*) in the capacity of bacterial association?. The results can give a evidence if *P. ixocarpa* plants can be used as a reservoir of bacterial communities native of the zone and can be used to restore contaminated soils that have lost functions by the excessive application of pesticides.

Our objectives were: 1) Determinate the differences in the rhizobiomes among two *Physalis* species (*ixocarpa* and *philadelphica*), the maize (*Zea mays*), and the soil without plants. 2) Estimate the differences in the potential function of the rhizobiomes of three plant species in a milpa system (maize, *P. ixocarpa*, and *P. philadelphica*) with respect to soil without plants. We expect that *P. ixocarpa* can harbor in their roots some bacterial species a high number of genera presents in the soil and harbor genus that no are present in the other plants. This can be an indication of the adaptation to soil conditions and the potential use of this plant to introduce new functions to the milpa system.

2. Materials and Methods

2.1. Description of the study area

The experiments were established in the municipality of Jiquilpan, Michoacan. This municipality is ubicated at 1600 masl in the northwestern of the Balsas Basin. The climate of this region is semi-arid; the annual average temperature is 19 °C, and the annual precipitation of 800 mm. In this municipality, the agricultural activity is not extensive, the production of most farms is dedicated to own-consume, and the sown of traditional red corn is common. These farms associate many species inside the crop, such as squash, bean, and husk tomatoe and tomatoe milpero. The farm selected does not use herbicides as a weed control method, and the presence of some weeds is allowed because such species are consumed as medicinal or condiments.

Previous to the establishment of the experiments, during the land preparation for the sown, a sample of soil was analysed for physicochemical analyses (texture, organic matter, pH, electric conductivity, apparent density, total Nitrogen, Phosphorus (Bray y Kurtz), and Potassium), and the germination of seedlings. The soil samples were processed in the soil laboratory in the CIIDIR-IPN, Unidad Michoacán. The experimental design had four treatments: Soil without roots (hereafter soil), rhizospheric maize (*Zea mays*), *P. ixocarpa* and *P. philadelphica*, each treatment had four replicates.

Seeds of *P. ixocarpa* and *P. philadelphica* were sterilized with hypochlorite of sodium at 10% for 10 minutes and washed with distillate water; this process was repeated three times. Next, the seeds were placed in Petri dished with filter paper and distillate water. When the apparition of the radicle was superior to 3 mm, the seedlings were placed in pots that contained the soil from the study site. Seedlings were maintained with constant soil humidity until plants grew to 10 cm. A the moment of the planting, the maize presented 40 cm of height. Seedlings were planted inside the crop site avoiding contact with other plant species such as maize or weeds. Seedling survival was monitored during the crop cycle, and the germination of weeds around plants used for the study was avoided.

2.2. Total DNA extraction and metagenomic sequencing

Collection of rhizospheric soil occurred at the flowering of three studied species, maize and *P. ixocarpa*, and *P. philadelphica*. Samples were taken at a depth of 30 cm. The soil near the roots was taken and introduced to Eppendorf tubes of 2 mL. A DNA shield was added to each tube to preserve the samples' DNA. The samples were maintained to -20 °C until DNA extraction. The DNA extraction was performed using a commercial kit for microbiome DNA-extraction (ZymoBIOMICS 96 DNA Kit) following the instructions of the fabricant. The quality of samples was verified by agarose gel and nanodrop quantification. Samples with a ratio of 1.8 to 2.0 (260/280 nm relation) were used for NGS sequencing. The 16S-V3-V4 region was sequenced in HiSeq platform PE300 by BGI Americas Corp.

2.3. Bioinformatic analyses

Raw data were processed in Mothur [11], where the data were trimmed, screened, aligned sequences, calculated distances, assigned sequences to operational taxonomic units using the Silva database. The remotion of potential chimeras was made with VSEARCH [13] in the same environment (Mothur). The final result of Mothur was a spreadsheet in format CSV where all Phyla, Class, Order, Family, and Genera of all samples are listed with the respective abundances. Abundance analyses were made at Phyla and Genera levels, at genera level, the diversity indices of Jaccard, Simpson and Shannon-Wiener were estimated. Exploratory data analyses were performed to determine the phylogenetic diversity and detect exclusive and shared Phylum and Genera among treatments.

IVikodak platform [12], https://web.rniapps.net/iVikodak/) was used for Inferred Functions of all microbiomes studied. The modules used were Global and Local mapper.

Global Mapper was used to identifying the most important functions according to KEGG (Kyoto Encyclopedia of Genes and Genomes, https://www.genome.jp/kegg). The functions more important were analyzed, and the principal sub-levels were arranged in relation to importance (number of functions). The Local mapper module inferred the participation of each genus and the metabolic pathway reconstruction in the primary metabolic process. Heatmap and Venn diagrams were used to select the most important functions and separate the most important genus in the metabolic participation in metabolic pathways.

For the Reconstruction of the most important pathway in each treatment, the information of the Local mapper module was uploaded to KEGG Mapper Module (https://www.genome.jp/kegg/mapper) to visualize the respective Color Pathway Maps. Exploratory data analyses, tables, and graphs were performed using RStudio (ver. 2021.09.1)

3. Results

3.1. Soil determinations

According to physicochemical analyses performed on Soil, this is clay loam, with content of silt medium and low salinity. In the same way, the contents of N, P, and K are in medium levels (Table 1). Therefore, this Soil does not show degradation caused by extensive agricultural practices.

Table 1. Physicochemical parameters of the soil farm previous to the study establishment.

Parameter	Value
Texture	40% Clay, 30 % Silt, 30% Sand
Organic Matter (MO)	1.6 %
pH	6.4
Electric Conductivity	1.9 dS m^{-1}
Aparent Density	1.09 g cm^{-3}
Total Nitrogen	0.096%
Phosphorus (Bray y Kurt z)	17 mg kg^{-1}
Potassium (K)	0.7 Cmol kg^{-1}

3.2. Structure of bacterial communities

Jaccard index showed that the of the microbiome Soil and maize rhizobiome are more similar (80% of similarity) than the two *Physalis* species. Contrary to expected, the *P. ixocarpa* rhizobiome is more similar to maize than *P. philadelphica* (76 versus 67% of similarity, respectively). In the case of similarity of maize and both *Physalis* species studied, we did not find differences (Table 2).

Table 2. Distance matrix of distance according to Jaccard index among rhizobiomes of Soil, *P. ixocarpa, P. philadelphica,* and *Zea mays* in a milpa system in Mexico. Under diagonal represent Jaccard distance, Below diagonal are the standard error.

	Soil	*P. ixocarpa*	*P. philadelphica*	*Zea mays* L.
Soil	*	0.001	0.001	0.001
P. ixocarpa	0.71	*	0.001	0.001
P. philadelphica	0.73	0.67	*	0.001
Zea mays	0.8	0.76	0.76	*

The two diversity indices estimated (Simpson and Shannon-Wiener), Soil had the lowest diversity in index Simpson ($p < 0.05$, Table 3). In the other treatments, we did not find statistical differences among them. Shannon-Wiener index showed a contrary trend;

in this index, the Soil is the most diverse, and no statistical differences were found among the other treatments.

Table 3. Simpson and Shannon-Wiener indices estimated for the bacterial rhizobiomes of Soil, *P. ixocarpa*, *P. philadelphica*, and *Zea mays* in a milpa system in Mexico. Mean ± standard error (n=4). Different letters indicates significant differences (Tukey ≤ 0.05)..

	Simpson	Shannon -Wiener
Soil	0.00414 ± 0.000167 b	6.83 ± 0.0448 a
P. ixocarpa	0.00927 ± 0.00103 a	6.43 ± 0.0345 b
P. philadelphica	0.00705 ± 0.000403 a	6.46 ± 0.0625 ab
Zea mays	0.00716 ± 0.000244 a	6.4 ± 0.123 b

Concerning phylogenetic diversity at the Phylum level in the different treatments, we found 34 Phyla. Figure 1A shows the 19 Phyla with higher abundance; the phyla that do not represent the least of five percent of abundance in the samples and not for this figure (The complete list of taxonomy is contained in the supplementary material). In all samples, the three principal Phylum in abundance were Proteobacteria (around 27%), Acidobacteria (23 to 25%), and Actinobacteria (12.4 to 14.7%). The taxonomic analyses were unclassified around 10 to 13% of bacterias (Figure 1A). The order in the abundance of the subsequent phyla had variations among treatments. The Bacteroidetes phylum was the fifth more abundant, followed by Planctomycetes for soil conditions. In the rest of the treatments, a swift tendency was observed.
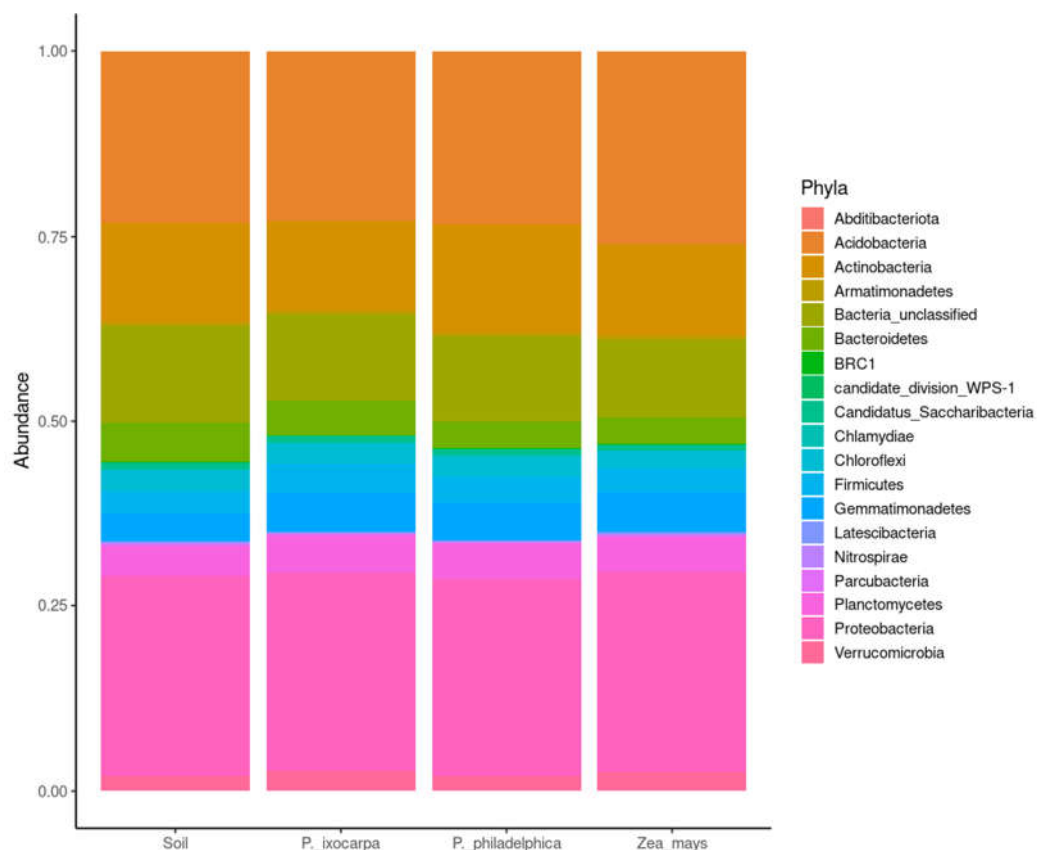

Figure 1. Relative abundance of Phylum presents in different rhizobiomes in a traditional milpa system in Michoacan state, Mexico.

The number of shared Phyla among treatments was 21 (Figure 2). However, some treatments showed the presence of exclusive Phyla as the Soil that presents two exclusive

Phyla: Campilobacterota and Tenericutes; *P. ixocarpa*: Thermotogae, *P. philadelphica*: Cloacimonetes; and *Zea mays*: Cyanobacteria/Chloroplast. In addition, *P. ixocarpa* was the unique species that shared an exclusive association with Soil of one Phylum: Microgenomates.
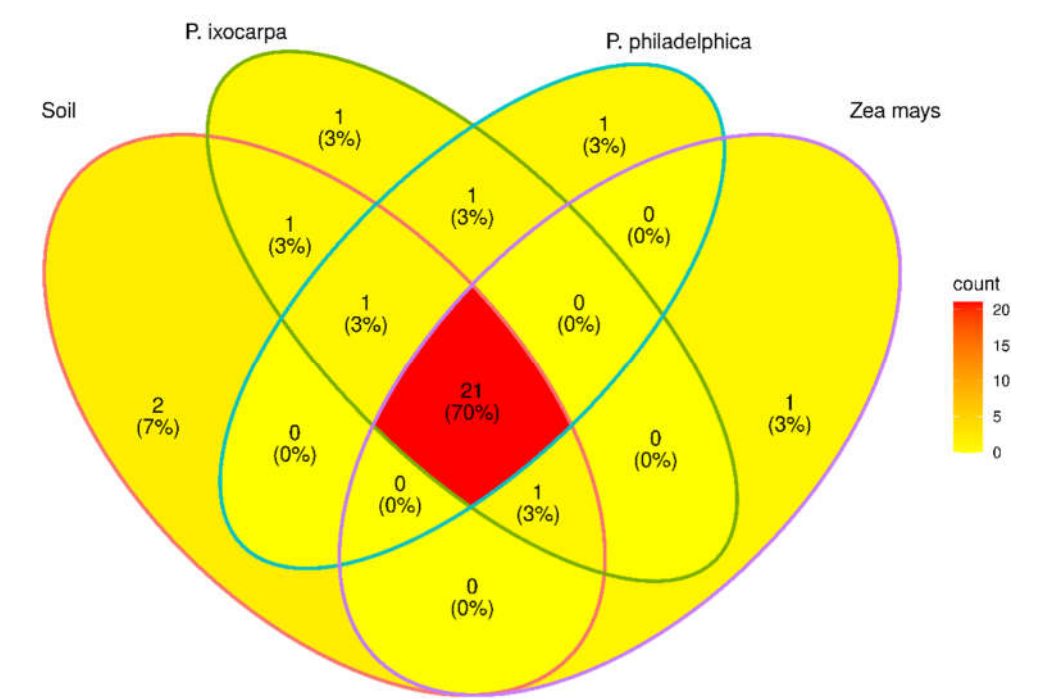

Figure 2. Venn diagram of Phyla found in different rhizobiomes in a traditional milpa system in Michoacan state, Mexico.

At the genera level, a total of 795 genera were identified; of this, 386 are shared among the treatments (Figure 3). The Soil had the highest number of exclusive genera (87 genera), followed by *P. philadelphica* (50 genera), *Z. mays* (38 genera), and *P. ixocarpa* (37 genera). *P. philadelphica* had the highest number of genera exclusive association with Soil (29). On the other hand, *P. ixocarpa* had more genera in exclusive association with maize than *P. philadelphica* (12).
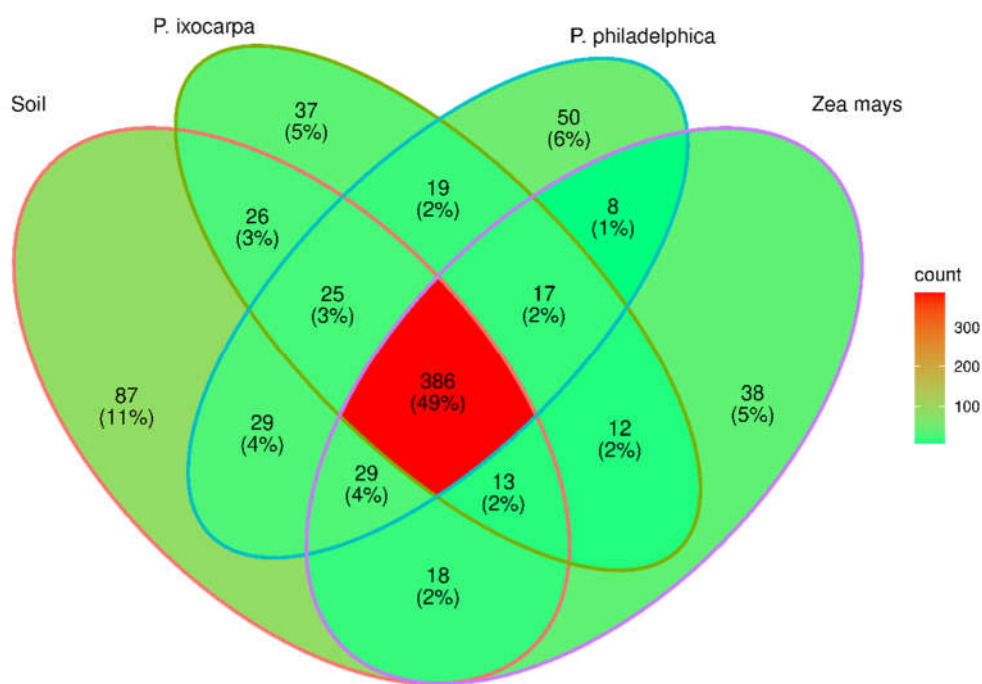
Figure 3. Venn diagram of genera found in different rhizobiomes in a traditional milpa system in Michoacan state, Mexico.

3.3. *Inferred Functions of bacterial on the system*

The inferred functions of microbiomes are presented as the abundance of function according to KEGG pathway categories. In all treatments, the metabolism is the most represented (166 022 functions for maize), followed by Human diseases. The least abundance of functions was found for Environmental Information Processing (2500) (Figure 4). In the metabolic functions, maize had the highest abundance function for metabolism with respect to other treatments.
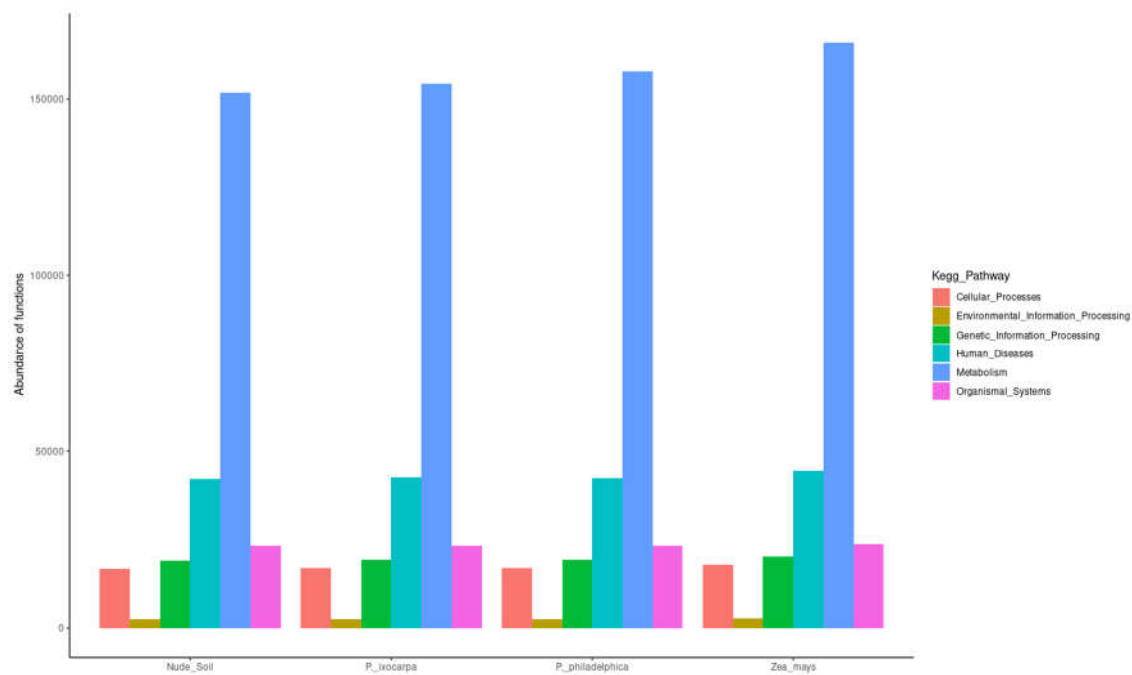
Figure 4. Abundance of functions inferred by Ivikodak for rhizobiomes in a traditional milpa system in Michoacan state, Mexico.

The most important functions in the metabolism are represented in a heatmap (Figure 5). The heatmap was constructed with the 30 functions more important according to the global mapper module by iVikodak. The superior dendrogram shows two groups, the first where Soil is the unique treatment and shows considerable distance with respect to other branches. The second branch includes the rhizobiomes of three studied species, and both *Physalis* species were grouped as sister groups. The lateral dendrogram on the right side shows the grouping of metabolic functions. The dendrogram shows the formation of two principal branches. The down branch contains only the Purine metabolism function. This function was the most important for all rhizobiomes studied. The superior branch is represented by amino acids and nucleic acid pathways, principally.
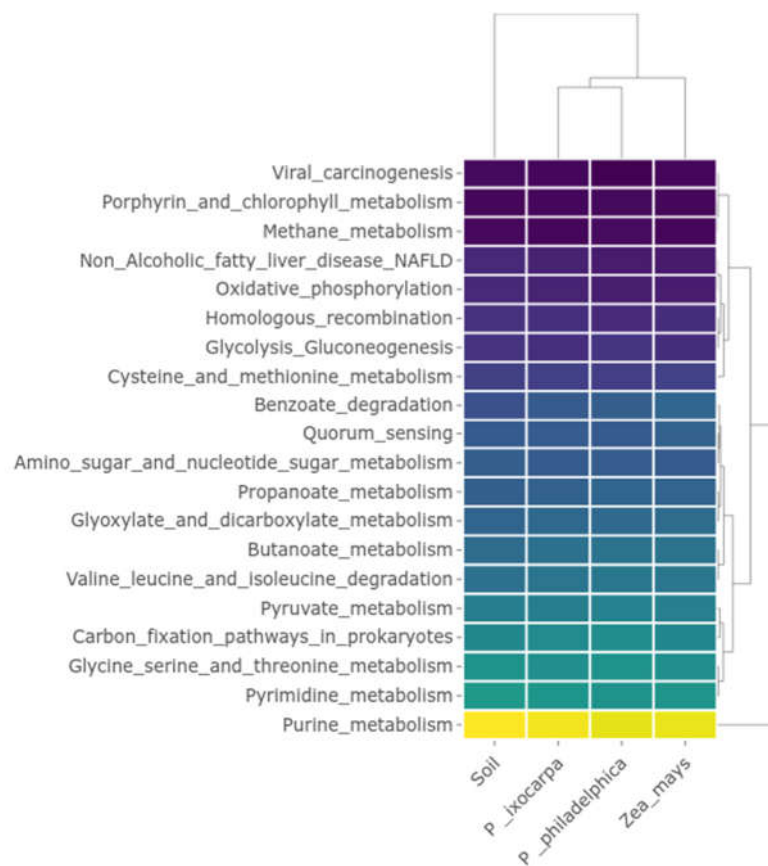
Figure 5. Heatmap of the 20 principal metabolic pathways by Ivikodak for rhizobiomes in a traditional milpa system in Michoacan state, Mexico.

To obtain more details about the Purine metabolism in the rhizobiomes, we used the local mapper module of Ivikodak to estimate the participation of each genus and reconstruct this metabolic pathway. The participation of the genera in Purine metabolism was different among species. One hundred genera were shared in all treatments; Soil had 161 genera (20 exclusives), *P. ixocarpa* 151 (11 exclusives), *P. philadelphica* 159 (14 exclusives), *Z. mays* 145 (13 exclusives) (Figure 6).

The importance of each genus in the participation in Purine metabolism is distinct in each treatment. Table 4 shows the genera that participate with the 50% for purine production. The genus *Sphingomonas* was the most important for *Zea mays* and Soil (14.91 and 11.97%, respectively), and *Sphingobium* was the principal genus in the purine metabolism in *P. ixocarpa* and *P. philadelphica* (14.5 and 13.93%, respectively). The genera *Nocardioides, Streptomyces, Pseudonocardia*, and *Solirubrobacter*, were shared among treatments; however, the participation of such genera is limited, the percentages of participation are around 2.9 as minimum and 5.56% as maximum. The majority of the purine metabolism was distributed in different genera, and each species showed distinct genera for this (Table 4).
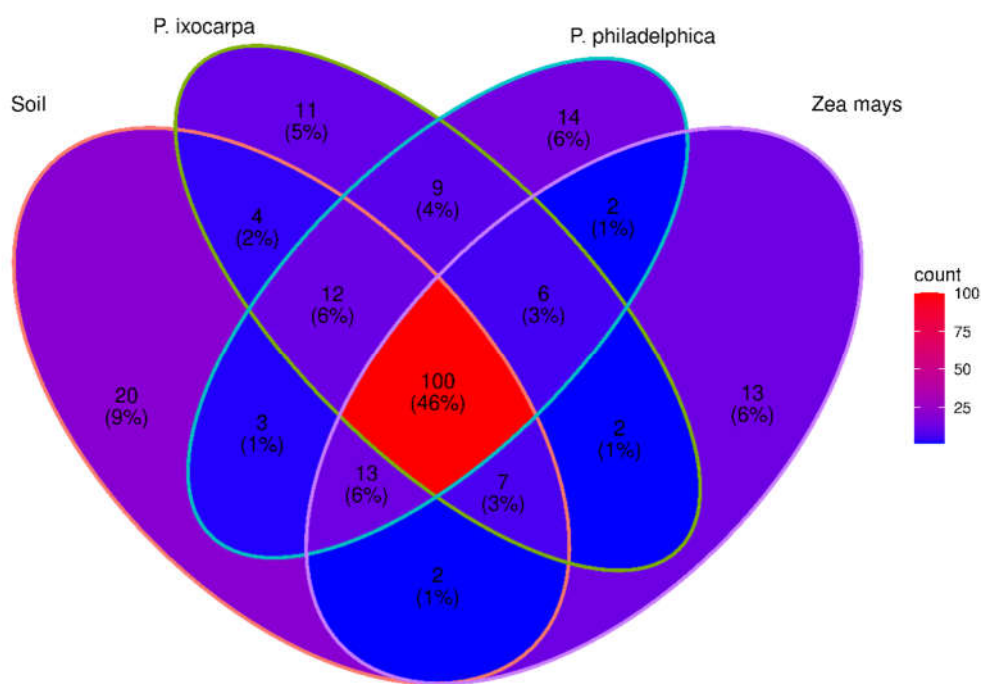
Figure 6. Venn diagram of genera found that participate in Purine metabolism pathway in a traditional milpa system in Michoacan state, Mexico.

Table 4. Participation of genus in the Purine metabolism (50% of the total) infered by Local maper of Ivikodack.

Soil			P. ixocarpa			P. philadelphica			Zea mays		
Genus	%	cumulated percentage	Genus	%	cumulated percentage	Genus	%	cumulated percentage	Genus	%	cumulated percentage
Sphingomonas	12.0	12.0	Sphingobium	14.5	14.5	Sphingobium	13.9	13.9	Sphingomonas	14.9	14.9
Nocardioides	5.6	17.5	Archangium	4.8	19.2	Pseudorhodoferax	6.3	20.2	Ramlibacter	5.5	20.4
Streptomyces	4.8	22.4	Skermanella	3.8	23.0	Streptomyces	5.7	25.9	Pseudonocardia	4.5	24.9
Ramlibacter	3.6	26.0	Pelomonas	3.6	26.6	Solirubrobacter	3.9	29.8	Nocardioides	4.0	28.8
Pseudonocardia	3.6	29.5	Streptomyces	3.6	30.2	Archangium	3.8	33.6	Streptomyces	3.9	32.8
Reyranella	3.0	32.5	Solirubrobacter	3.5	33.8	Nocardioides	3.8	37.4	Solirubrobacter	3.8	36.6
Paenibacillus	3.0	35.5	Nocardioides	3.4	37.1	Skermanella	3.4	40.8	Reyranella	3.4	40.0
Solirubrobacter	2.9	38.4	Nitrospira	3.2	40.3	Flavisolibacter	3.3	44.1	Singulisphaera	3.1	43.1
Labilithrix	2.8	41.2	Tumebacillus	3.1	43.5	Pseudonocardia	3.1	47.2	Flavisolibacter	2.7	45.7
Singulisphaera	2.3	43.5	Flavisolibacter	3.0	46.4	Nitrospira	2.6	49.8	Phenylobacterium	2.6	48.3
Massilia	2.3	45.7	Pseudonocardia	2.9	49.3						
Chryseolinea	2.2	48.0									
Lysobacter	2.2	50.2									

For the reconstruction of the Purine metabolism pathway (map00230), Ivikodack estimates the participation in 97 enzymes. The metabolic map of each treatment does not show marked differences among them (Figure 7). Results showed that the principal participation of enzymes is principally in the Pentose phosphate pathway, alanine, aspartate and glutamate metabolism, thiamine metabolism, histidine metabolism, riboflavin metabolism, folate biosynthesis, and glyoxylate metabolism. For glycine, serine, and threonine metabolism no participation of enzymes was detected in any treatments.
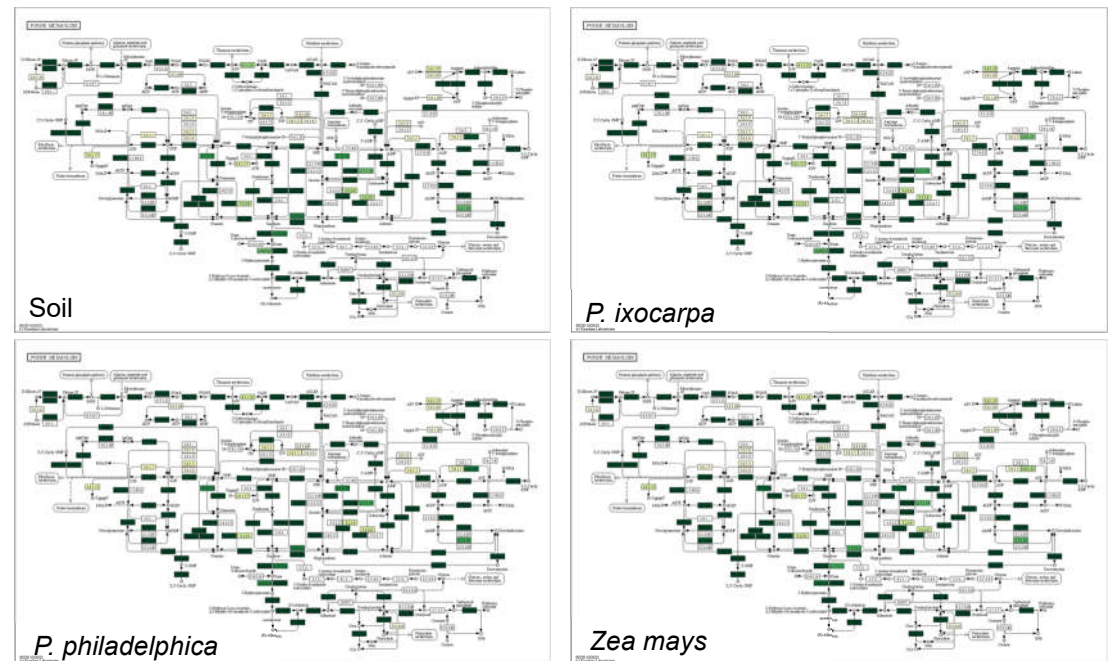
Figure 7. Metabolic reconstuction of Purie pathway infered by Ivikodack for rhizobiomes present in a traditional milpa system in Michoacan state, Mexico.

4. Discussion

The rhizobiomes of the plants present in the milpa system studied showed differences in structure and diversity. Each species of *Physalis* genus had Phyla and genera exclusive, also the exclusive association with Soil and maize. According to the Jaccard index, such differences had a 33% maximum of differentiation. However, the rhizobiomes of all plants and Soil had the metabolism of Purine as principal functions, and each plant species preferred the distinct genera for this function. This can result from adaptive evolution caused by the domestication process in the milpa systems, which results in the complementarity of functions among plants and microbiomes in this ecological crop system. This scope can be employed to introduce native plants as *P. ixocarpa* in degraded agricultural Soils with lost metabolic processes by applying agrochemicals in extensive agriculture systems.

The initial soil conditions showed an excellent conservation state with medium pH and nutrient concentration (N, P, and K). These conditions allow the establishment of soil microbes that is an essential component in the soils due they are involved in nutrient cycling, thus maintaining soil fertility [14]. Some estimations of the distribution of bacteria in Soil assumes that pH, nutrient concentration, and C/N ratio are essential to predict their potential diversity. It has been well documented that fertilizers and pesticides affect the soil micro-organisms [15]. The agricultural practices in the region include the application of organic fertilizers and the manual control of weeds avoiding the addition of agrochemicals, thus maintaining the soil properties for bacterial proliferation [14].

In terms of diversity measured with the Simpson index, Soil showed the lowest diversity; however, the Shannon-Wiener index considered the Soil the most diverse microbiome. Low values in Simpson are due to the high proportion of a low number of species with high abundance and many species with low abundances. The Soil had an increased number of exclusive genera that are low abundant; this index is sensitive to differences in dominance. However, in general terms, the Simpson index in the milpa systems studied is superior to reported as global media estimate (0.003) [16]. On the other hand, Shannon-Wiener places a greater weight on species richness [17]. In this case, the Soil had the highest diversity value with respect to other treatments.

The phylogenetic diversity of the rhizobiomes showed differences among them; the Soil and maize had the highest similarity (80%). Molecular and anthropological evidence

indicates that maize probably had its center of origin and domestication in the Balsas Basin; thus, it can explain that the root physiology is also adapted to the region's soil microbiome [18, 19]. The same case occurs in the *Physalis spp*. Evidence indicates that the genus *Physalis* has been domesticated in this region [9, 20,21]; for this reason, the presence of some Phyla with exclusive association with Soil and maize can be explained.

The phylogenetic diversity found in this study corresponds to reported for farms that have been cultivated under organic agriculture methods in other regions of the world [22, 23]. The Phylum Proteobacteria is the most abundant in the soils due to its capacity to generate Antibiotic Resistance Genes (ARG) to defend Fungi. In this phylum, some genera are responsible for nitrogen fixation [24]. Nevertheless, this phylum includes pathogenic genera, such as *Escherichia, Salmonella, Helicobacter*; however, the presence of these genera is reduced in abundance. Another important phylum in abundance and functionality is Acidobacteria; species of this phylum can be found in soil and decomposing wood. This phylum is particularly abundant in soil habitats representing up to 52% of the total bacterial community. The third phylum in abundance was Actinobacteria; this phylum has ecological and economic importance. Genera of this phylum help decompose the organic matter, are symbiotic, and fix nitrogen. Also is reported that it contributes to the biological buffering of soils and produces many antibiotics. According to the dominance of Phyla, we assume that this soil maintains the conditions for the correct development of plants and bacterial communities, due to that when existing contaminants affect the soil, the dominance of Phyla changes. For example in deposit uranium, the proportion of phyla was Firmicutes (24.2%), Fusobacteriota (23.0%), Proteobacteria (18.7%), Actinobacteriota (15.5%) and Bacteroidota (9.0%) [22].

The presence of exclusive Phyla in plant rhizobiomes is an important trait because seeds contain seed-borne bacteria compatible with soil conditions of the Balsas Basin region. Such bacteria are compatible with the region's soil conditions and can establish reservoirs of beneficial bacteria that improve the soil functions where such plants are introduced [25]. In this sense, the two species exclusive of soil have important functions in the biogeochemical cycles in the soil, Campilobacterota Phylum has been associated with functions in sulfide-oxidizing [26], and Tenericutes have functions in carbohydrate storage carbon fixation [27]. In the same way, the *Physalis* species had an exclusive association with Phyla that are involved in functions of stress resistance and metabolization of toxic compounds. The Thermotogae Phylum found exclusive in the *P. ixocarpa* roots shows tolerance to adverse conditions such as salinity or temperature and is also involved in the metabolization of carbohydrates [14]. On the other hand, *P. philadelphica*: had Cloacimonetes as an exclusive phylum; this phylum has been recently described in syntrophic degradation of propionic acid, a compound toxic for bacterial communities. *P. ixocarpa* was the unique species that shared an exclusive association with the Soil of one Phylum: Microgenomates. About this Phyla, no information has been found because the presence of this has been obtained with NGS. At this moment is not possibly cultivated in lab conditions. However, the first report of this phylum was in a Yellowstone Hot Spring [28], which shows extremophilic characteristics.

The functions found in the core microbiome in this study were focused on the primary metabolism. Purine pathway had a remarked preference in the metabolic functions. This pathway is involved in essential functions in the metabolism, such as the synthesis of amino acids such as histidine, alanine, aspartate, and glutamate, and other essential compounds such as thiamine, riboflavin, and folate biosynthesis. Another important pathway in the Purine metabolism is the Pentose phosphate pathway. This pathway maintains carbon homeostasis, provides precursors for nucleotide and amino acid biosynthesis, reduces molecules for anabolism, and defeating oxidative stress [29]. Finally, we also found evidence of sugar metabolism through Glyoxylate metabolism.

Using the same methods (Ivikodak) to evaluate the potential function of diverse environments contaminated with uranium, petroleum hydrocarbons, or Polystyrene, the principal functions are more related to the degradation of xenobiotics [22, 30, 31]. For example, in the case of uranium deposits in Russia, the main functions were: pathways of

carbohydrate, nitrogen, and sulfur metabolism, degradation of xenobiotics, benzoate, polycyclic aromatic hydrocarbons, and chlorinated organic compounds [22]. On the other hand, when the cause of contamination of soil was the petroleum hydrocarbons, the bacterial communities had a higher abundance of xenobiotic biodegradation, in specific: xylene, benzoate, toluene, halogenated compounds, fluorobenzoate, chloroalkane, chlorocyclohexane, and chlorobenzene [30].

The principal function of all studied microbiomes was the Purine metabolism, specifically the metabolism. However, the list of the most important genus in the Purine metabolism showed that the principal genus involved in this function is different according to each microbiome. The genus *Sphingomonas* was the most important for Soil and *Z. mays*. However, this genus only participates in 12 to 15 % of Purine production. In addition, the average participation in all genera was around 3 to 4%. This means that not only one genus is the most important and that exists a redundancy for this function. Redundancy is a concept that has been employed in microbial communities to indicate that a community contains multiple species that serve similar roles [8]. This has been described as insurance for events where the diversity can be affected by external conditions that eliminate a part of the bacterial community. In this sense, bacteria from *P. ixocarpa* or *P. philadelphica* can be used in cases where the diversity of soil has been reduced by applying pesticide that modify the bacterial communities [32-34]. This complementarity can result from the domestication process that has been developed for many years and the adaptive continuum process of these plants in a milpa system.

The metabolic maps of Purine metabolism did not show marked differences in the enzyme participation in each species even when the metabolism participation of each genus was different among treatments. Soil microbial enzymatic activities are the indicators of soil biological health, fertility, and chemical status [14].

In the milpa systems studied the presence of native plants showed a complementarity in functions compatible with the biological functions in the Soil. The use of native plants for soil restoration is a viable alternative in zones where the agricultural practices have modified the structure of microbial communities. In addition, *P. ixocarpa* is a plant that represents a genetic resource for the production of edible fruits that represents an income alternative source and can help to maintain the metabolic functions in the soil where is planted.

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