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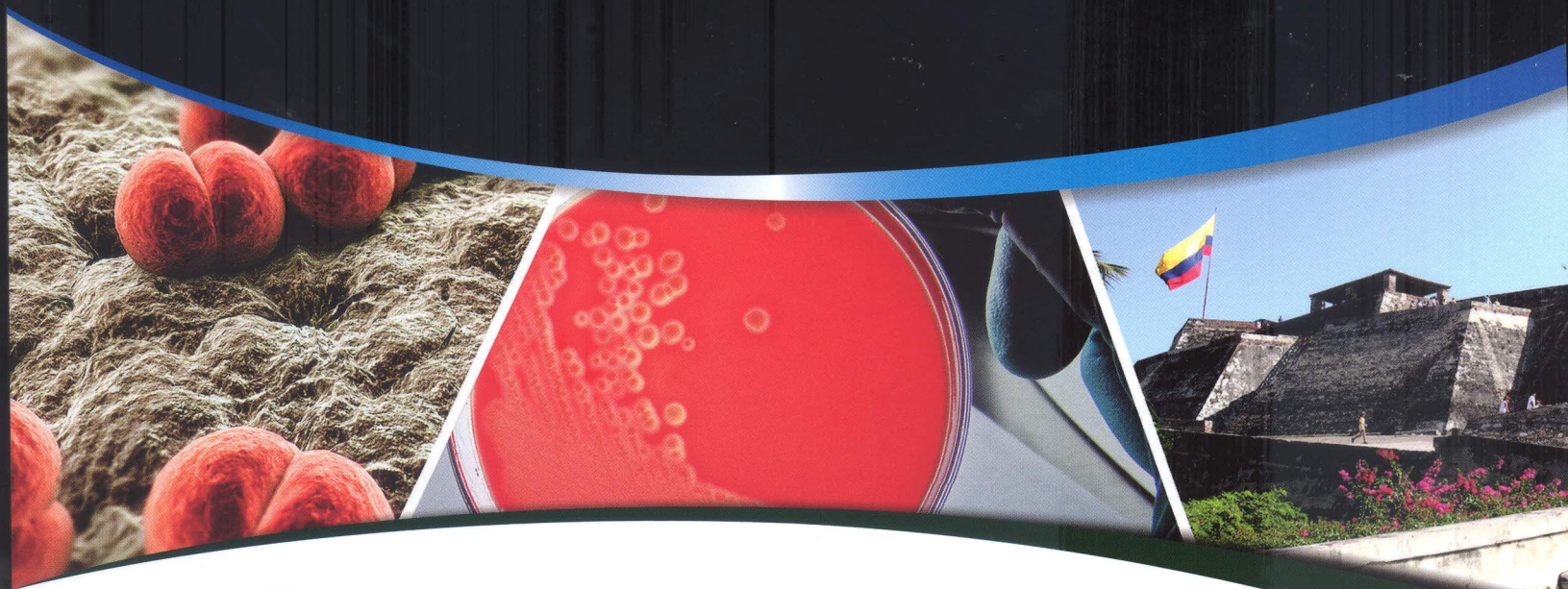

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te trabalho tem como principal objetivo avaliar e comparar microbiologicamente a água de poços rústicos em bairros do município de breu branco/pa, sendo coletada uma (01) amostra por bairro, totalizando dez bairros.
Materiais e métodos. Para a determinação dos coliformes totais e termotolerantes presentes na água analisada, utilizou-se a técnica do número mais provável (nmp), cujas etapas consistiram em: teste presuntivo (caldo LST – Caldo Lauril Sulfato Triptose), teste confirmativo para coliformes totais (caldo VB – Caldo Verde Brilhante) e teste confirmativo para coliformes fecais/termotolerantes (caldo EC – Caldo *Escherichia coli*).
Resultados. Todas as amostras dos bairros do centro do município (4, 100%) apresentaram resultados negativos para ambas as análises, coliformes totais e fecais, respectivamente com valores inferiores a 0,3 nmp/mL e ausência, conforme preconiza a referida legislação. Todas as amostras da periferia (6, 1000%) foram positivas para coliformes totais apresentando valores entre 0,91 nmp/mL e 16 nmp/ml. Destas, duas amostras (2, 33,3%) foram positivas para coliformes termotolerantes com valores de 0,36 nmp/mL e 0,91 nmp/ml, mostrando-se fora dos padrões estabelecidos por conterem coliformes de origem fecal.
Conclusões. Conforme as análises obtidas, sugere-se um tratamento adequado da água para o consumo humano nos bairros analisados da periferia, bem como medidas sanitárias e de higiene.

TLP-131. Micro-organismos promotores do crescimento vegetal na aclimatização de plantas de mangabeira micropropagadas

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Introdução. A inoculação de micro-organismos promotores do crescimento vegetal, na fase de aclimatização de mudas, favorece sua formação precoce, assim como maior crescimento e uniformidade do sistema radicular e da parte aérea. A mangabeira (hancornia speciosa gomes) se destaca como uma promissora árvore frutífera para programas de exploração sustentável.avaliar o efeito da inoculação de microorganismos solubilizadores de fosfato (msf) e do fungo micorrízico arbuscular (fma) *Glomus clarum*, na aclimatização de plântulas micropropagadas de mangabeira, sob diferentes substratos, na presença e ausência de câmara úmida.
Materiais e métodos. Plântulas de mangabeira micropropagadas foram aclimatizadas em três substratos: solo; solo + areia + vermiculita (1:1:1) e Bioplant®, na ausência ou presença de câmara úmida, em vasos e com quatro tratamentos de inoculação: msf; fma; msf + fma e controle.
Resultados. As plantas de mangabeira inoculadas com fma, quando aclimatizadas em câmara úmida, utilizando Bioplant®, tiveram maior formação de raízes adventícias e folhas expandidas. As plantas inoculadas com msf tiveram menor comprimento de parte aérea e formação de folhas expandidas, porém, maior formação de raízes adventícias e volume destas no Bioplant®. Em solo + areia + vermiculita, as plantas obtidas estavam menores, com folhas expandidas e menor número de raízes adventícias. As plantas aclimatizadas em solo + areia + vermiculita + fma continham folhas expandidas menores com pigmentação vermelha e formação de raízes, enquanto que em solo + areia + vermiculita + msf, as plantas eram parecidas com as descritas anteriormente, porém o volume de raízes foi menor. A co-inoculação de fma + msf se sobressaiu, na ausência de câmara úmida.
Conclusões. A inoculação de fma e msf foi adequada para aclimatização de plântulas micropropagadas de mangabeira com a utilização de câmara úmida, utilizando os substratos Bioplant® e solo + areia + vermiculita.

TLP-132. Isolation and production of cellulose by filamentous fungi isolated from composting process at São Paulo zoo park foundation

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Introduction. There is a large search of microorganisms for producing hydrolytic enzymes like cellulases that can be employed as an increment for second generation bioethanol processes. Composting organic waste process represent a promising habitat to recovery microorganisms with special capabilities. The improvement of this process with sugar cane filter cake as substrate can enhance the chances to have efficient microorganisms with cellulolytic potential. The present study had as goals the isolation and screening of fungi strains with cellulase activity from the composting process at São Paulo zoo park foundation (fpzsp).
Materials and methods. The composting cells were filled by sugar cane filter cake mixed with soil, trees and leaves debris from the atlantic forest park. The first sampling was done in august 2012. After twenty and forty days, it was collected the second and third sampling. About 10 grams of composting soil were collected. The samples were serially diluted in sterilized distilled water. Aliquots of 100 µl were inoculated onto petri dishes containing the media mea and pda. The petri dishes were incubated at 28 °C during 1-4 weeks. The obtained isolates were conserved by cryopreservation (-80 °C) and castellani (4°C). Micromorphology analyses were done. Enzymatic screening was done by culturing of strains into 50 mL of liquid medium me supplemented by 10 g l-1 Celuflok 100®. The activity was quantified by the using of the commercial kit azo-cm-cellulose (Megazyme®).
Results. One hundred and ninety filamentous fungi were isolated. Most of them were affiliated to phylum ascomycota followed by Zygomycota. Sixteen strains were selected as efficient cellulase producers. The strain fpzsp3 15 (not identified), showed cellulase activity of 70.80 u ml^{-1}; followed by the fpzsp3 1 (70.60 u ml^{-1}), identified as *Aspergillus sp*.
Conclusions. The results showed the importance of filamentous fungi from composting and their potential for future use in second generation ethanol production.

TLP-133. Comparative characterization of the archaeal community in the amazon forest: Native forest and oil palm plantation by high-throughput sequencing

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Introduction. The introduction of monocultures modifies not only the physicochemical properties of the soil, but also the composition of the microbial communities. This study aims to compare the archaea microbial communities of an amazon native forest soil and soil cultivated with oil palm.
Materials and methods. Archaeal microbial communities were characterized using 16s rRNA genes pyrosequencing.
Results. More than 700,000 sequences were analyzed in this work. Despite this relatively large sequencing effort, it was not possible to cover all the archaeal biodiversity in either the native forest or the oil palm cultivated area. In total 977 otus were obtained, 681 from the native forest and 455 belonging to the oil palm cultivated area, 159 otus being were shared by the native forest and oil palm cultivated area communities. The predominant phylum in both areas was the euryachaeota, followed by thaumarchaeota; sequences belonging to other phyla were not found. However, the native forest soil archaeal community showed more than twice the number of thaumarchaeota otus than the oil palm cultivated area. Within the phylum euryarchaeota the predominant classes identified were Halobacteria, Methanomicrobia and Thermoplasmata, in that order; the latter two being significantly more abundant in the oil palm cultivated area. In the phylum Thaumarchaeota, south african gold mine gp 1, terrestrial group and soil Crenarcheotic group were the main classes identified, the latter two classes being significantly more abundant in the native forest soil. We also found more rare archaeal genera in the native forest soil than in the soil cultivated with oil palm. Examples of such genera are *Methanimicrococcus*, *Methanospirillum*, *Methanoregula* and *Methanoculleus*.
Conclusions. There is a decrease in the richness of the archaeal community in soils of the amazon native forest when the land is used for agricultural purposes such as for oil palm farming.

TLP-312. Influence of pH and glycerol concentration in growth and production of recombinant *Plasmodium vivax* antigen in *Pichia pastoris*

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Introduction. The *Methylotrophic* yeast Pichia Pastoris is widely used as a eukaryote system to express recombinant proteins. The control of culture conditions (e.g. Ph, temperature, concentrations of inducer, *Carbon* and *Nitrogen* sources, etc.) Are important factors for both yeast growth and protein production. Therefore, the goal of this study was to evaluate the influence of Ph (range 3.0 to 8.0) and *Glycerol* concentration (0 to 60 g l-1) on p. Pastoris growth and *Plasmodium vixax* chimeric antigen (pvama166-msp119) production.
Materiales y métodos: submerged cultures were performed in baffled erlenmeyer flasks in orbital shaker (250 rpm), at 30 °C (for growth stage, 24 h) and 20 °C (for induction stage, 48 h) employing buffered glycerol-complex medium (bmgy), with 1% (v/v) methanol addition every 24 h.
Resultados: the results showed the highest biomass concentration (~12 g l-1) at initial ph between 6.5 and 7.5, and lowest biomass concentration (~9 g l-1) in acidic medium, between 3.0 and 5.0. Cell growth and final ph decrease were directly proportional to the initial *Glycerol* concentration. However, we observed growth inhibition at 60 g l-1 *Glycerol*. Higher biomass production (~29 g l-1) was obtained at 50 g l-1 glycerol, and at this condition we obtained yx/s = 0.7 g g-1. Additionally, experiments were carried out with combinations of initial ph (6.0, 7.0 and 8.0) and *Glycerol* concentration (10, 30 and 50 g l-1). The best condition for pvama166-msp119 production was observed at initial ph 7.0 and 30 g l-1 *Glycerol*.
Conclusiones: acidification during cultivation may have been an inhibitory factor for cell growth and pvama166-msp119 production. In order to increase biomass and obtain a higher expression of recombinant protein cultivations will be performed in bench bioreactor with automated ph control.

TLP-313. Imobilização e estabilização da lipase de *Thermomyces lanuginosa* em suportes iônicos

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Introdução. A técnica de imobilização de enzimas apresenta vantagens tais como: reutilização do biocatalizador, fácil e eficiente separação destas moléculas do meio reacional, melhor estabilidade da enzima em presença de solventes, inibidores e outros agentes desnaturantes e também pode melhorar a estabilidade ao ph e temperatura. Iônicos. Objetivo: imobilização da *Lipase* de *Thermomyces lanuginosa* (tll) em suportes iônicos.
Materiales y métodos: a lipase foi imobilizada por meio da sua dissolução em de tampão *Fosfato* 10 mm ph 7,0 juntamente com os respectivos suportes iônicos (deae, pei-agarose, q-sepharose, manae). A atividade hidrolítica foi analisada por meio da hidrólise do butirato de *p-nitrofenila*.
Resultados: a enzima tll foi 100 % imobilizada em todos os suportes no período de 30 minutos, porém a atividade hidrolítica recuperada dos derivados foi baixa em relação à atividade da enzima livre, apresentando apenas 13, 15, 14 e 31 % de atividade em relação à enzima livre para os derivados de deae, pei-agarose, q-sepharose e manae respectivamente. Após a imobilização os derivados foram submetidos a diferentes concentrações de nacl para dessorver a enzima, sendo necessário as concentrações de 200, 250 e 300 mm de nacl para dessorver a lipase dos derivados deae, q-sepharose e manae respectivamente. A lipase não se dessorveu do suporte pei-agarose, se mostrando mais fortemente ligada a este suporte. A enzima livre e as imobilizadas foram mantidos a 65°C para a verificação da estabilidade térmica. O derivado pei apresentou uma hiperativação durante os primeiros 20 minutos de encubação. Também foi realizada a estabilidade em solução de etanol 50 %, onde o derivado pei apresentou maior estabilidade durante 6 horas de incubação.
Conclusiones: conclusão: o derivado de pei-agarose se mostrou mais estável, não sendo possível dessorver a enzima e manteve 65 % da atividade hidrolítica após 6 horas em solução de etanol.

TLP-314. Caracterización *in silico* del proceso de acoplamiento molecular entre una enzima lacasa diseñada y el *Orto-dihidroxibenceno*

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Introducción. Las enzimas lacasas catalizan la oxidación de compuestos aromáticos reduciendo o2 a h2o. Estructuralmente poseen tres dominios cupredoxina, pero existen reportes de lacasas de dos dominios y evolutivamente se cree que provienen de una oxidasa ancestral de dos dominios *Cupredoxina*. Se presume que estas enzimas pueden prescindir del dominio 2 para realizar sus funciones catalíticas. Objetivo: realizar el diseño in silico de una lacasa de dos dominios tomando como base la lacasa cueo de escherichia coli y evaluar las energías de interacción con el *Orto-dihidroxibenceno* utilizando técnicas de acoplamiento-molecular.
Materiales y métodos: se identificaron los dominios funcionales de cueo (búsqueda en cdd-ncbi) y se realizaron deleciones del segundo dominio y secuencias conectoras. Las estructuras tridimensionales de los mutantes se modelaron en swiss-model y fueron comparadas con respecto a la enzima lacasa de dos dominios de streptomyces coelicolor. En swiss-dock se realizaron simulaciones de acoplamiento molecular entre la lacasa de dos dominios y los mutantes utilizando *Orto-dihidroxibenceno* como ligando.
Resultados: se generaron cuatro secuencias mutantes de cueo: l2 (del-asp179-pro321), l3 (del-asp164-pro321), l4 (del-asp179-his405) y l5 (del-asp165-his405). Por medio de comparaciones con respecto a la lacasa de dos dominios, se encontró que los porcentajes de identidad estuvieron representados por l5>l4>l3>l2, el rmsd l3.
Conclusiones: los análisis desarrollados en este trabajo, permiten seleccionar como candidatos para el diseño de una lacasa de dos dominios y la realización de simulaciones de dinámica molecular a los mutantes l2 y l5 por sus particulares procesos de interacción con el ligando de interés.

TLP-315. Culture-independent assessment of the microbial diversity in the sugarcane-ethanol production process

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Introduction. Brazil is the major producer of sugarcane and the second largest producer of ethanol. Although the *Ethanol* production process is well established, since it does not occur in sterile conditions, microbial contamination can be a problem resulting in decreased ethanol productivity.the aim of this work was to study the microbial diversity of contaminants in six stages of the industrial ethanol production process using culture-independent techniques.
Materiales y métodos: triplicate samples from different stages of the ethanol production process were collected and evaluated: sugarcane juice, mixed juice, clarified juice, evaporated juice, must and wine. Dna extraction of samples was performed, and used for pyrosequencing of bacteria and archaea 16s rrna genes, and fungi its (internal transcribed spacer) region.
Resultados: pyrosequecing of all samples resulted, in 356 groups at genus level for bacteria, 19 for archaea and 203 for the kingdom fungi. Analysis of bacterial sequences showed that community changes are related to the increase of temperature n certain stages of the ethanol production process. After fermentation, *Lactobacillus* and unclassified *Lactobacillales* account for nearly 100% of the sequences. For fungi as well as archaea, sequence data were obtained only for sugarcane juice and mixed juice. The predominant fungi groups identified were unclassified fungi, meyerozima and candida. For archaea, the predominant group identified was an unclassified soil crenarchaeotic group. Rarefaction curves showed that the number of sequences analyzed was not sufficiently high to describe the existing diversity for all the steps of the

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Introducción. la aplicación de proteasas sobre el tamo de arroz tiene un efecto positivo sobre su degradación y aumenta la disponibilidad de materia orgánica en el suelo. Los bacilos aerobios formadores de endoesporas (bafes) son reconocidos por su producción de proteasas y por tanto su inoculación sobre el tamo podría favorecer la descomposición y reincorporación del residuo al suelo. Por tanto, se evaluó la actividad hidrolítica de bafes usando tamo de arroz como sustrato en fermentación liquida y sólida.
Materiales y métodos: se evaluó la actividad proteolítica, celulolítica y xilanolítica de 33 bafes en medios diferenciales. Se determinó el crecimiento y la cinética enzimática de 10 aislamientos seleccionados, en fermentaciones liquidas con tamo de arroz como sustrato durante 20 días. Se establecieron cinco consorcios entre bafes y un hongo del género trichoderma, evaluando la liberación de co2, nh4 y reducción de peso seco, después de 24 días de fermentación sólida sobre tamo de arroz.
Resultados: el 81.25% de las cepas mostraron halos de proteólisis mientras que el 12.5% presentaron actividad celulolítica y xilanolítica. En medio de cultivo líquido, la cinética proteolítica estuvo asociada a la cinética de crecimiento; las cepas ibun13a02, 8a05, 15a03, 15a09 y 7a06 fueron significativamente mayores al control experimental. En la fermentación sólida, los tratamientos con mayor liberación de co2 fueron los consorcios trichoderma sp.+ibun13a02, 8a05 y 15a09. El contenido de amonio estuvo en un rango de 0,083-0,151 mg nh4.g tamo seco-1. Los consorcios con las cepas 8a05 y 7a06 presentaron las mayores actividades enzimáticas, mientras que las cepas ibun15a09 y 8a05 mostraron mayor reducción en peso seco respecto del control.
Conclusiones: nuestros resultados revelan el potencial uso de bafes en procesos de degradación del tamo de arroz y evidencian la necesidad de la aplicación de inoculantes mixtos para la descomposición mas eficiente del residuo vegetal.

TLP-424. Estructura de las comunidades rizobacterianas de dos plantas antárticas mediante el uso de pirosecuenciación

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Introducción. con la llegada de la tecnología de pirosecuenciación, ahora es posible examinar con una mayor resolución la estructura y diversidad de las comunidades rizobacterianas. La rizósfera de plantas de la antártica ha sido escasamente estudiada y prácticamente nada se sabe cómo las rizobacterias pueden contribuir a la adaptación, salud y nutrición de las plantas en tan extremas condiciones. Objetivos: determinar y comparar la estructura de las comunidades rizobacterianas de dos plantas vasculares nativas del ecosistema antártico chileno
Materiales y métodos: muestras de rizósfera de pasto antartico (deschampsia antarctica) y clavel antártico (colobanthus quitensis) fueron tomadas en las islas shetland del sur durante febrero de 2014. La composición taxonómica y abundancia relativa (ar) de las comunidades rizobacterianas fueron determinadas y comparadas mediante el software visualization tool for taxonomic compositions of microbial community (vitcomic), que pueden analizar millones de secuencias bacterianas del gen 16s rrna.
Resultados: en ambas especies vegetales, los resultados revelaron mayores ar de las filas proteobacteria (29-55%) y actinobacteria (12-36%). En proteobacteria, las mayores ar fueron de los órdenes rhodobacterales (11-32%; alphaproteobacteria), *Xanthomonadales* (7-23%; gammaproteobacteria) y burkholderiales (4-18%; betaproteobacteria). En actinobacteria el order con mayor ar correspondió a actinomycetales (12-31%). Al comparar las estructuras de las comunidades con el software vitcomic, no se observó diferencias significativas tanto en la composición taxonómica como en la diversidad entre las especies vegetales muestreadas
Conclusiones: diferencias significativas en los grupos dominantes, estructura y diversidad de las comunidades no fueron detectadas entre las dos plantas estudiadas. El presente trabajo representa uno de los escasos estudios en profundidad sobre la estructura de las comunidades *Rizobacterianas* asociadas a plantas adaptadas a ambientes extremos de chile, como es la antártica.agradecimientos: apoyo al desarrollo de proyectos de investigación chile-ee.uu. (usa2013-0010) e inach (rt_14-12)

TLP-425. Bacterial communities associated with honeybees

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Introduction. the honeybee *Apis mellifera* is the most representative species from hymenoptera order. The immune system in apis comprises both individual and social mechanisms. A relevant but yet underexplored portion of the social defenses is the microbiota associated with honeybees, which may play a relevant role in the health maintenance, protecting the host against pathogens. A comprehensive survey of associations between bacteria and honeybees was the aim of this research.
Materiales y métodos: sampling events were performed in 2011 in carmo da mata, minas gerais, brazil, in five hives of africanized *A.Mellifera* and in 2013 in beltsville, maryland, united states, in 4 hives of *A.Mellifera* ligustica. Pollen, bee bread, hive debris, body surfaces of nurse and forager bees and intestinal contents were evaluated by inoculation on eugon and mrs agar plates, for isolation of general and latic acid bacteria, respectively. The isolates were identified by the sequencing of 16s rrna gene.
Resultados: the substrates with higher species richness were the hive debris in brazil and surface of forager honeybees in the united states. A total of 283 bacterial isolates were identified, comprising 61 species mainly within *Firmicutes phylum*. Bacillus was the most prevalent genus, representing 47% (133) of the isolates. A total of 71 (25%) isolates was identified as acid lactic bacteria, with *Lactobacillus kunkeii* as the prevalent species. Only twelve species were common in brazil and us, demonstrating a different microbiota associated with honeybees between both environments. The species saccharibacter sp., observed from bee bread and surface of nurse bees, probably represents a new bacterium species, closely related to *Saccharibacter floricola*.
Conclusiones: the results suggest that honeybees have their microbiota largely composed of bacterial species from the foraging environment, acting as a vector of micro-organisms among plant species and reflecting distinct bacterial communities between brazil and united states.

TLP-426. Assessment of the microbial diversity in the cerrado savanna as a temporal response to moisture variation

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Introduction. The biome cerrado, a tropical savanna, is found only in brazil. It is characterized by sharp differences between seasons, where summers are wet and winters are dry. Soils in the cerrado are weathered, with high acidity and clay content. The cerrado vegetation varies considerably in physiognomy. This worked aimed at determining the microbial assembly variations, taxonomically and functionally, according to changes in water availability during the wet and dry seasons in soils from savanna-like.
Materiales y métodos: the composition of microbial communities of four different cerrado vegetation physiognomies were studied using high-throughput sequencing of ribosomal rna and metagenomica dna.
Resultados: changes in bacterial, archaeal, and fungal community structures in the cerrado denso, cerrado sensu stricto, campo sujo, and gallery forest soils strongly correlated with seasonal patterns of water availability. The relative abundance of phyla ad3, wps-2, *Planctomycetes*, thermoprotei, *Glomeromycota typically* decreased in the rainy season, while the relative abundance of proteobacteria and ascomycota increased. Analysis of metagenomic data showed that there is a signi-