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Meeting report

Microbiome-MX 2018: microbiota and microbiome opportunities in Mexico, a megadiverse country

A B S T R A C T

A weekly conference series paired with lectures entitled “Microbiome-MX: exploring the Microbiota and Microbiome Research in Mexico” was organized to provide a multidisciplinary overview of the most recent research done in Mexico using high-throughput sequencing. Scientists and postgraduate students from several disciplines such as microbiology, bioinformatics, virology, immunology, nutrition, and medical genomics gathered to discuss state of the art in each of their respective subjects of expertise, as well as advances, applications and new opportunities on microbiota/microbiome research. In particular, high-throughput sequencing is a crucial tool to understand the challenges of a megadiverse developing country as Mexico, and moreover to know the scientific capital and capabilities available for collaboration. The conference series addressed three main topics important for Mexico: i) the complex role of microbiota in health and prevalent diseases such as obesity, diabetes, inflammatory bowel disease, tuberculosis, HIV, autoimmune diseases and gastric cancer; ii) the use of local, traditional and pre-hispanic products as pre/probiotics to modulate the microbiota and improve human health; and iii) the impact of the microbiota in shaping the biodiversity of economically important terrestrial and marine ecosystems. Herein, we summarize the contributions that Mexican microbiota/microbiome research is making to the global trends, describing the highlights of the conferences and lectures, rather than a review of the state-of-the-art of this research. This meeting report also presents the efforts of a multidisciplinary group of scientist to encourage collaborations and bringing this research field closer for younger generations.

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1. Introduction

Microorganisms co-inhabit all corners and environments on the Earth, including all forms of life and even inside tissues and organs that were considered bacteria-free just a few years ago [1–3]. In the beginning, their role was fundamental during the initial geochemical and biochemical events on Earth's atmosphere, and today they influence on all chemical processes that sustain life in our planet as we know it. Moreover, in the last decade, we began to understand that microorganisms are an inherent part of our nature as humans and that their changes largely determines the balance between health and disease. The recent discoveries derived from the Human Microbiome Project are generating new paradigms in biology, in which microorganisms are now significant players in different areas such as medicine, agriculture, environment, pharmaceutical, and food industries [4,5]. The relevance of this new field has encouraged several governments of developed countries such as England, United States of America, Spain, and France to invest significant funds in creating new research departments, institutes and startups-ventures focused on microbiota/microbiome research. Furthermore, these new microbiota/microbiome discoveries are essential to improve government policies in different areas such as environmental management, efficient agriculture and the generation of future personalized medical healthcare strategies [6,7].

For each new development in science, education becomes a fundamental aspect for promoting awareness in young scientists, students, and the general population. Thus, to foster an interest in the study of the microbiota/microbiome among postgraduate students and scientists, we organized this microbiota/microbiome conference series. Every week during four months, one of the thirteen invited scientists presented a conference about their area of expertise to a multidisciplinary scientific community. Then, selected postgraduate UNAM students enjoyed more detailed lectures where besides learning from the researcher's experience in the field, they learned about the current experimental and analysis techniques available for this research [8]. These conference series was hosted at the Institute of Biotechnology of the National Autonomous University of Mexico (UNAM) in Cuernavaca, Morelos, from January 31st to May 14th, 2018.

This article describes the highlights of the conference series as an overview of the state-of-the-art of the microbiota/microbiome research in Mexico from the perspective of some of the Mexican leaders in the field. This series aimed to bring together a multidisciplinary group of scientist working across Mexico (Fig. 1) to

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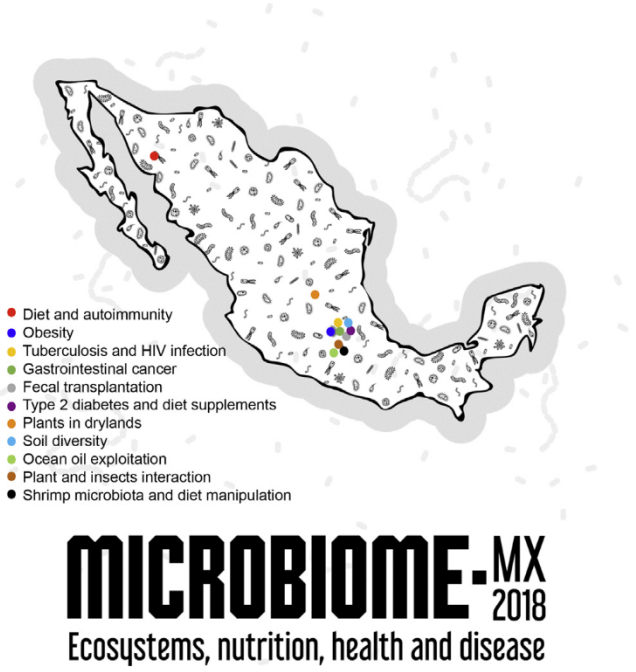

Fig. 1. Microbiome-MX 2018. The conference series looked to gather a multidisciplinary research community around Mexico revealing significant new opportunities and conclusions for the global community.

encourage collaborations and share and discuss their advances and contributions.

2. General highlights

a. Impact of the human microbiome and microbiota in health and disease

Microbial diversity and structure between commensal and pathobionts are fundamental to maintain the host homeostasis [9]. Under changing physiological conditions one or several bacteria, usually, pathobionts, get out of balance generating a dysbiosis [10]. Non-communicable diseases such as diabetes and obesity are serious public health problems, and their pathogenesis is usually associated with diet and genetic factors. Mexico has one of the highest diabetes and obesity prevalence among the Organization for Economic Cooperation and Development (OECD) member countries [11]. Importantly, genome variants only found in Mexican individuals have been associated as risk factors for Type 2 diabetes [12].

The ethnic origin of individuals is an essential factor to consider in microbiome research given that ethnicity contributes to explain several dissimilarities in gut microbiota [13]. The demographic and adaptive processes that occurred in Mexico's population shaped their genetic architecture and could have implications in the biological processes of today's Native Americans and Mestizos [14]. All this makes Mexico a unique niche to study the impact of the microbiome and microbiota in health and disease in a mestizo population.

Dr. Ana María Calderón de la Barca from the Research Center for Food and Development A.C. (CIAD), from Hermosillo, Sonora, found that the microbiota dysbiosis is associated with some dietary components increasing paracellular permeability, and facilitating the exposure to different antigens at *lamina propria,* where the immune system starts an abnormal response inducing the production of autoimmune antibodies [15]. As a consequence, autoimmune diseases such as Type 1 diabetes (T1D) or celiac disease may develop [15]. She observed that in children at the onset of T1D, the fecal microbiota structure showed an increase of *Bacteroides* from 12.3 to 44.7% compared to healthy individuals, while after two years of treatment it decreased to 23.1% [15]. On the other hand, *Bacteroides* dominates the fecal microbiota of celiac disease patients, while *Prevotella*, *Paraprevotella,* and *Bifidobacterium* decreased as compared to healthy children (Calderón de la Barca et al., unpublished), suggesting that a dietary modulation could aid in preventing autoimmunity development in infants and children at high-risk.

Whether altered gut microbiota is a cause or consequence for obesity remains a matter of controversy; however, recent research suggests that gut microbiome of obese individuals has an increased capacity of energy harvest and metabolites such as branched-chain amino acids could participate in the obesity-related metabolic complications [16]. Mexican-Hispanic children are recognized as a high-risk ethnic group [17] with a high prevalence of metabolic syndrome and its components [18]. These findings show the relevance of preventing and treating obesity in the early stages of life to decrease the incidence rates of cardiovascular disease and Type 2 diabetes mellitus [18]. Dr. Samuel Canizales-Quinteros from the School of Chemistry, UNAM, and the National Institute of Genomic Medicine, in Mexico City, discussed his findings between the association of gut microbiota and children obesity [19]. Interestingly, the *Christensenellaceae* abundance was higher in healthy weight children, as was previously observed in European adults [20]. This *Christensenellaceae* overabundance correlates with lower serum levels of amino acids such as phenylalanine, commonly found in increased concentrations in obese patients [19]. Given the high prevalence of Type-2 Diabetes (T2D) in the Mexican population, these findings in children deserve further study with longitudinal cohorts [19] and could be useful to design dietary interventions directed to prevent obesity and its complications [21].

Despite significant advances for treating infectious diseases, these are still present as critical global health challenges. Moreover, the interaction between the microbiota, pathogens, and the immune system is an intriguing and complex open question that is being actively addressed [22]. In this regard, Dr. Xavier Soberón from the National Institute of Genomic Medicine and Dr. Sandra Pinto from the Research Center in Infectious Diseases (CIENI) at the National Institute of Respiratory Diseases, both in Mexico City, focus their research on the impact of the microbiota in tuberculosis and HIV infection, respectively, two of the most prevalent infectious diseases in Mexico [23].

At the meeting, Dr. Xavier Soberón explained the importance of how lung microbiota influences the development of tuberculosis, a poorly understood aspect of this disease [22], and pointed the importance of developing new diagnostic methods using massive sequencing [24]. Preliminary results obtained in his lab using a gene panel (Sure Select; Agilent Technologies) containing 88 regions of the *Mycobacterium tuberculosis* genome, provided a protocol able to identify mutations in the majority of the genes implicated in antibiotic resistance directly from sputum samples, based both in known and newly discovered variants (Soberón et al., unpublished results). Dr. Soberón highlighted that special bioinformatic pipelines were required to accurately assign variants to *M. tuberculosis*, which was not straightforward for the more highly conserved genes involved in drug resistance (Soberón et al., unpublished results). Further, he pointed out that more massive databases and validated processes with well-characterized clinical samples are necessary to afford a reliable alternative to current molecular diagnostics methods focused on the detection of a few selected mutations, such as GeneXpert.

Overall, initial results from the Dr. Soberón Lab indicated that massive sequencing is a feasible approach to obtain the drug resistance and the virulence profiles of the strains circulating in Mexican tuberculosis patients.

Nowadays, HIV infection is a chronic disease where antiretroviral therapy has changed the lives of countless infected people; however, low-grade inflammation persists in some patients despite complete viral suppression, rendering a negative impact on their quality and life expectancy. Indeed, people living with HIV have an increased risk of acquiring non-communicable diseases compared to those HIV-negative ones. Recent studies have pointed out that gut microbiome could contribute toward fueling inflammation and chronic activation [25]. Dr. Pinto-Cardoso results have shown that microbial diversity is lower in HIV-positive than in HIV-negative individuals, and that specific commensals (in particular bacteria capable of producing butyrate) are depleted. Furthermore, the depletion of these commensals negatively correlated with intestinal gut dysfunction [26,27].

Gastric cancer is a critical public health problem with a high mortality rate in Mexico, representing one of the top causes of death due to all cancers [28]. Dr. Javier Torres, from the Mexican Social Security Institute (IMSS) in Mexico City, has studied the association between the stomach microbiota and the progress of gastric cancer. His results show that the microbiota diversity decreased as a function of disease severity, from non-atrophic gastritis to preneoplasia and cancer [29]. Proteobacteria and *Helicobacter pylori* were the most abundant taxa, in agreement with its known role as the main risk to develop gastric cancer [30]. Also, comparative analyses showed a higher variation and different functional profiles of the stomach microbiota than in other body sites [31]. Dr. Torres also studied the association between the bile duct microbiota and the development of biliary tract cancer. Interestingly, his studies revealed the presence of extremophilic bacteria in the bile duct of diseased humans, which were previously reported in sea sediments and deserts [32]. Results of his work are in line with previous studies suggesting that *H. pylori* may also be associated with cancer of the biliary tract [32].

Dr. Yolanda López-Vidal from the School of Medicine at UNAM in Mexico City talked about her initial studies on Crohn's and irritable bowel disease (IBS) aimed to determine the microbial composition differences between diseased and healthy subjects and the potential association with intestinal alterations that can affect essential host functions (López-Vidal et al., unpublished). Additionally, she talked about two notable efforts to study and treat infectious diseases in Mexico. The first, is the analysis of the microbiota/microbiome resistome, a study part of the Plan for Control of Antimicrobial Resistance at the UNAM, a network that generates information and strategies to counteract the advance of antimicrobial resistance in humans, animals and the environment [33]. The second project is the study of a new product for fecal transplantation named Trabiota® developed from the feces of a healthy donor carefully monitored for a long time. The use of fecal transplantation is not a standard treatment in Mexico but has proved to be very useful for treating recurrent *Clostridium difficile* infection and reestablishing a healthy microbiota [34]. So far, the first studies in Mexico show that Trabiota® was successful in treating several patients with *C. difficile* infection resistant to antibiotics and were still infection-free after up to 2 years after treatment (López-Vidal et al., unpublished results).

b. Microbiota modulation to improve human health

The results of the Human Microbiome Project (HMP) have facilitated the identification of microbiota taxonomic and functional factors associated with several diseases [5]. Currently, some scientists are looking to re-engineer the disturbed microbial composition through diet, drugs, prebiotics/probiotics, and postbiotics, improving the host health. Mexico has one of the richest pre-Hispanic and modern diets in the world. Indeed the United Nations Educational, Scientific and Cultural Organization (UNESCO) declared the Mexican cuisine in the List of the Intangible Cultural Heritage of Humanity [35]. In this sense, pulque is a crucial reference regarding Mexican culture, tradition, and cuisine and it was an essential element for the UNESCO decision in 2010. Recent Mexican research has suggested several health benefits to pulque consumption, which are associated with diverse probiotic lactic acid bacteria isolated from the beverage microbiota [36]. Additionally, since ancient times Mexicans have used plants as remedies for headaches, stomachache, wound healing, among others [37,38]. Therefore, it is not surprising that Mexican scientists are now interested in knowing if the traditional pre-Hispanic foods do the beneficial effects via modulation of the gut microbiota.

One of the most common plants used in the Mexican cuisine since the pre-Hispanic era is the prickly pear or nopal (*Opuntia ficus-indica*) and Dr. Nimbe Torres from the National Institute of Medical Sciences and Nutrition, in Mexico City, talked about its use as a prebiotic rich in polyphenols, vitamin C, and soluble and insoluble fiber. She found that Nopal consumption modified gut microbiota in rats and it was associated with a decrease in metabolic endotoxemia, glucose insulinotropic peptide, glucose intolerance, lipogenesis, and metabolic inflexibility [39]. Her studies support the use of nopal as a functional food and prebiotic for its ability to modify the gut microbiota and to reduce metabolic endotoxemia and other obesity-related biochemical abnormalities [39]. Dr. Nimbe Torres also talked about the gut microbiota in patients with T2D (characterized by an increase in *Prevotella copri*), in which a dietary intervention using functional foods significantly increase the alpha diversity and changed the abundance of specific bacteria, independently of the use of anti-diabetic drugs [40]. She also found a decrease in the abundance of *P. copri* and an increase of *Faecalibacterium prausnitzii* and *Akkermansia muciniphila,* two bacterial species known for their anti-inflammatory effects [40]. Additionally, the patients exhibited a significant reduction in blood glucose, total and LDL cholesterol, free fatty acids, HbA1c, triglycerides, C-reactive protein and an increase in antioxidant activity [40].

c. Impact of the microbiota in shaping the biodiversity of particular ecosystems

The characteristics of every ecosystem are determined by the flora and fauna that inhabits it, as well as by the complex network of interactions within the microbial communities and the hosts. According to the National Commission for the Knowledge and Use of Biodiversity (CONABIO), the Mexican territory represents only 1% of the earth's surface, but it harbors more than 10% of the world's biological diversity [41]. Due to its geographic location, Mexico has a wide diversity of terrestrial and aquatic ecosystems inhabited by a broad variety of flora and fauna species, many of them regionally endemic and that contribute importantly to the economic development of coastal wetland areas in Mexico [42]. One of this species is *Litopenaeus vannamei* (Pacific whiteleg shrimp) which is one of the most cultured shrimp species worldwide. Now, in the light of the microbiome revolution, the characterization of the microbial communities and the dynamics that impact the health, growth, and survival of essential organisms becomes very important [43]. Recent Mexican research on the shrimp microbiome was also discussed in the lectures as an example of the opportunity that microbiome-based knowledge offers to control outbreaks enhancing shrimp productivity [43,44] one of the most economically important species cultivated in Mexico.

Dr. Laila Partida-Martínez, from the Department of Genetic Engineering at CINVESTAV in Irapuato, Guanajuato, discussed the plant microbiota in drylands, focusing in two essential but taxonomically unrelated plant lineages native to the American continent, the genus *Agave* (family *Asparagaceae*) and the family *Cactaceae*. These plant lineages share the *Crassulacean Acid Metabolism* (CAM), a form of photosynthesis that prevents water evaporation, which along with other morphological characteristics help them to live and succeed in arid ecosystems around the globe. Phylogenetic profiling of the bacterial, archaeal and fungal communities associated with the soils, rhizospheres, roots, leaves endosphere, and phyllosphere of the cultivated *Agave tequilana* (used mainly for tequila production), and the wild *A. deserti, A. salmiana, Myrtillocactus geometrizans* and *Opuntia robusta*, revealed a high microbial diversity, where the plant compartment and the biogeography of the plant species played the most promising roles in the assembly of the plant microbiome [45–47]. Moreover, taxonomical, metagenomic and network analyses support the notion that the analyzed CAM-plants share a core microbiome [48], which functions, including the production of microbial volatile organic compounds (mVOCs), improve plant performance and development of model and native plant hosts [49]. Altogether, these studies highlight the potential of using microbiome-based knowledge to enhance the productivity and sustainability of agriculture in the arid and semi-arid regions of the world.

Mexico hosts 26 out of 30 soil types according to the Food and Agriculture Organization (FAO) classification (FAO-ISRIC-ISSS, 1998) [50], which constitutes a great system to test the microbiological diversity of the soils and its role in the plant's microbiome. In this regard, Dr. Luis David Alcaráz, from the Faculty of Sciences, UNAM in Mexico City, presented his research about the soil microbiota as the primary source in plant rhizosphere community structure. His lab is testing other microbiome structuring factors as plant genotype (wild plants and agricultural varieties) and reducing environmental variables by common-garden experiments. The research group described the microbiome of two sympatric *Marchantia* liverworts species, which represent one of the first groups of land plants [51]. They identified metabolic complementation between the microbiome and *Utricularia gibba* L, in which the microorganism inhabiting *U. gibba* traps are complementing hydrolytic enzymes activity, ROS detoxification, nutrient uptake, and assimilation [52]. They are currently tracking multiple sources and interactions to test the role of the plant discriminating friends from foes and on the engineering of microbial communities to promote plant growth and aid phytostabilization, which is an *in-situ* stabilization of contaminated soils and mine-tailings [53].

Dr. Liliana Pardo, from the Institute of Biotechnology, UNAM in Cuernavaca, discussed her most recent project as one of the leaders of the Gulf of Mexico Research Consortium. Her primary research interest is to analyze the microbiome inhabiting the areas assigned for exploitation by oil companies and to characterize the metabolic potential given by the microbiome that inhabits the ocean. The aim is to establish contingency plans in case of future oil spills that endanger the flora and fauna in this environment. Her group conducted fieldwork at 74 sampling sites in the Southwestern Gulf of Mexico (swGoM) in an area encompassing ~150,000 km^2. Dr. Pardo's group analyzed bacterial populations from sediment samples and determined their relationships with 79 different abiotic variables, including metals, organic matter, depth, and in particular, the concentration of aromatic hydrocarbons [54]. They described, for the first time, a distinctive bacterial population structure from sediments of the swGoM as compared to worldwide sediments and correlate it with hydrocarbon levels [55]. This knowledge contributes to the understanding of how microbial communities are shaped under chronic exposure to hydrocarbons in the swGoM.

Dr. Esperanza Martínez-Romero, from the Center for Genomics Sciences, UNAM in Cuernavaca, explained the importance of studying the relationship between bacteria and insects. Of all animals, insects have the most specialized microbiota with selected bacteria inhabiting inside insect cells called bacteriocytes. In some insects, bacteriocytes may constitute organs called bacteriomes in the abdominal region [56]. Symbionts inhabiting the bacteriome have reduced genomes and are inherited from mother to offspring. In the wax cochineal from Chiapas, Dr. Martínez's group discovered a co-symbiont [57] and a novel flavobacterial endosymbiont with a reduced genome that is capable of producing essential amino acids for the host [58]. In contrast, they found a novel nitrogen-fixing bacterium with a large genome named *Dactylopiibacterium* [59] inherited to the offspring in the carmine cochineal, which is used for producing carmine, a red dye for food and cosmetics [60]. Microbiome and functional genomic studies performed by her group, highlight the outstanding characteristics of *Dactylopiibacterium* from the carmine cochineal [61].

3. Conclusions

This first edition of Microbiome-MX conference series meant for many of the attendees a first glimpse at the microbiota/microbiome research field and its enormous potential in Mexico and Latin-America. The idea of combining conferences followed by lectures imparted by experts in the different microbiota/microbiome fields was designed to create interest, informal discussions and encourage postgraduate students to consider a career within the area. Microbiome research is a growing field with the tremendous potential to improve human and animal health and other aspects of our daily life. To address novel questions of microbiota/microbiome research, we need collaborations among different area specialists such as epidemiologists, microbiologists, gastroenterologists, bioinformaticians, statisticians, among others.

The advancement of knowledge in microbiome research is in many cases limited by the small size of cohorts and cross-sectional studies that only report associations but not infer causality. Much larger cohorts, preferably prospective and longitudinal with detailed metadata, are needed to understand the contribution of the microbiota to disease onset and to promote open-access biorepositories [62]. Research in Mexico about microbiota/microbiome also needs appropriate funding. With this in mind, scientist and students consider it imperative to create a Mexican network of Microbiome and Microbiota Researchers. The objective is to bring together scientists working in the field to share knowledge and resources, and join efforts to create government policies to promote this research in the country. Mexico is a particularly rich and unique country with a mixed-ethnicity human population [14] exposed to a broad diversity of environments and diets which impact on the high incidence of specific diseases now related with a microbiota dysbiosis, such as obesity and diabetes. Thus, the study of the microbiota in this specific country emerges as an incredibly exciting field that should be foster and further explored.

Conflicts of interest

All authors declare no conflict of interest.

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