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A Step Forward to Empower Global Microbiome Research Through Local Leadership

Victor S. Pylro,[1,*] Tsai S. Mui,[2] Jorge L.M. Rodrigues,[3] Fernando D. Andreote,[4] Luiz F.W. Roesch,[5,*] and the Working Group Supporting the INCT Microbiome[6]

Obtaining the full microbial potential to benefit local communities and citizens, as well as ongoing conservation efforts, is a major challenge for Brazil and other developing countries. We propose policies and priorities for organizing microbiome studies locally and worldwide, aiming for a comprehensive catalogue of microbiomes, as recently urged.

Microorganisms are recognized as a fundamental resource for creating a fast and efficient strategy for ecosystem management and scientific and technological development. Microbial community assembly and functions are tightly linked to local geographic and environmental features [1]. Developing countries, such as Brazil, house biomes evolving under specific environmental conditions that likely harbor unique microbiomes. Challenges in profiling microbial diversity include a lack of standardized methods and metadata collection that precludes robust interstudy comparisons, limiting the value of these studies [2–4]. Advances in sequencing technologies and associated bioinformatics approaches should now enable comparison of the diversity, abundance, and function of microbial communities at much greater resolution than was previously possible [5]. Recently, three papers have pointed to the need for microbiome research coordination [4,6,7]. These proposals envisioned guidelines for intellectual-property rights, research priorities for funding (including a long-term interagency funding strategy), the development of new analytical tools, training programs, data integration through an in-country distributed data center and policies on data sharing. We argue here that there is a need for developing local leadership in microbiome research [8] and propose establishing an initiative to foster international collaborations in order to marshal microbiome research in Brazil. Recently, the US government, along with the private sector, has announced a significant budget to launch the National Microbiome Initiative (NMI) [9]. Considering geography as an important part of the microbiome puzzle, the development of local initiatives will strengthen the NMI and provide a basis for a global microbiome effort [6].

In 2008, the Brazilian Ministry of Science and Technology consolidated an important strategy to restructure policies of scientific development, implementing the National Institutes of Science and Technology (INCT in Portuguese). The main objectives of the INCT are to strengthen national research, increase training, internationalize groups, and transfer knowledge to society, business sectors, and government when appropriate. Despite a direct relationship with the strategic themes established by public policies of the Brazilian government, the systematic study of microbiomes and their biotechnological potential remain limited. We have proposed a systematic study of Brazil's microbial resources as a valuable new focus area for the INCT efforts. Although pressed by the economic slump, political crisis, and their consequences for science and technology [10], Brazil is moving forward on microbiome research. The National Council of Technological and Scientific Development (CNPq) has recommended the creation of a National Institute of Science and Technology to advance microbial research–the INCT Microbiome[i]. During the next months, a combined evaluation by the CNPq and other Brazilian agencies supporting research, such as CAPES, FINEP and FAPESP (São Paulo Research Foundation), will forge a definitive agreement for assembling this institute, potentially awarding it a budget of up to 10 million Brazilian Reais (US$ 2.8 million). The INCT Microbiome aims to be a center of international excellence seeking to coordinate and provide guidance to microbiome researchers, besides balancing inequality of research opportunities in Brazil. Low- and middle-income countries are usually prevented from performing state-of-the-art microbiome research due to a lack of structure or to a lack of well-trained people. Local initiatives might benefit from a core center housing all the necessary infrastructure and trained scientists, to provide high-quality project design, cutting-edge technology (sample processing, sequencing, etc.) and training on data analyses to deliver the best solutions to collaborators. The benefits brought by such an endeavor will be essential to consolidate the local initiative envisioned to advance Brazilian microbial studies [8]. We hope that this initiative can serve as a template for leadership of microbiome research in other developing countries.

The Brazilian Microbiome Project (BMP)[ii] [11] was set up in 2013, and this will form the basis for the INCT Microbiome. Now, Brazil is expected to advance on it, uniting microbiome investigators to support the development of an unprecedented knowledge base of microbial resources. In 2012, Brazil moved forward in the assessment and use of biodiversity by joining the Global Biodiversity Information Facility (GBIF) and by creating the Biodiversity Portal[iii] in 2015. However, microbial information has been ignored, as has happened in previous attempts to explore biological resources in Brazil [12]. A new database is required to combine Brazilian microbial data with other biodiversity data, including GBIF, and in agreement with the Brazilian laws of biodiversity data sharing (normative instruction 02/2015 of the

Chico Mendes Institute for Biodiversity Conservation–ICMBio).

Four key issues have been identified as priorities for scientific research: (i) standardization of methods and integration with existing groups developing protocols and standards, such as the Earth Microbiome Project (EMP)[iv], TerraGenome[v] and QIITA[vi], to ensure that studies are systematic and comparable, allowing the development of a dynamic and constantly expanding database that can provide real-time information for all users; (ii) characterizing microbiomes under natural conditions, which is of fundamental importance for establishing comparative models; (iii) developing microbial interventions that allow the maintenance of the quality and productivity of the environment or host; and (iv) develop/expand partnership among academic studies, agriculture, medicine, and industry. This well-defined approach is expected to increase the understanding of Brazil's microbial resources with the goal of developing strategies to: mitigate environmental pollution; increase the activity of beneficial microorganisms from soils; suppress pathogenic microorganisms in plants and humans; and create an efficient strategy for scientific and technological bioprospecting. To achieve these goals, the INCT Microbiome needs to be inherently collaborative. We propose an organizational model that represents specific scientific research domains, and strategic committees focused on training and transfer of knowledge and technology (Figure 1). Research domain committees will be thematic, addressing microbial interactions with plants, animals, soils, aquatic environments, and humans as well as on bioprospecting. Each theme will consider research drivers, scan the horizon, and translate research into socioeconomic relevance. Promising translational areas include the effects of pollution and land use change, improving agricultural

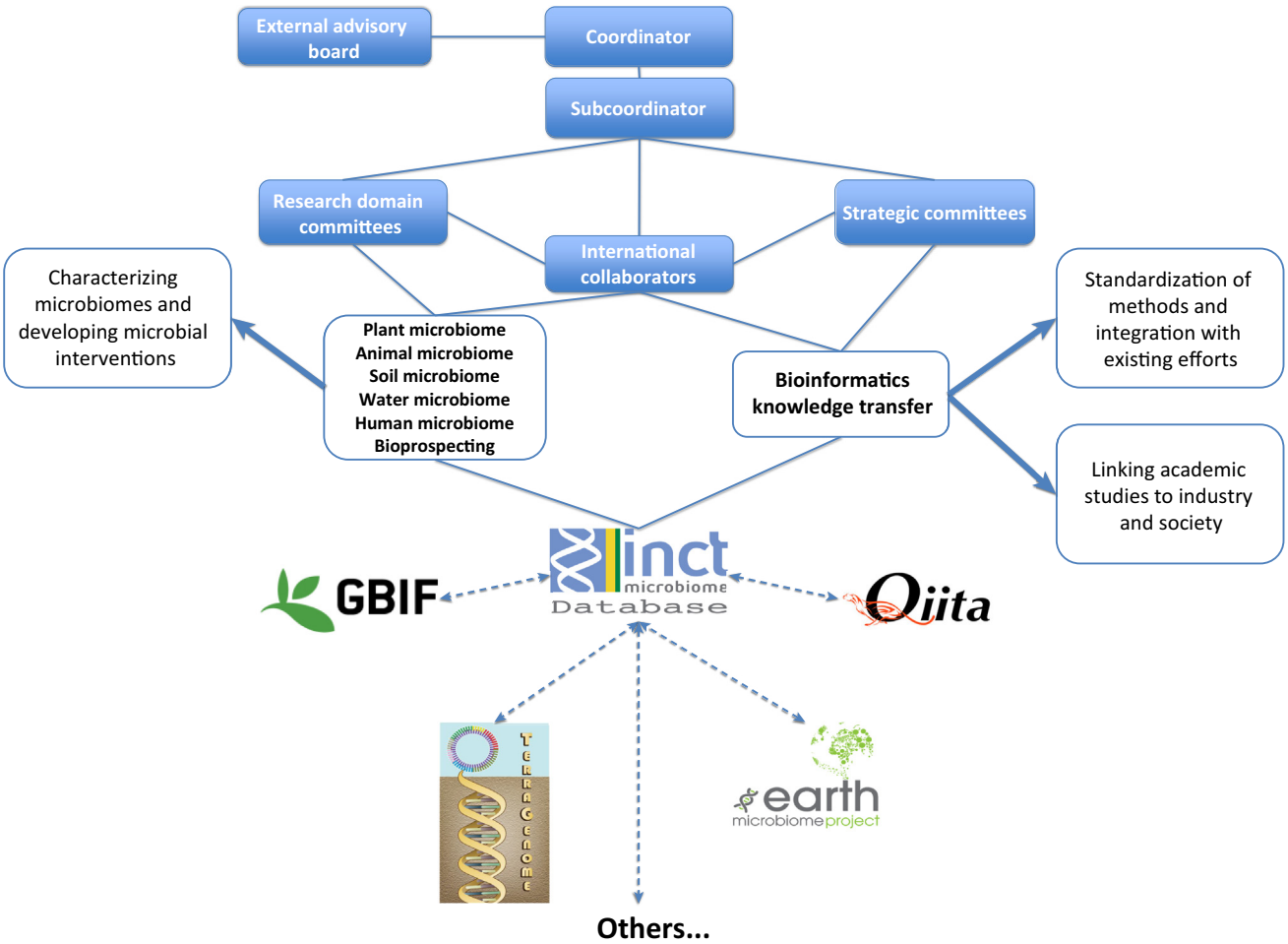

Figure 1. Organizational and Functional Structure of the INCT Microbiome. Broken lines represent possible future interactions of the INCT Database with other initiatives underway.

Box 1. Primary Habitats and Hosts Currently Being Addressed by the INCT Microbiome Collaborators

A microbiome is defined as 'the entire habitat, including the microbes (bacteria, archaea, lower and higher eukaryotes, and viruses), their genomes (i.e., genes), and the surrounding environmental conditions' [14]. Megadiverse countries represent promising scenarios for biotechnological advances, which can be enhanced by the local researcher's microbiome consortia. A catalogue of targets for microbial prospection, permeating the entire Brazilian territory, ranging from agricultural improvements to human health promotion, is provided below as an example:

(i) Microbiomes associated with plants

- The microbiome of wheat cultivars and their ancestors: many microorganisms associated with roots can benefit plants on nutrition and tolerance to biotic and abiotic stress factors. For over a century, breeders have explored plant genes to improve their performance and productivity, but the rhizosphere microbiome has been neglected. Exploring the microbiome of wheat cultivars and their ancestors will allow for the development of new approaches applying microorganisms to sustainably improve plant development and health.
- The microbiome of sugarcane: plants seek the help of microbesEvidence suggests that plants under attack 'ask for help' from the microbiome and actively recruit beneficial microbes for their health. As a result, soil becomes suppressive to diseases. Characterizing the microbiome of sugarcane with contrasting levels of resistance might contribute to empowering biological control through specific microbe activities.

(ii) Microbiomes associated with animals

- The cattle's intestinal and skin microbiome: ruminants' microbiomes are now being explored as sources of biomass-degrading enzymes to be applied in the industrial production of biofuels. Also, tick susceptibility in cattle has now been related to volatile compounds produced by specific skin microbiome profiles, making its modulation a hopeful strategy for promoting resistance to ectoparasites. The comparisons of microbiomes of different cattle breeds, in different geographical regions and subject to different management practices, will allow verification of the degree of contribution of genetic makeup and the environment. The knowledge generated will enable the development of innovative and sustainable technologies for tick control, such as probiotics and/or repellents.
- Intestinal microbiota associated with productivity performance in broiler chickens: the use of antibiotics in animal feed as growth promoters has resulted in increased susceptibility to enteric diseases and dysbiosis, and caused a negative effect on the productivity of broiler chickens. Research has shown various beneficial effects of using probiotics, prebiotics, symbiotic, organic acids and plant extracts in the diets of birds. The sum of the results of these studies will allow the nutritionist to adopt optimized feeding strategies, in order to modulate the intestinal microbiota of broiler chickens for higher performance.

(iii) Microbiomes associated with soils

- Microbiomes of mangrove soils: mangroves are highly productive and have a rich associated biodiversity. They are subject to constant anthropic impacts, which are the main cause of deterioration of these ecosystems. This line of research seeks to access the microbiome of mangrove soils of the North, Northeast, Southeast, and Southern regions of Brazil, correlating them with environmental variables to better understanding the microbial taxonomy and functioning in mangroves.
- Microbiome of Caatinga biome soils (arid soils): the Caatinga biome suffers constant limitations related to water deficit. In recent years, aridity of the Caatinga has been increasing due to human activities, resulting in increased erosion of soils, microclimate changes, and desertification. Obtaining information regarding the effects of these habitats on the diversity and function of the microbial community is essential to guide sustainable recovery practices for this biome. At the same time, this region is a promising target to unravel novel strategies of plant drought resistance mediated by microbes.
- Microbiome of agricultural soils: defining the organizing principles that control the assembly of soil microbial communities under land-use change will help to provide the basis to understand how individual microbes and microbial communities function in response to changing environmental parameters (at a molecular scale) and vice versa. Microbial-community-mediated biological processes will be linked to environmental parameters, allowing us to build predictive models of metabolic and regulatory processes related to the changes caused by agriculture and adopting strategies for mitigating global heating.

(iv) Microbiomes associated with aquatic environments

- Characterizing the microbiome of water and sediments of the rivers and water reservoirs: environmental pollution is also an important issue, and initiatives to mitigate it are highly important to preserve rivers and water reservoirs. Increasing anthropogenic activity can lead to ecological imbalance of water bodies and the extinction of key microbial species. A comprehensive metagenomic study correlating the microbiome and the trophic conditions allow for proposing solutions for improving the quality of water available in the reservoirs.

(v) Microbiomes associated with human health

- Biocontrol of human vector-borne diseases: in the tropics, several emerging maladies and vector-borne viral diseases have the potential to cause epidemics, such as malaria, dengue fever, chikungunya, and more recently the Zika virus. Combating these vectors and pathogenic agents is somehow ineffective by traditional approaches, but novel interventions such as the introduction of a *Wolbachia* strain in *Aedes aegypti* has reduced the infection with dengue, chikungunya, and Zika viruses as well as *Plasmodium* parasites. Biological control and its ecological implications are promising for solving public health issues.
- Association among fetal microbiota, prematurity, and preterm morbidities: the biological causes for preterm deliveries remain poorly defined. Multiple lines of evidence indicate that the microbiome in expectant mothers can trigger premature delivery. Identifying such a mechanism would have important implications for designing nutritional interventions in pregnant mothers aimed at establishing a microbiota with lower risk for preterm delivery.

(vi) Bioprospecting

- One of the most promising approaches for the discovery of new bioactive compounds is the use of metagenomics in the search for new biosynthetic genes for cloning and expression in a heterologous or homologous host. Bioprospecting allows for the development of new biotechnological sustainable processes related to bioremediation, biorefining, mining bioleaching, oil recovery, or cleaning of oil storage tanks, based on the isolation of microorganisms capable of performing these functions.

(vii) Bioinformatics

- Data analysis and pipelines: computational resources can be difficult to handle, and the effective execution and reproducibility of analyses is a challenge given the amount and nature of the data. Metagenomic analyses have been described as one of the least reproducible NGS applications, mainly due to the lack of integrated and standardized solutions. The recent development of standard methods and protocols allowed proper data analysis and interpretation. Up to now, this initiative provided the community with a web platform for data analysis, a Linux-based Operational System dedicated to data analyses and standard methods, and data analyses pipelines[ii]. More than 10 000 users from 112 countries (most from Brazil, the USA, and the UK) have already benefited.

practices, water treatment and management, animal breeding, and microbial effects on human health (Box 1 gives for a detailed list of primary targets in Brazil).

Expanding capacity in bioinformatics is critical because the current bottleneck for biosciences is how to analyze 'big data' [4,13]. In-depth analysis of the growing number of completely sequenced microbial genomes in public databases is providing fascinating contributions to our understanding of how microbes are genetically tailored to their lifestyles. The quality of this information critically depends on high-quality genome annotation. Accurate annotation is challenging, and perhaps even more important to new understanding of microbes than the development of new sequencing technologies. For example, mobile genomic elements (MGEs) are important for pathogenicity and antibiotic resistance, but are often poorly understood because they are not well annotated. To address the annotation and other related issues, an effective Bioinformatics Committee should be assembled to support satellite bioinformatics laboratories for training collaborative teams. This effort is paramount to improve data interpretation and reproducibility, and to foster the development of new protocols to meet the demands of a diverse group of collaborators. Studies of microbiomes in particular are under-funded in Brazil, and ties to international efforts and to industry will be important for developing appropriate bioinformatics techniques.

A Committee for Knowledge Transfer is essential to integrate all other groups and to link university/research institutions and society. In addition to providing resources for improving basic education, local leaderships will make scientific knowledge accessible to all, transferring information generated by the different research groups to society. Intellectual property (IP) issues represent a major concern for developing countries, and the improvement of already established IP organizations should be addressed. Strong IP protection systems benefit the country's economy by attracting investors and fostering innovation. The restricted budget of such countries reflects the lack of IP expertise hindering patent deposition. In agreement with the recently approved Congressional Law Project (PLC) 77/2015, which regulates long-term partnerships between the public and private sectors in Brazil, local activities allow better integration of scientific data with entrepreneurs. Eliminating these bureaucratic obstacles promotes innovation and technological advances for exploring Brazilian biodiversity. The challenge here includes the creation of a 'one-stop shopping service' providing a single point of contact between public and private sectors, capable of addressing individual challenges, to offer both sides the most appropriate IP solutions. Protecting the particular interests of traditional societies (e.g., indigenous people) and sharing the benefits generated from the use of traditional knowledge in biotechnological development fairly is also an important and ongoing discussion, now supported by the Brazilian Biodiversity Law 13.123/2015 and its recent regulatory decree.

We urge support of the integrative solutions proposed here, through the creation of the INCT Microbiome. This local microbiome initiative will not only address the Brazilian problems of imbalance of microbiome studies and data fragmentation, but will serve as a model helping to guide future interagency funding efforts worldwide, besides ensuring appropriate project alignment with other international efforts [6,7]. We aim to empower local research to solve our problems at home, generating knowledge and technology to further address global questions.

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Disclaimer Statement

The authors declare that they have no competing interests.

Resources

[i] http://inct-microbiome.org
[ii] http://brmicrobiome.org
[iii] http://portaldabiodiversidade.icmbio.gov.br
[iv] http://earthmicrobiome.org
[v] http://terragenome.org
[vi] http://qiita.ucsd.edu

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[1]Biosystems Informatics and Genomics Group, René Rachou Research Center–CPqRR, Av. Augusto de Lima, 1715, 30190-002, Belo Horizonte, Minas Gerais, Brazil
[2]Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture–CENA, University of Sao Paulo USP, Piracicaba, São Paulo, Brazil
[3]Department of Land, Air and Water Resources, College of Agricultural and Environmental Sciences, University of California–Davis, Davis, California, USA
[4]Department of Soil Science, "Luiz de Queiroz" College of Agriculture, University of São Paulo–ESALQ/USP, Piracicaba, São Paulo, Brazil
[5]Federal University of Pampa–UNIPAMPA, Campus São Gabriel, São Gabriel, RS, Brazil
[6]For a full list of authors, please see Table S1 in the supplemental material online.

*Correspondence: victor.pylro@brmicrobiome.org (V.S. Pylro) and luizroesch@unipampa.edu.br (Luiz F.W. Roesch).

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Spotlight

Antibiotic Methylation: A New Mechanism of Antimicrobial Resistance

Kerri M. Malone[1] and Stephen V. Gordon[1,2,3,4,*]

In new research on *Mycobacterium tuberculosis*, the causative agent of tuberculosis, Warrier and colleagues have discovered a novel mode of bacterial drug resistance, namely antibiotic inactivation via *N*-methylation.

By 2050, 10 million lives a year and a cumulative US$100 trillion of economic output are at risk due to the rise of drug-resistant infections[i]. Tuberculosis (TB) represents just one of the infectious diseases that we must find new ways to control if this trend is to be halted and reversed. TB kills someone every ~25 seconds, a shocking statistic that makes *Mycobacterium tuberculosis* one of the deadliest pathogens on the planet [1]. *M. tuberculosis* was one of the first pathogens to be identified as the aetiological agent of an infectious disease by Robert Koch in 1882. Bacillus Calmette–Guérin (BCG), the TB vaccine, is the most widely used vaccine in the world, given to over 100 million children every year, while cheap, effective drugs are available for treatment of drug-sensitive infections. So why does TB continue to exact such a heavy toll of morbidity and mortality?

Control of TB is complicated by the unique biology of *M. tuberculosis*, the variable efficacy provided by the BCG vaccine to protect those immunized against disease, restricted healthcare access for those affected, HIV coinfection, and prolonged drug therapy that is driving the emergence of drug resistance [1]. It is predicted that over 2 billion people harbor latent *M. tuberculosis* infection, providing a vast reservoir of infection [1]. These hurdles have not, however, limited the vision of TB-control policies with the World Health Organization (WHO) End TB Strategy seeking to end the global TB epidemic by 2035 through achieving a 95% decline in TB deaths and a 90% reduction in disease incidence as compared to 2015[ii]. Delivery of this vision will need progress on multiple fronts, and will hinge on the development of new anti-TB drugs.

The drugs currently available for the treatment of TB are mainly the products of antibiotic discovery efforts in the 1940s–1960s. Only since the declaration of TB as a global public health emergency by the WHO in 1993 did the TB drug development pipeline sputter back to life. However, decades of neglect, increased regulations, and a lack of interest from pharmaceutical companies has meant that the pipeline contains a mere trickle of new compounds; further research is desperately needed to identify novel drug candidates and to unravel drug-resistance mechanisms of the TB bacillus.

Investigations into the antimycobacterial properties of imidazopyridine- and pyrido[1,2-α]benzimidazole-containing compounds have shown that these compounds are active against *M. tuberculosis*, and against multidrug-resistant (MDR)- and extensively drug resistant (XDR)-TB clinical isolates [2–5]. Through the characterization of one such compound containing a pyrido-benzimidazole core designated '14' [4], Warrier *et al.* have described an entirely novel mode of drug resistance for bacteria, namely inactivation of an antibiotic via *N*-methylation [6].

To elucidate the mode of action of '14', Warrier and colleagues isolated multiple *M. tuberculosis* clones resistant to the compound. Whole-genome sequencing of these clones identified 16 unique candidate resistance-conferring mutations, with nine of these mutations mapping to the *rv2887* gene alone [6]. The Rv2887 protein belongs to the MarR family of transcription factors, with the resistance-conferring mutations predicted to affect the ability of Rv2887 to bind DNA. Binding assays showed that Rv2887 bound directly to the promoter region upstream of *rv0560c*, a gene encoding a putative methyltransferase. *rv0560c* was found to be expressed at ~400-fold higher levels in resistant *M. tuberculosis* clones than in sensitive wild-type *M. tuberculosis*, while exposure of wild-type *M. tuberculosis* to '14' caused consistent upregulation of *rv0560c* expression. Based on these findings it was deduced that Rv2887 was a negative regulator of *rv0560c* expression, with resistance-conferring mutations in *rv2887* found to inhibit the ability of the Rv2887 transcription factor to bind DNA, resulting in the upregulated expression of *rv0560c* in resistant clones [6].

The role of Rv0560c in resistance of *M. tuberculosis* to '14' was next explored. As it was reported that Rv0560c was nonessential to the survival of *M. tuberculosis* [7], it was unlikely that Rv0560c was the target of '14' but rather that its enzymatic activity must render '14' inactive in some manner. Characterization of the methyltransferase activity of recombinant Rv0560c showed that it directly methylated '14' at the N-5 position, leading to the loss of 14's antimycobacterial