



ILSI 2021 Annual Symposium Session 2: The Microbiome beyond the Gut

Transcript of the presentation, Microbiomes, Ecosystem Functions and Sustainable Agriculture, Brajesh Singh, PhD, Global Centre for Land-Based Innovation, Australia

Good morning or good afternoon everybody. Let me start with today to apologize for not being presenting live. Personal set of circumstances prevented me to come along. I would like to thank the organizers to ask me to come and share some of the work I'm doing in this area. As you can see, the presentations I'm going to make today is on the microbiome, ecosystem functions, and sustainable agriculture.

Let's remind ourself, the soil and the soil microbiomes, what are the key ecosystem functions and services they provide. For the human point of view, one of the key ecosystem functions is of crop productions. And that is being influenced by a number of processes, including the availability of plant-available nutrients, which is a link to enhanced biological activity in the soils and increase cation exchange capacity. The other key functions is filtrations in the buffering, soils [inaudible 00:01:20] want to play one of the most important role is filtering the water and buffering the water in the soils that is available for the plant uptake, and as a result, the plant re-productivity, but also of buffering and filtering for the better groundwater, which then ultimately supply back to in the stream and provide the water for agriculture irrigations, but also the drinking and other recreational purposes.

Soils have a critical role in the climate regulations, mainly through the emissions and mitigations or the absorptions, conjunctions of the greenhouse gas. And all these functions are mainly carried out by the soil microbes. But there are other drivers of the research in the microbiome, soil microbiome and the plant microbiome. Including the requirement to increase the food productivity productions by 50 to 70% by 2050. The other driver is that the farm productivity has been in a structural decline since the last few years, particularly in the developed country. What that means, the additions of additional inputs, the fertilizers or the pesticides, does not result into proportions in creating the productivity.

Agriculture is now being increasingly called for to contribute towards climate change mitigation. There have been a loss of agrochemicals and the plant breeding program in terms of productivity gain. And there are negative impacts of the quality of the human health, but I guess there's two types of the rational. One is a policy top-down, where the in government and inter-government organizations requires the sustainable productions, but also the bottom-ups, where the consumers are demanding for the quality food, but also the environmental protections. So low use of agrochemicals, particularly the pesticides in agriculture production system.

If you look at the old analysis that has been done over the last couple of decades, that where this gain of productivity at the same time minimizes the environmental protections, challenge can be made. It

comes from two aspects. And this is a diagram mainly produced by Professor Fusuo Zhang of Chinese Agricultural University in Beijing, where this suggests that the increasing soil health alone can increase the farm productivity by 15 to 20%. And if we combine and that the soil health with the resource efficient crop varieties, then we can gain 30 to 50% without further increase in the resource input in the soils. So, it's going to be increasing the productivity without further damaging our environment. And I will argue that both of these requirements soil health and resource efficient variety of crops will require the explicit considerations of soil and the plant microbiome.

Another driver is that the microbial product is, the market itself is a driver. If you look at the global perspective of the microbiome contributions to the human economy, it sits around \$1 trillion. And this does not include a lot of antibiotics that are mainly produced from the microbial communities. Then if you just look for the agricultural products, it sits here it is biological product, not just microbial product, of its total biological production sits around today around 5 to \$6 billion. Out of that, the four billion are the biopesticides. Out of half of that, more than half, 60% of that agriculture products are the microbial nature. The microbial product in agriculture is expected to increase to 12 billion within next five years. And so, the increase in the value is 3.5 times faster than the chemical pesticides. And looking because today's sits very high in terms of dollars, it's annual sales around 250 billion. Out of that is 55 billion is the pesticides.

And it is estimated that in the very near future, the microbial pesticides will be similar market size as the chemical pesticides. So, the market itself driving the research and innovation in this area. So, in my presentations, what I'm going to cover very briefly of three aspects. The soil microbial diversity, particularly focusing with what we call the most wanted list or the most dominant species globally microbes, and what role they play in the ecosystem function. So, providing some evidence in this area. The second point then I will talk about the plant microbiome and sustainable agriculture. And the third I will end my presentations with a few of the slides that talk about a global initiative that we launched to address some of the gaps in the fundamental knowledge in the plant and soil microbiome that we need to be addressed in order to advance this discipline and advance this industry.

So let us start with the first, which is the dominant microbial communities. If you look at the the ecological arc, we will see that I will use the words ecological principle and laws in my presentations, all through these presentations, because I believe that if the ecological principle exists, that has to be unifying in most cases. Not in all cases, but most cases. So, if there is a ecological theory it should be applicable across the different organisms. And from the plant ecology and the animal ecology, we have a good understanding that there's hundreds and thousands and millions of these species of plants and animals. But if you look at the global distributions, only few species really dominate globally. And we thought, if this is the case in the plant and animal, you expect similar sort of dominance in the microbiome communities.

This is the one studies that is carried out by Manuel Delgado Baquerizo. He was in the postdoc my lab, now is a well-known scientist himself in Spain. Where we sampled across the globe. You can see that where the sampling has been done in these slides. And what we did is to look at the microbial communities, both the bacteria and the fungal communities, and measure all other aspects of the ecosystem property, that's where it come from is temperate forest or the tropical forest, boreal or subtropical agriculture or dryland, and look at the multiple functions as well in those soils where those samples were collected. So, the first things to look at is there a dominant species of, here in case it is a bacterium. And so, we define the dominant species, again, sticking some knowledge from the plant

ecology that is present in the most of the samples. So, 75% and half of number of samples. And it is among the top 50 in terms of the relative abundance.

What do you see in this diagram below? There's only 2% of OTUs, or I guess a lot of people will [inaudible 00:10:24] the general species regulations. Only 2% of the total, which is equivalent to 551 phylotypes, was at the dominant microbial taxa across the globe. But on the right side, if you look at the same pie chart, you'll see that those 2% account for the 41% of the relative abundance of the total bacteria community. Giving the evidence of the presence of the dominant bacterial phylotypes across the globe. We went on to pursue and look for the fungal dominant species. Again, you see there is only 0.1% that was dominant. But accounted for the 18% of relative abundance. And what that then allows this sort of analysis is to predict the distributions of this microbial community across the globe.

And here on the right side, you see that for the fungal sort of things, fungal communities, we predicted that this is where fungal species are distributed across the different ecosystem, the forest ecosystem, because the globe dry lands and the Mesic Forest ecosystems. The other utility of this approach is, is to predict the economically important microbes. In this case, we look at the distributions of the soil born fungal pathogens response to global warming. And so, what we do is we look at the distribution of microbiomes across the globe, the soil born fungal pathogens. And we look at which are the key drivers. And what we find is there's two important factors, the moisture level and the soil temperature. And then we use the IBCC model to look at what will happen in 2035 and 2050 and projected what will be the distributions of those fungal pathogens.

You see, this is Phoma, Venturian, Fusariums, well-known taxa in terms of the fungal pathogens. And you see that, what do you see? That instantly, that most of the species, obviously the data here is only for three, but we've done a lot more analysis for like [inaudible 00:12:50] and so on and so forth. But what you see on the middle side, is that projected in 2050, you'll see that the abundance of these organisms or plant pathogens will dramatically increase under the projected climate change in 2050. But these are the projections. These are the modeling. People always ask us that we need some experiment evidence. So, what do we need that we took the samples are from the long-term climate change experiments? And where we look at the control, which is ambient temperatures and the warming temperatures, and look for using this times the qPCR to look for the abundance of these organisms. Sorry, the limited abundance of these organisms.

And what you see that is a consistent with our projection in almost every case, the limited abundance of this fungal pathogen phylotypes would increase by at least four under the climate change within the 10 years of the warming experiment. So that's the utility. This is just a soil born fungal pathogens for the plant. You can do the same things for the bacterial pathogens, for the human pathogens and animal pathogens and so on. Second aspect is that one of the real questions have been in the microbial ecology is that because microbes are so diverse, if you lose some diversity, will really have impact on the ecosystem functions. And the second question has always been the up-scaling because microbes are microscopic, so can that have impact on the ecosystems at the global level?

And this is a study we've done a lot earlier in the dryland. And here you see that the two sort of database, one is a dryland, global dryland, which come from the biocom experiments. And there's another database, which is Scotland, where I was before I came to UK. And we'll look again the bacterial and fungal community, but also look for the 16 functions we measure. And then we take the indices of those 16 measures into one measure, what we call the ecosystem multi-functionality. And so, they did do by different matters. One approach is averaging those functions into one indices. And this is a

structural equation modeling that's done to link the microbial community. And we chose these two data sets, again, to define that if there is a relationship between microbial diversity ecosystem functions like other organisms. So, we know there is for the plant diversity ecosystem functions. Then it has to be consistent against in the different ecosystems types.

And the drylands and the Scotland's are really contrasting ecosystems. In fact, I would say they're orange and apple. Drylands are really limited basalt, carbon, high pH. It is a very low carbon, most of the Scotland soils have a very high range for very high organic matter competitions and very acidic soils. But you see in both these cases, ecosystem multi-functionality was directly linked by the microbial diversity. It is almost 0.4% variation ecosystem multi-functionality was explained by the microbial diversity near dry land and similarly ecosystem functions in the Scotland. Saying that the impact of the relationship between microbial diversity multi-functionality is consistent irrespective of the ecosystem types. And after that, we have shown that in multiple ecosystems, including Australia, agricultural ecosystems and so on and so forth, but also at all levels from microscopic level to the global level.

So that was the relations in ecosystem function and multi-functionality. The second part of my discussions today, on my talk today, the agriculture microbiome. And as I say, there is a lot of demand for the two explicit considerations of the microbiome and development on harnessing of microbiome tools to increase the farm productivity to meet multiple sustainable development goals of the United Nations. This is because we have very good understanding now that the soil and plant microbes play a very critical role, particularly the soil microbes in terms of the provision of the nutrients through the nitrogen fixations and nitrogen and phosphorous utilizations and productions of the phytohormones. But also, both the microbes of the soils and the plant microbes play a crucial role in the plant defense against the pests and pathogens.

The other aspects is that they're having, I guess, so there is a lot of push, I'll discuss briefly, both from the policy point of view, but also from the consumer demands. And this has resulted into driven by some of the real translational success stories. I guess you guys probably heard that the European Union's Green Deal requires that 50% of the chemical pesticides and 30% of chemical fertilizers to be reduced in the use in agriculture by 20, 39 years from today. And that's probably will need to be replaced by the biological products or the microbial products. And this has been significantly driven the investment both by the government agencies, but also the multinational and the startup companies.

And that is one, the real excitement is there. There's a lot of to be discovered in the microbiome tools in agriculture. For example, if you look at the human medicine, between 50 to 60% of human medicines have a natural origin, either for the plants and majority of them from the microbes. But if you look at the chemical pesticides we use today it's only 11 percent of pesticides having that natural origin. So there's really a lot of things there that are ready to be discovered and employed in the agriculture. And that has driven the microbiome industry in really a really accelerated growth, from farm productivity to food security and quality of the food to human and environmental health. It is a one of the fastest growing industry in the world today.

But the use of microbial inoculants in agriculture is not new. We have been using for decades. In fact, rhizoBMs have been used more than a hundred years ago. But the problem is the efficacy of the microbial inoculants in the fields are inconsistent. It works in some ecosystems, some soils, some farm, but does not work in others. If you look at the product availability, there's the lack of product diversity. If you look at in what is in the market today, it mainly come in bacteria. It mainly comes from either from bacillus genus or now some streptomyces. Otherwise very few from other bacterial taxa. In fungi, it's as

tri-chordoma, and a couple of more, but there is a limited number of the taxa that is being used either as a growth promoters or as to control the pest and pathogen.

The third thing is the sustainability of microbes. Microbes are chemical that you live it and after a certain period of time going through. The formulations have to be such a way that microbes survive. This is a real constraint. And there are other factors that can influence the activities we did not talk about, is the competitions and the desiccations, the fermentations, so on, follow up it. But another example is there is a lot of product in the market, but there's no regulatory agency in the regulatory framework. To district is particularly what we call the plant good promoting microbes to distinguish between genuine and the snake soils.

And this then requires to have a better understanding of the plant microbiome assembly. I will be talking about that in a minute. Why? One of the reasons is there is a significant knowledge gaps in the fundamental and applied side of the plant microbiome interactions. Fundamental, I think we talk about that. One of the things we do not understand the relations, eco-evolutionary relationship that are eco-evolutionary process that determines the microbiome assembly. Particularly in the plant root regions or inside the plant tissues. We have a very poor understanding of what are the healthy and unhealthy microbiome. So, we say there's healthy soil and unhealthy soils, but we have no understanding what these means in terms of the microbiome, and on any biological activities.

Distinguishing correlations from the cause. And if you look at the another aspect that we have a little understanding is the microbiome beyond rhizosphere of bacterial communities is very less known, other than some of the fungal side of things. Both in the gut and microbiome, so just like in that animals, also in the plant. And on [inaudible 00:23:41], the applied research gaps in which we're in fact is that inoculate industry have been using this organisms, started from the microbes, fermented it, put it in formulation, throw back in the soil, and expected them to grow. But this microbe is going into the foreign environment, alien environment, and they need to compete with the indigenous micro-flora and have to able to utilize the resources that is being available. But to have this access, we need to understand the ecological requirement of those [inaudible 00:24:16] for the colonizations of the new environment.

Honestly understand, what are those ecological requirements. It will work in some area where the condition is favorable, and it will fail in other area where condition is not favorable. We also need to understand the interplay between the introduced microbiome with the host genetics and immune systems. We have very good industry understanding in animal, more known, but also increasingly in the plant more now that the plant physiology and plant immune systems play a very important role which microbes will be able to colonize each surface or inside the tissues. And we need to understand better for the microbial inoculants to grow the plant, the first thing it needs to do it needs to colonize the plants. And we need to understand how that introduce microbes that interacts with the plant immune system. And then other sort of things that can help going forward is one of the tools to manipulate the indigenous micro-flora. If you have the ability to switch on activities of some of the plant microbiome without introducing new microbes, or just by providing some chemical signals, that can achieve a lot better results than other way around.

So, address these issues, what we propose is what we call the system-based approaches, that looks into... Some [inaudible 00:25:49] look into two aspects. One is eco-evolutionary interactions between plant and microbes, but not with all microbes, what we call the core microbiome. And the core microbiota or the keystone microbiota. And we are defining the core microbiomes are those that are

present in particular plant species in all or most samples, irrespective of the climate conditions, soil types and management practice, put in the perspective. And because these microbes are considered to be inherited, either vertically, or have ability to attract those selective microbes very, very good. So, they are dominant in most of the environment. And if you put that plant, that plant specifically with a [inaudible 00:26:41] microbiome. So, there is a mutual recognition making the plant, and that that microbiota. And so, if you target that microbiota as a bio [inaudible 00:26:53], then the chances of those microbiota to colonize the plant is significantly higher as the regional phenotype will increase.

And we also say that there is another aspect that need to look into the multidisciplinary research, public partnerships and coordinated global approaches. But does the core microbiome exist? There is no significant evidence it does. I'll just give you one example that we looked at in the sugarcane across Australia. So, we sampled around 1600-kilometer radius where all the sugarcane are grown, and we look at the leaf and the root, and rhizosphere core microbiota. This is just an example that if you look at there, there's only 0.2% of phylotypes or OTUs fit into the sugarcane microbiota core microbiomes. And if you look at the rate of illness, those 0.2% is 35%. So, there's only a few species or phylotypes, sorry, that just satisfy the core microbiome definitions, but they are dominant.

And that's goes both for fungi and bacteria, both in leaf and roots. One of the questions that people always ask. And then again, you're still talking about the hundreds of species, and then we use the network analysis to identify the hub microbiome. And what is a hub microbiota? Microbiota that is linked to most of other, statistically at least, most of the other species or the phylotype of that leaf or the root system. And we come back with the 12 species or phylotypes of the microbes. There's always questions that, what is this statistical analysis, and you can't have cause... So, this is the correlations, and you can't say this have a role in the plant [inaudible 00:28:55].

To test that what we did, we take out one off the phylotypes by using antibiotics and grow the plants. What do you see here? There's a 50% decline by just knocking out one of the hub microbiota from the [inaudible 00:29:15]. That's give you the evidence. This microbiota is not just this big because of statistical dominance but did have a critical host function. And if you remove one, it has a direct consequence for health and fitness of the plant. So that's really exciting news, but I guess we need to... And as I said that another excitement comes from what we have understood in terms of the human microbiomes, and how the human microbiome influences every aspect of the human health, including our physiology, metabolism, disease progressions, our behavior and so on and so forth.

Plant microbiomes do the similar role. But our understanding of the plant microbiome is very limited in comparison to the human microbiome. We have a little understanding beyond the rhizosphere microbiome. Although the research is increasing to look at the interfitting micro-organisms. And as I said that microbiome beyond the bacterial community, except for the fungi or to microbial fungi, sorry, the microbial fungi is limited. And we need to move beyond that. We look at the fungi and the whole fungi, [inaudible 00:30:35] and other things like protists. They also play an important tool in the plant health.

So, moving forward, what we believe that we need to have a system-based approach, including predator and prey relationship, and the public private partnership for the research inception to market and the coordinated global approach. I think I said that before. That approach is for the improved outcomes. And to address these particular questions, we have established what we call the global initiatives of the crop microbiomes system agriculture. The short-term aim is to define the core and hub microbiota of these crops. And what are the process that influences the colonization of the rhizosphere in the plant endophytes. And how these microbes that contribute to the overall performance of the plants and the

yield. And the long-term of course, have a policy deliverance in terms of economic growth, it will create a new industry in the inoculations, it has a critical role in sustainability, in biodiversity, climate mitigation and so forth and so forth.

So, what we are doing in this project, we have identified five crops that these are the wheat, rice, corn, potatoes, and cotton, in the first instance. For each crop, we will sample 250 sites across the globe, and then will sample the rhizosphere and the bulk soils, but also the natural sites of 250 sites. And then for each plant samples, we will have leaf, stalk and roots samples. And then we will do the microbial analysis along with the soil properties, soil functions, plant and nutritional properties and plant traits and so on.

And this activity is already in the second year. Currently we have 178 scientific groups from across the globe who have signed on to work with us to drive this research. You see the distributions and this moment on the paper, it looks quite good. We, in fact, we already have received samples from the 27 countries, our target is 73. And we are working in a partnership with the multiple organizations, including the global soil bio-diversity initiatives and the microbiome support and bio-deserts and agriculture microbiomes. I believe that Linda Linco is speaking in this atmosphere. So, with that, I would like to finish my presentations with the acknowledgement of the key people who have contributed to some of the data presented today on the top of the slides is my external collaborators comes from the USA and Europe, particularly, and the bottom two lines is in the post-doc PhD students of my lab who carried out most of this. Thank you very much for your time, and I wish you have a wonderful session of the symposium. Thank you.