NUS National University of Singapore | Faculty of Science

Advances in Science

Volume 27 Number 1 June 2022

A special issue on Agri-food sciences: From the farm to the plate

FEATURES

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RESEARCH CENTRES

RESEARCH FEATURES

Advances in Science

The Faculty of Science conducts basic and applied experimental, theoretical and simulation research over a broad spectrum of science, mathematics and technology domains. We cover most of the key fields in biological sciences, chemistry, physics, pharmacy, food sciences, natural history, mathematics, statistics and data science.

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On the cover: The indoor farming research facility at the Research Centre on Sustainable Urban Farming is equipped to grow plants under different light wavelengths.

Research Centre on Sustainable Urban Farming (SUrF)

Enhancing food security and consumer health through research innovations

Introduction

Rapid urbanisation imposes unprecedented pressure on cities to feed their residents. By the year 2050, it is projected that over 60% of the world population will live in urban areas, and many of the large cities are located in Asia. Additionally, consumers have become more discerning and they expect higher quality fresh products with added health and nutritional benefits. Collectively, these pressing considerations highlight the urgent need to develop science-based solutions and technological innovations to shape future urban farming systems.

NUS has established a Faculty Research Centre on Sustainable Urban Farming (SUrF) in January 2022 to facilitate multidisciplinary research in the field of urban farming. This centre will leverage on the wide spectrum of expertise available at NUS and form highly effective interdisciplinary teams to address the research needs in this emerging area (see Figure 1).

The mission and vision of setting-up SUrF are as follows:

Mission: to leverage on the diverse expertise within NUS to provide a platform for undertaking highly integrated multidisciplinary research that deepens the scientific understanding to drive innovative, sustainable and transformational solutions for the urban farming value chain.

Vision: to create a globally competitive research program in the sustainable urban farming space that incorporates smart agriculture solutions for diverse stakeholders.

Broad research scope of SUrF@NUS

SUrF seeks to work on the following topics to address major scientific and technological gaps and challenges in the urban farming space:

(i) Improve crop varieties for indoor farming. The available varieties are not ideal for indoor farming because they were developed for outdoor cultivation.

(ii) Identify better growth substrates. Growth substrates increase waste disposal needs. Also, in hydroponics cultivation, there is a lack of root sensing of the solid/ soil surfaces. This can affect plant metabolism, their flavor compounds, texture and stress tolerance.

(iii) Address knowledge gaps in nutrients and nutraceuticals specific for indoor crops.

(iv) Better understand the effect of light emitting diodes (LEDs) on growth, nutrient enhancement and decontamination. The use of different LED wavelengths spans production and post-production phases, including for decontamination purposes. The effects of LED illumination on plants beyond biomass yield, such as on quality (i.e. nutrients and nutraceuticals) is not available.

(v) Improve understanding of beneficial microbiomes for plants. Natural microbial communities (microbiomes) around plants influence growth and plant products, almost to the same extent as what their genes provide the plants. Hence, it is necessary to better understand the roles of these plant microbiomes.

(vi) Advance post-production science to benefit consumer health. The post-production processing and handling of food has profound effects on consumer health because they can influence nutrient composition and food product safety. Also, the shelf-life of products can be extended by proper processing technologies.

(vii) Develop data science and artificial intelligence (AI) solutions for indoor farms. There are currently no effective data-driven solutions for optimising plant growth and metabolism. Thus, it is essential to identify such solutions.

(viii) Develop sensing technologies and robotics for agricultural automation. Smart sensors can help to monitor real-time growth and the health of plants during various stages of cultivation. They can also provide valuable data for the optimisation of the production system. Innovative robotics solutions can facilitate automation of the different stages, such as pollination, harvesting and post-production handling.

Partnering with stakeholders in the urban farming value chain

The establishment of SUrF is expected to enhance the on-going partnerships between NUS researchers and Singapore-based agribusinesses. The partners could include both upstream producers of multiple large-scale agri products (e.g. RGE group / Asia Pacific Resources International Limited

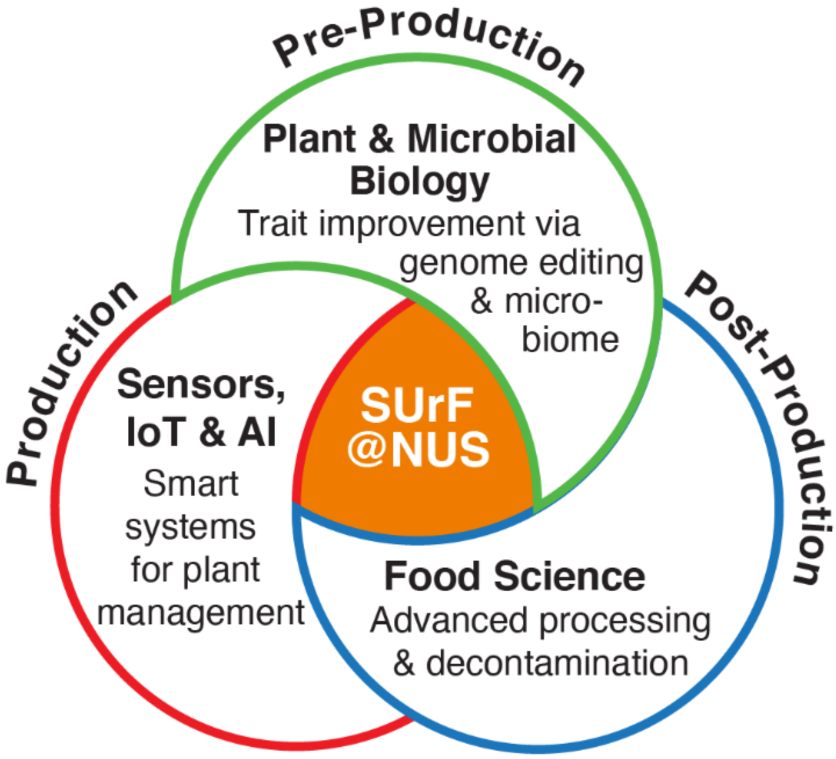

Figure 1: The overall concept of SUrF. A highly multidisciplinary research approach will address the three key stages of urban farm production.

(APRIL), Indofood Agri Resources, New Agri Seed, etc.) and also downstream players (e.g. Archisen, Sustenir, Vertivegies, SinGrow), which focus more on modular urban farm systems.

SUrF will operate as a virtual centre at participating NUS laboratories. A new indoor farming facility is currently being constructed for research purposes within the Faculty of Science, NUS. This core research facility will be managed by SUrF. It will house several pieces of equipment such as plant phenotyping tools and various sensors, to support research activities and facilitate the multidisciplinary projects undertaken by PIs associated with SUrF and their industrial collaborators.

What is sustainable urban farming?

Our broad definition of sustainable urban farming includes all aspects associated with the cultivation of food plants in indoor farms. This includes the production of novel biotechnology products (e.g. nutraceuticals, proteins and novel biomaterials) via such "plant factories". An important point is that it is essential to integrate good environmental and farming practices and limit potential pollutants for the long-term sustainability of urban farming and for promoting consumer health.

Why focus on urban farming research?

Farming practices and technologies have undergone remarkable development and intensification during the past several centuries. In the face of challenges posed by climate change and population increases as well as potential disruptions caused by the pandemic and natural calamities, there is a need to significantly change the way we grow crops to provide adequate food in the coming decades.

The concept of urban farming has evolved with the idea of producing food plants under controlled environments. This technology combines a low environmental footprint through reduced energy and water resources needed for crop cultivation. It also eliminates uncertainties associated with outdoor farming that traditionally rely heavily on farmland availability, good weather and adequate water supply.

Mere adoption of currently available solutions from traditional farming techniques is inadequate for indoor farming because this sector has its own special needs. Therefore, novel solutions that integrate capabilities coming from multiple disciplines based on the core fundamental sciences need to be developed. This requires multidisciplinary specialist teams to work seamlessly together.

Based on industry trends, we expect that stakeholders (e.g. growers, food processors, distributors, retailers, consumers and waste managers) will rapidly adopt novel scientific breakthroughs and solutions when these become available. In Singapore alone, a number of start-up companies in this space have sprung up in the recent past, indicating a strong interest in urban farming activities locally. Such "plant factories" could also be production sites for higher value nutraceuticals and biotechnological products. Through SUrF, we hope to be able to make novel contributions towards successful sustainable urban farming in Singapore.

For more details, please visit: https://www.dbs.nus.edu.sg/surf/

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Nature-derived solutions for climate-resilient food production

Reaffirming the ages-old associations between plant crops and their companion microbes

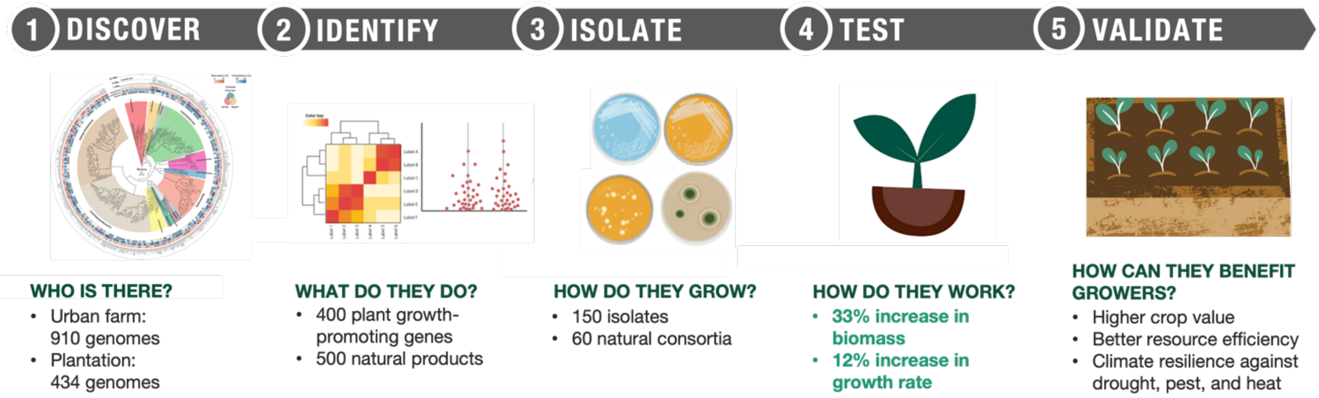

Figure 1: Microbiome-based nature-derived solution for food production.

Introduction

In an uncertain era of environmental change, it is crucial to re-evaluate our interactions with the environment. Against the backdrop of impending climate adversity and rapid urbanisation, sustaining food production using conventional means will be a key challenge in the future. Although the agricultural advancements from the first Green Revolution (1950–1970) have served us well, we recognise the need to adapt and transform existing practices to intensify crop yields—this time with sustainability ethos taking center stage once again. By adopting practices that optimise resource use and by investing back into ecosystem health, nature-derived and nature-based solutions seek to limit the impacts of food production and ensure the longevity of natural resources.

In resource-limited Singapore, food and nutritional security is a perennial issue. To meet the '30-by-30' goal of providing 30% of nutritional needs

locally by 2030, local food production would need to increase multiple-fold, while also investing in sustainable, ecology-inspired technologies and innovations within the agri-food ecosystem. By drawing cues from nature, we seek to develop new climate-resilient and resource-efficient technologies that can be integrated into urban farm systems (see Figure 1).

Our nature-derived approach is inspired by multi-trophic ecological associations thriving within the local ecosystems. It is centered around the discovery and application of microbial associations found in nature. Over millions of years, plants and their associated microbiomes have formed highly complex, inter-dependent relationships that heavily influence plant growth, productivity, and resilience. Using knowledge of microbial associations, we can then develop sustainable nature-derived solutions to fulfil a variety of needs in the agri-food ecosystem, such as rejuvenating soil health, enhancing crop nutrition and building climate resilience.

Nature-derived solutions

Nature-based practices provide sustainable means to tackle the challenges of the current agricultural climate, including land degradation, pollution, and climate change. These problems might also be consequences of poor agricultural practices and land management. By integrating biological associations found in nature, we can efficiently draw upon more of the naturally available resources and ecosystem services to boost agricultural productivity. In practice, we can then reduce carbon, chemical and energy footprint that is central to current practices in conventional agriculture.

In Singapore's context, nature-derived technologies will help to build circularity and sustainability for our nation's future food security. Implementing these technologies can benefit stakeholders at all levels, ranging from tech-driven urban farms to conventional field-based farming. Currently, we are focused on developing products and services derived from microbial associations to enhance crop performance, while minimising the nutrient and chemical inputs to reduce carbon footprints. By tapping into our understanding of both plant and microbial systems, we can couple crop systems with suitable microbial partners depending on the needs of growers and consumers, for example, by promoting plant growth, increasing crop nutritional density, improving fertilizer use-efficiency, or conferring pest/ pathogen resistance.

Most importantly, nature-derived solutions can find applications in any agricultural system, be it controlled

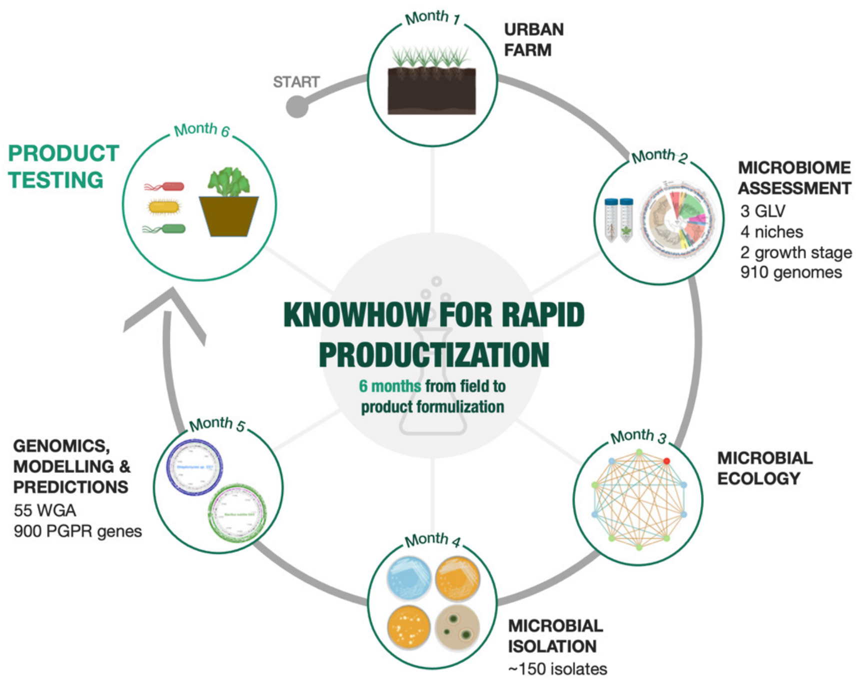

Figure 2: Bringing lab ventures to the table, our framework for rapid engineering of microbiome solutions.

greenhouse environments or conventional open-air farms. The opportunities afforded by nature-derived practices could extend to an entire regenerative farming ecosystem. Microbial associations from nearly every component of a natural ecosystem can be drawn upon and incorporated into analogous roles within agricultural systems, including wastewater treatment and post-harvest recycling.

Pushing the frontiers

In no small measure, our nature-derived approach has been driven by recent technological advances, which afford the opportunity for us to comprehend the inner workings of microbial associations in nature. However, the field is relatively young, and more efforts are necessary to process large quantities of high-fidelity data, deconvolute complex microbiome networks, and then harness the benefits of selected microbes. For rapid development of effective microbiomes for sustainable agriculture, an engineering-based framework has proven to be highly suitable (see Figure 2).

After isolating microbes from the environment, a crucial part of our approach pivots on the ability to generate in-depth understanding of their roles and mechanisms. To achieve this, diverse computational pipelines will be required to process large quantities of omics data. Next, genome-scale metabolic models have been developed to efficiently simulate the interactions and dynamics of key microbial members. This knowledge will allow us to assemble consortia with synergistic microbial members, which would have stronger, more lasting effects on soil health. Then, by examining how environmental parameters influence microbial members, we can then derive best management practices to maintain microbiome health. We would thus obtain a thorough functional understanding of our selected microbiome composition and may then proceed to conduct field trials.

A few more bottlenecks must be resolved for widespread commercial adoption to proceed, such as uncertainties regarding colonisation efficacy and persistence of microbiomes in field conditions. Another more pressing concern lies at the interface of science and policy and any innovation must be attractive from a business point of view to ensure wider buy-in from farmers and consumers.

In conclusion, nature-derived solutions, supported by data science approaches, offer an adaptive tool to meet upcoming challenges of global food production. Through the adoption of targeted microbiome-based practices, we can adopt a more sustainable vision of regenerative agriculture, by reducing ecological footprints while raising resilience to climate uncertainty.

For more details, please visit: https://smbl.nus.edu.sg/

Sanjay SWARUP is an Associate Professor with the Department of Biological Sciences, NUS. He is also the Deputy Director of NUS Environmental Research Institute and Deputy Research Director at the Singapore Centre for Environmental Life Sciences Engineering. He obtained his M.Sc. and Ph.D. in genetics from the Indian Agricultural Research Institute and another Ph.D. in plant pathology from the University of Florida. His research interest includes studying the ecology of highly complex microbial communities in the environment and in agriculture, from genes to ecosystems.

Darren SIM is a research fellow developing different approaches to harness the potential of plant microbiomes for enhancing the productivity of different food crops. Shruti PAVAGADHI is managing local and regional level research programs under the agri-food sustainability umbrella. Nura WONG is a Ph.D. student working on data analytics in urban agriculture.

Members of the research team (from left to right): Dr Darren Sim, Dr Shruti Pavagadhi, A/P Sanjay Swarup and Ms Nura Wong.

Breath control for urban farming

Changing stomatal numbers in plants for optimised growth and improved shelf-life

Introduction

Food security has become a major focus for many nations. This is largely due to the impact of climate change on food production, but the COVID-19 pandemic and its disruption to supply chains also highlighted the danger of relying solely on food imports. As Singapore currently imports over 90% of its food supply, it is particularly susceptible to the instability of global food supply. To address the challenge, the Singapore government has launched the "30 by 30" initiative in 2019, which aims to produce 30% of the country's nutritional needs locally by 2030. This would entail a substantial increase in local food production, and thus, the need to develop and adopt agri-tech that can boost production efficiency in the land-scarce nation.

In my lab at the Department of Biological Sciences, National University of Singapore, we study how an essential cell type called the stomatal guard cells are formed in plants, and explore the potential of developing novel plant varieties that are better suited for urban farming.

Importance of stomata for plants

If you take the greens from your salad bowl and put them under a microscope, you will observe many pore-like structures that are distributed throughout the leaf surface (see Figure 1). These microscopic pores are called stomata, and they are formed by a pair of guard cells. Stomata are critically important for plants because they allow plants to "breathe", i.e., the exchange of gases between plants and the atmosphere. The most notable gas that plants need to take in is carbon dioxide, which is fixed in a light-dependent process known as photosynthesis and is converted to sugars to support plant growth. A

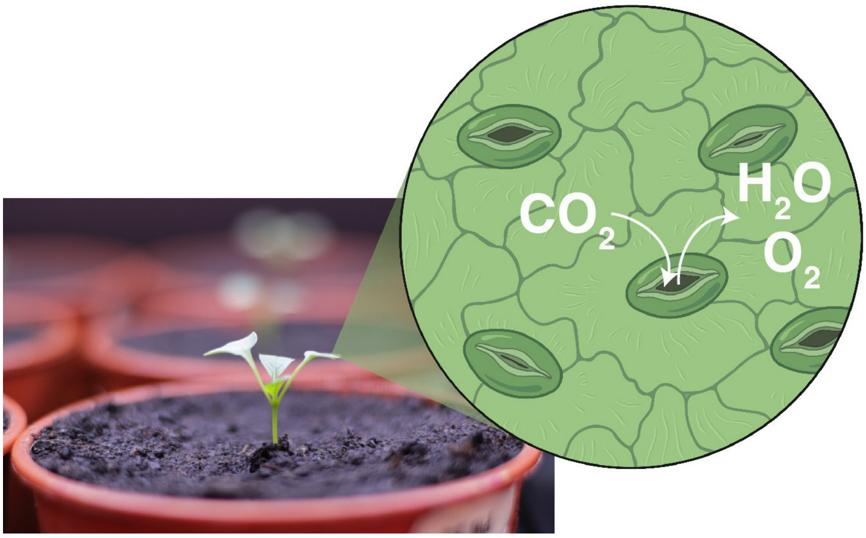

Figure 1: Stomata are microscopic pores on the surface of leaves that mediate critical gas exchange between plants and the environment. [Created with BioRender.com]

"side-effect" of this exchange is the loss of water through the stomatal pores, in the form of water vapour. Thus, this gas exchange process is a trade-off for plants, where they need to balance carbon uptake with water loss. To achieve this, plants not only control the opening and closing of the stomatal pores, but also modulate the number of stomata that they produce. Understanding the underlying genetic programs on stomatal production, therefore, would enable scientists and urban farmers to optimise yield and water use-efficiency of plants for urban farms.

Understanding how stomatal production is controlled

Surprisingly, the logic of how guard cells are made in plants is similar to how specific cell types, such as muscle cells, are produced in the human body. Specialised cells begin as cell type-specific stem cells, divide multiple times, and eventually "mature" to form the final cell products. Research in the past fifteen years have identified the key regulatory proteins and signaling molecules that mediate each of these steps in the formation of guard cells. For example, the master regulatory protein named SPEECHLESS (SPCH) is responsible for producing the stomatal stem cells, and without it (e.g. in the mutant spch), the plant cannot generate stomata and will die at the seedling stage (see Figure 2). Further, how environmental signals can influence the activity of the stomatal stem cells, which leads to altered stomatal numbers, is starting to be elucidated. For example, we recently discovered a mechanism by which light promotes the production of stomata (see Figure 3). We found that a secreted peptide called STOMAGEN is induced by light and it stabilises SPCH and enhances stomatal production. Thus, with this knowledge, we now have a few genetic access points where we can manipulate stomatal numbers and their response to varying growth conditions.

Controlling stomatal number for improved plant traits

How is altering stomatal numbers

useful for urban farming? As mentioned earlier, stomata impact carbon uptake and water loss in plants. By increasing the number of stomata in crops, one could boost their carbon uptake, providing more "fuels" for photosynthesis and drives plant growth. Given water resource is not a major constrain in indoor farms and that the level of light intensity and carbon dioxide can be elevated artificially, this is an attractive approach to improve yield through engineering novel varieties. Alternatively, in more traditional farming settings, e.g. rooftop and outdoor farms, where water is a limiting factor, plants with reduced stomatal number shall be more resilient to drought and utilise less water during cultivation. Another potential useful application is related to the post-harvest shelf life of plant products. After harvest, vegetables, fruits and even flowers continue to lose water through their stomata, but with no access to water, they will wilt in days. Creating varieties that have lower stomatal numbers in the specific plant parts can help reduce water loss and extend their shelf-life.

While the application of stomatal control is still in its infancy, it holds considerable potential for optimising plants to the "new" growth environment of the urban farms. The advancement of gene-editing technology, sensor systems and artificial intelligence (AI)-enabled monitoring and modeling of plant growth shall help create and select new generation of crops with stomatal traits tailored for urban farming.

For more details, please visit: https://www.dbs.nus.edu.sg/staffs/lau-on-sun/

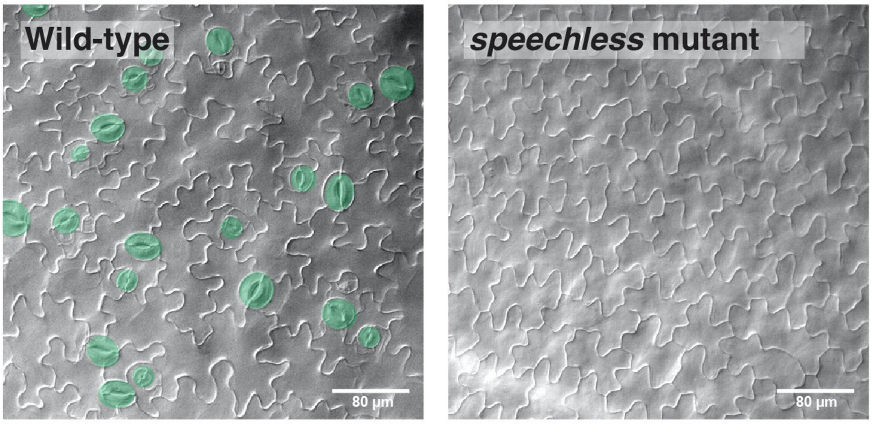

Figure 2: The *speechless* mutant plant cannot make stomatal guard cells on the leaf surface. [Images are adapted from Wang et al., Nat. Commun. 2021 under Creative Common licence (http://creativecommons.org/licenses/by/4.0/)]

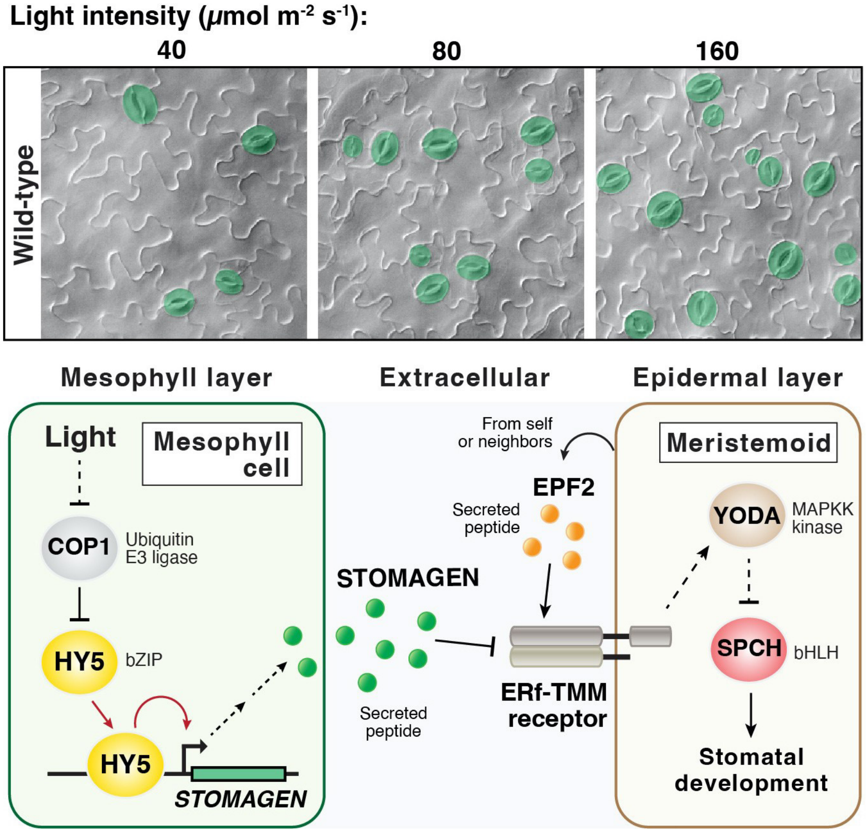

Figure 3: Light promotes the production of stomata in plants. Our lab has recently identified the underlying mechanism of this process. [Images are adapted from Wang et al., Nat. Commun. 2021 under Creative Common licence (http://creativecommons.org/licenses/by/4.0/)]

LAU On Sun is an Assistant Professor at the Department of Biological Sciences, NUS, and Associate Director of the Research Centre on Sustainable Urban Farming (SUrF). He received his Ph.D. from Yale University, USA, and did his postdoctoral research at Stanford University, USA. His research focuses on plant developmental biology and plant environmental responses, using the development of stomatal guard cells as a model system.

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A holistic approach on food microbial safety

Reducing obstacles on the road towards a more sustainable agri-fresh produce supply chain

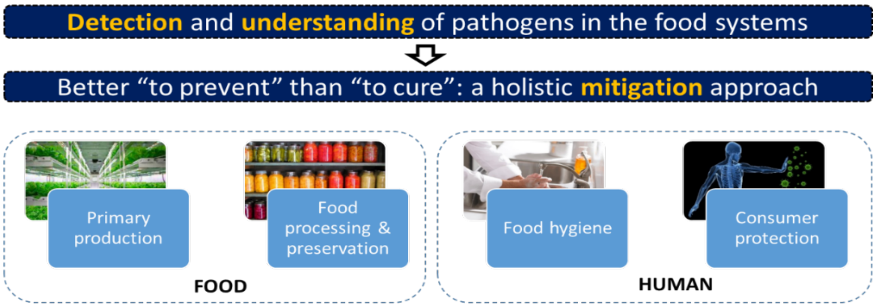

Figure 1: Illustration of our holistic research scope on food microbial safety.

Introduction

Safer food saves lives. Billions of people are at risk. Millions fall ill every year, and many die from consuming unsafe food. According to the World Health Organization (WHO) estimation in 2010, [1], of approximately 600 million cases of illness caused by 31 foodborne hazards, infectious (referring to microbiological) agents that cause diarrhoeal diseases accounted for the vast majority. However, food safety is often taken for granted. Food safety concerns usually come to the fore when there is media coverage of incidents with public health, trade and economic impacts. Moreover, food safety regulations and concerns are sometimes perceived as conflicting with, or hindering changes in the food system for more sustainable food production.

The mission of our research group is to understand and mitigate foodborne microbial threats in food systems and to adapt them to the unique urban food system of Singapore as a city-state. Typically, a food microbiologist focuses on the detection and mitigation of foodborne pathogens in food products. This is also our primary research direction. However, in recent years, we realised that it is more effective to adopt a preventive approach rather than one focusing on "curing". This leads to our research direction on the microbial safety of emerging urban agriculture systems and other alternative food production systems. Also, as one is aware, it is not possible to have zero risk when it comes to food safety. Coupled with lessons from the Covid-19 pandemic on better protecting vulnerable groups in our population, the third research direction of our group is therefore on the effectiveness of functional food components in conferring protection from foodborne illnesses for this group of people.

Here, we present two representative studies by our research group as an overview of our holistic food microbial safety research (see Figure 1).

Sustainable options of urban farming systems: A mixed blessing?

Due to limited land space, there is a strong emphasis on indoor vertical farm technology in Singapore to achieve its food security goal. Most indoor vertical farms employ hydroponic systems, where plants are grown in a water-based, nutrient-rich solution. The roots of the plants are supported by substrates such as peat moss, clay pellets, perlite or rockwool. Soil or other soil-like substrates are sometimes also used in indoor farming. Environmental variables such as temperature, humidity, carbon dioxide levels and lighting conditions are carefully calibrated and controlled. This allows for higher crop yield with the same amount of space, compared to traditional farming techniques. It also maximises the efficiency of water usage and allows the flexibility of having multi-trophic farming systems such as aquaponics. However, indoor farming has its own share of food safety issues. For instance, in July 2021, eight varieties of hydroponic greenhouse-grown leafy green vegetables from the BrightFarms brand were linked to a *Salmonella* outbreak in the United States. Eleven people became ill, two were hospitalised, and there were likely more unreported cases of illness (estimated to be 30 times of the reported cases) during this outbreak.

The majority of indoor farms in Singapore use recirculating irrigation systems. These systems are typically used for a few years and they are not replaced unless a malfunction occurs. Undesired microorganisms can be introduced to the system from multiple sources, including water (e.g. water reservoirs from recirculating irrigation system), the growth substrate (e.g. coco peat, clay pebbles, hydroponic sponges) and/or the seeds/ seedlings of plants. Our recent study demonstrated that once *Salmonella* contamination is introduced, it spreads rapidly to the whole hydroponic system, likely by the circulation of the nutrient solution [2].

Our group also investigated the potential of applying photodynamic inactivation (PDI) as a preventive measure to enhance the microbial safety of hydroponic farming systems [2]. The findings show that when PDI is applied, the *Salmonella* population decreased at comparable levels in both the seeds and the seedlings. However, the PDI treatment is effective only for seedlings as the residual *Salmonella* on the treated seeds proliferated to high levels after germination. This demonstrates the vital importance of technical knowledge when applying food safety mitigation strategies in indoor agricultural systems.

Easy home fermentation? Hot culinary trends? Watch out for the pitfalls!

Fermentation, especially spontaneous fermentation, has evolved from an ancient food preservation method to a stylish cooking trend recently. Accordingly, the associated food safety aspect should be revisited, particularly since inexperienced people are increasingly performing spontaneous fermentation on an ad hoc basis. Not only are restaurants creating "new flavours", but consumers, especially the young generation, are influenced by social media to devise their own "creations".

Recently, our study compared spontaneous fermentation of carrots with a probiotic starter culture *Lactiplantibacillus* plantarum 299v [3]. According to our results, spontaneous fermentation may compromise on food safety as it depends primarily on raw materials, utensils used and the fermentation method. This creates many uncertainties. *Enterobacteriaceae* are commonly found in fresh produce. Many microbes belonging to the *Enterobacteriaceae* family are known to be human pathogens or opportunistic pathogens, including *Salmonella*, (pathogenic) *E. coli*, and *Shigella*. During the fermentation of vegetables, *Enterobacteriaceae* may also increase the possibility of spoilage. On the other hand, *L. plantarum* 299v, as the starter culture, could suppress possible *Salmonella* contamination and *Enterobacteriaceae* levels in vegetable fermentation systems (see Figure 2).

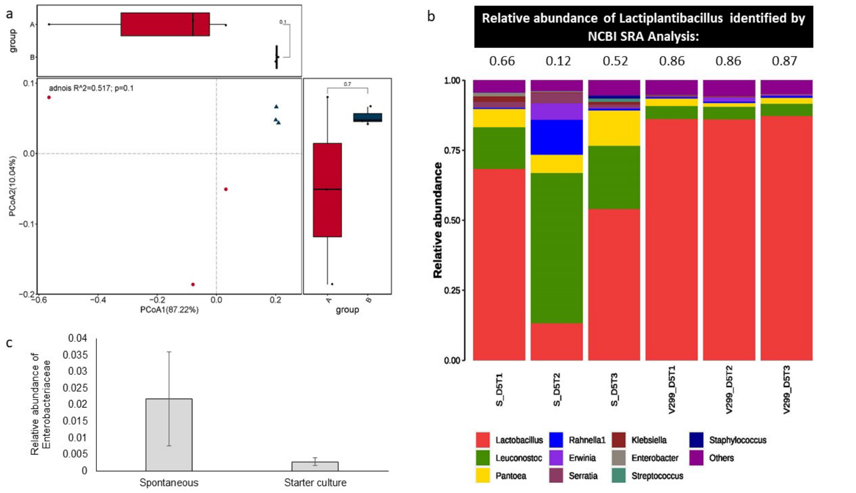

Figure 2: Taxonomy analysis of the microbiome in carrot fermentation using the probiotic *Lactiplantibacillus plantarum* 299v as a starter culture and in-parallel spontaneous fermentation. (a) Principal coordinate analysis (PCoA) at operational taxonomic unit (OTU) level (the first two components PCoA1 and PCoA2) revealed greater diversities of samples from spontaneous fermentation (group A, red dots) than fermentation using *L. plantarum* 299v as the starter culture (group B, blue triangles). (b) The identified genera consist > 1 % of the total microbiome in one or multiple samples. S_D5T1, S_D5T2 and S_D5T3 represent samples from spontaneous fermentation. V299_D5T1, V299_D5T2 and V299_D5T3 represent samples from fermentation using *L. plantarum* 299v as starter culture. (c) The relative abundances of *Enterobacteriaceae* family between the samples from the two groups.

The concept of using probiotics as the starter culture in fermentation could also increase the health benefits of fermented products.

To test the feasibility of using *L. plantarum* 299V as a starter culture to ferment household vegetables, we organised a small-scale study involving two inexperienced middle school students. They purchased *L. plantarum* 299V capsules (Jarrow Formulas) and performed six independent carrot fermentation trials at home using *L. plantarum* 299V as a starter culture, with spontaneous fermentation running in parallel. The fermented products were brought to our laboratory for microbial analysis and the results were consistent with those from our study. This project also won the Merit Award at the Singapore Science and Engineering Fair (SSEF) 2021.

Our research group hopes to gain more insights to mitigate the microbial contamination in our food chains from farm to fork. This will help improve the capacity to prevent and control foodborne diseases to benefit the community.

For more details, please visit: https://www.fst.nus.edu.sg/our_people/faculty-members/li-dan/

Dan LI is an Assistant Professor at the Department of Food Science and Technology, NUS. She is also the Associate Director of the Research Centre on Sustainable Urban Farming (SUrF) at the Faculty of Science. Prof Li obtained her Ph.D. in Applied Biological Science from Ghent University, Belgium in 2012 and worked as a postdoc researcher at Ghent University from 2013 to 2018. Her research focuses on food microbiology and food safety.

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