For a Healthier and Wealthier Developing World

By SONG Jianlan (Staff Reporter)

Offering lectures both on science frontier and practical skills, a training course on food biotechnology specially tailored for developing countries showed how biotechnology can upgrade ancient approaches to food production, like fermentation.

Fermentation, an ancient method of food production, is able to improve foods and beverages with respect to flavor, texture and nutritional value. Showed are bubbles forming in the process of beer brewing. (Photo: Wikipedia)

"A small sachet of the seed starter can produce over one hundred of meals," Dr. Wilbert Sybesma waved a small package when introducing "Yoba", a yoghurt-like beverage based on local-sourced probiotic bacteria strains to the participants at a food biotechnology training course occurring in Beijing: "It is very very affordable," he asserted. When asked about the shelf life of the sachet, his answer stirred a wave of excitement: "At least three years under ambient environment."

Dr. Sybesma was teaching at the 2015 Food Biotechnology Training Course for Developing Countries occurring from November 26 to December 2, 2015 in Beijing, organized by the CAS-TWAS Centre of Excellence for Biotechnology (CoEBio, http://www.cas-twas-coebio.org/), which is based in the CAS Institute of Microbiology (IMCAS, http://www.im.cas.cn). Through dissemination of biotechnology, the organizers hoped to help promote science excellence in developing countries, targeting the needs of the local social and economic developments. The training course covered fundamentals of fermentation, including the dynamics and products of the metabolic process in microorganisms, the potential of fermentation to improve food quality and increase nutritional value, modern techniques of strain selection and optimization, and practical skills needed in lab experiments, grant application, academic publication and team building. On top of these, a practical workshop embedded in the training offered an exciting opportunity for the participants to turn their dreams true: they were encouraged to propose their ideas to develop new fermented foods for their local people, aiming at solving some local issues. The outstanding proposals would win financial and technical aids from the organizers.

Dr. Sybesma, currently working for Nestlé Research Center, co-founded the Yoba for Life Foundation, a non-profit organization active in East Africa that aims to improve health and wealth in developing countries through the development, production and distribution of Yoba. An important idea of this project involves establishing indigenous ability to produce this healthy, affordable beverage. For this sake Dr. Sybesma not only shared his team's experience of Yoba, but also the science involved in developing the starter yeasts, particularly modern biotechnology for strain selection and optimization.

Dr. Wilbert Sybesma tells the story of "Yoba". (Photo by SONG J.)


Trainees in class. (Photo by SONG J.)

Magic Powder

"The secret of its long shelf life is," answered Dr. Sybesma to the inquiry of a trainee: "We made it very dry — the drier the better, and stored it together with some dry starch, which supplies the strains with maintenance energy to survive at low metabolic rates."

This handy sachet starter consists of optimized strain combinations of probiotic bacteria identified and selected from the local environment of Uganda. Using local species and strains makes it easier for the microbial community to survive; also it promises better robustness in presence of adversity. "Another reason is the costs," Dr. Sybesma added: "Otherwise the end products could be too expensive for the local people, which would not be what we expected."

This magic powder interested the audience immediately. "Maybe we can find some local probiotic strains and make our own brand of 'Yoba'," said Furqana Khalid Chaghtai,

a trainee from University of Karachi, Pakistan. "It's better than importing the microbes from Uganda, at least for a better survival of the probiotics," she laughed. According to Afolake Atinuke Olanbiwoninu, trainee from University of Ibadan, Nigeria, they have in their country a fermented beverage similar to yoghurt. "Therefore Yoba is not exotic for us," she commented, agreeing it is easy to transplant the idea to her homeland, though geographically Nigeria is not so close to Uganda, where the "Yoba for Life" concept has thrived for years.

More important secrets of the starter for Yoba, however, lie in the science profoundly seated in the construction of the microbial community, which has demonstrated excellent quality, stability and robustness. To deliver the involved knowledge, Dr. Sybesma prepared three lectures, with the first focusing on the probiotic bacteria themselves, including the concept of such special species, the characters distinguishing themselves from other bacteria, health benefits of them for humans, how they survive the digestion process and the acid environment of human intestinal tracts to work alive at the depth of the guts, and methods of identifying the strains from their environment.

Valued Seeds

"What should you rescue if you could choose only one thing from your bakery when it caught fire? The dear piece of old sourdough." — An experienced baker

The value of a good leavening agent for a bakery could never be over-stated, but only very few people know the secret of the microbiota involved.

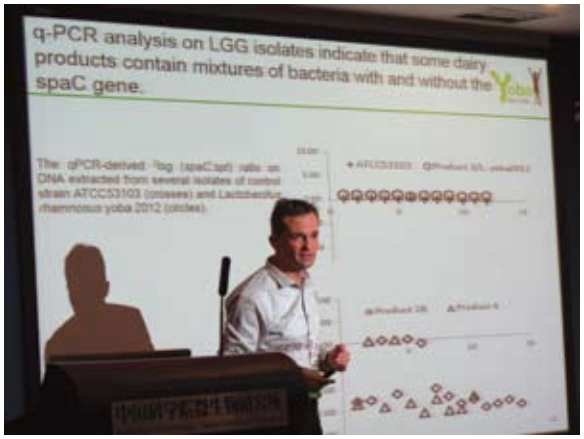
The microbial community in a starter culture for fermentation could be extremely complicated, as the result from a long evolution process of an ecosystem comprised of yeasts, lactic acid bacteria (LAB), and probably molds — which could be annals of wars among species and strains. This means the compositions, and the subtle equilibrium or the robust symbiosis among different actors in the community could be impossible to replicate. The same uniqueness constitutes the value of the seed starter of Yoba. How did they make it? Dr. Sybesma unraveled the secrets in the yeasts in his second lecture.

Particularly, Dr. Sybesma introduced techniques for strain construction and selection, illustrating how to meet different goals with aid from modern biotechnology, for example how to make the colons more acid tolerant (which is important for their survival in human guts), more stable at high temperatures, and more productive; and how to improve the nutrients in the end product through manipulating compositions of strains in the starter. For this sake Dr. Sybesma introduced multiple techniques, both genome modification (GM) and non-GM techniques involved in screening and selection of desired strains, including random mutagenesis (spontaneous or induced) and site directed mutagenesis, dominant selection schemes, and single-strained DNA recombineering. This arrangement provided alternative options for consumers who are concerned about genetically modified foods.

In his third lecture Dr. Sybesma introduced the concept of and the latest research on bacteriophages, which are seen as a possible therapy against multi-drug-resistant strains of many bacteria, as a potential way to combat bacterial infections in developing countries.

Dr. Herwig Bachmann from NIZO Food Research, Vrije Universiteit Amsterdam of the Netherlands also dealt with the choice of organisms and their improvement/selection for desired metabolic activities in food fermentation. He touched

A trainee-lecturer discussion. (Photo by SONG J.)



Dr. Wilbert Sybesma explains to the audience how to isolate probiotic bacteria from the environment. (Photo by SONG J.)

on more technical details, including how to characterize different organisms based on sequence information and high throughput phenotyping. With attention cast to the biodiversity of the ecosystem and interactions between strains, his introduction of quantitative methods like metabolic modeling and cases of growth strategies made his illustration of experimental evolution of industrially relevant traits more inspiring.

Sustainable Production

As showed in the case of Yoba, to reconstitute a microbial community for expected fermentation to produce foods of desired quality is difficult, but not impossible. With aid from modern biotechnology, scientists nowadays are able to determine the compositions and even manipulate the evolution of the ecosystem of the reconstituted microbial community, at least to some extent, to meet certain goals, say to assure good taste and texture, or even better, with enhanced healthy nutrients like folic acid, amino acids, low-calorie sugars, anti-microbial peptides like nisin *etc*., in the end products. Such developments have offered better opportunities for underdeveloped countries and regions to solve malnutrition problems and mitigate poverty with fermented foods; also they provide new opportunities for sustainable production of nutritional components and hence contribute to the sustainable development.

Prof. Jeroen Hugenholtz, Senior Scientist of Bioconversion at Food & Biobased Research at Wageningen UR of the Netherlands, an internationally acclaimed expert in food fermentation, has been working with the CoEBio for years to disseminate fermentation-related biotechnology. After introduction of fundamentals of fermentation, he shared various fermentative approaches to improving foods and beverages with respect to flavor, texture and nutritional value. Particularly he introduced a number of different fermentative microorganisms and their metabolic pathways to flavor compounds, exopolysaccharides or vitamins, suggesting solutions to increasing the flux through the mentioned pathways.

With emphasis on sustainable production of ingredients, Prof. Hugenholtz gave more details about how to produce essential ingredients like low-calorie sugars, natural colors, antifungals and even biobased/renewable plastics via fermentation, as alternatives for the food and beverage industry.

Echoing the idea to use fermentation as a substitute for industrial production, Dr. DU Guocheng from the School of Biotechnology of Jiangnan University, China shared with the audience his research on acid-

Prof. Jeroen Hugenholtz shares various fermentative approaches to improve foods and beverages with respect to flavor, texture and nutritional value. (Photo by courtesy of CoEBio)

tolerant mechanism and metabolic regulation of *Propionibacterium acidipropionici*, a family of bacteria able to synthesize propionic acid (PA). Given that the latter's calcium, sodium and potassium salts are widely used as preservatives in animal feeds and human foods due to their antimicrobial properties, and PA itself is an important chemical widely used in agriculture, food and pharmaceutical industries, more and more scientists are getting interested in R&D aimed at biosynthesis of PA, to solve the pollution and energy problems caused by the chemical synthesis process. Dr. DU is among them. His study aims at solving a paradox in the PA biosynthesis in the cell factory of these bacteria: the increase in concentration of PA produced in the cells inhibits the cell growth and PA synthesis itself. Inspired by the idea that rational regulation of metabolic pathways through genetic modifications might provide a solution, they performed a systematic analysis and investigation to understand the acid-tolerant mechanism of strains. Through comparing the wild-type *Propionibacterium acidipropionici* and its acid-tolerant mutants obtained by genome shuffling, they investigated the acid-tolerant mechanism of *Propionibacterium acidipropionici* at different sub-cellular levels, using metabolomics, proteomics, genomics and other modern methods. Based on this they succeeded in designing and constructing a gene expression system, to control and optimize the distribution of metabolic flux. As a result they successfully increased the production of PA with *Propionibacterium acidipropionici*.

Healthier World

Another expert, Prof. Dr Ing. Christophe Lacroix, Professor for Food Biotechnology of the Department of

Health Science and Technology, ETH-Zurich, brought a lecture on biopreservation and antifungal cultures as natural solutions to food spoilage or even diseases in humans. He shared knowledge on species that suppress fungi, for example antifungal LAB, propionic acid bacteria and combinations of them, highlighting recent achievements made by academia in antifungal metabolites and further inhibitory mechanisms. In his lecture he illustrated the role of combined high-throughput screening and comparative genomics in identifying the genes and functions that are responsible for antimicrobial activity.

In his second lecture, Prof. Lacroix turned to an interesting topic, the ironic effect of nutritional components on gut microbiota and human health. The complicated ecosystem of the innumerable different bacteria inhabited in human guts — which harbor over 3 million genes, as many as 100 times the coding capacity of human genome — is still poorly understood by scientists. The microbiota composition and metabolic activities of this largely unknown system are impacted by the diet, and in turn the host metabolism and metabolites also interact with the gut microbiota and diet, hence shaping a complex interaction network, which plays a previously neglected role in gut health and diseases including diabetes, malnutrition and obesity. In his lecture Prof. Lacroix shared his study in the absorption of iron (Fe), an important nutrient and its interaction with the gut microbiota. Previous research found that Fe is poorly absorbed and moreover, 90~95% of ingested Fe is "burnt out" in its interaction with the gut microbiota, and Fe supplementation or fortification could ironically increase the occurrence of infectious diseases, including diarrhea and dysentery. Prof. Lacroix introduced how his team developed integrated multi-scale strategies to understand the complex effects and mechanisms of dietary iron on infants' gut microbiota and inflammation of humans living in different environments, combining advanced *in vivo* and *in vitro* studies. They discovered that Fe supplementation is safe in the environment with low infectious disease, and iron can increase the activity of the gut microbiota and energy extraction from the diet. On the contrary, when Fe supplementation is administrated to infected and malnourished infants in Africa, enteropathogens could be promoted while beneficial bacteria repressed, causing imbalance of the microbiota and worsened gut inflammation. He also introduced safer iron supplementation strategies for infants in developing countries designed by his team.

Prof. Dr Ing. Christophe Lacroix's lectures focus on two topics: biopreservation and antifungal cultures, and effect of nutritional components on gut microbiota and human health. (Photo by SONG J.)

Practical Skills

In response to feedback from participants to the past trainings, organizers designed classes on practical skills, including those needed in grant application, scientific publishing and leadership building. The most attractive to the trainees among them are the sessions of workshop and lab experiment.

To offer trainees opportunities to exercise practical experiment techniques needed in modern research through real operation in the lab, the course gave the trainees assignment to PCR and sequence the LAB DNAs isolated from yogurt and pickled vegetables, and identify the strains via comparing the characterized DNA sequences with those in the "GenBank", an online database.

"We are gonna do it in a 'dirty' way," joked Dr. Sybesma when giving the participants induction to the lab experiment. They would need to isolate the DNAs directly from the fermented foods, rather than prepared, cleaned samples. Therefore the trainees were required to learn how to prepare the samples, including extracting the genomic DNA through centrifugal pumping and microwaving. Afterwards in the following lab modules occurring in two days during intermissions of the lectures, they practised designing the primers to be used in the PCR, performing PCR, running gel electrophoresis, sequencing the PCR products, and making blast analysis to finally identify the gene sequences of the LAB DNAs from the GenBank.

Apparently the trainees loved the experiment. "Great! We really enjoy it!" A trainee could not help expressing his excitement to the staff at the secretariat after the first session of the Lab Experiment.

Some of the trainees had never been exposed to such lab biological techniques, but they received help from more experienced teammates when working in groups. Due to the

Trainees learning how to extract the LAB strains from yogurt and pickled vegetables to prepare for PCR samples. (Photo by courtesy of CoEBio)

complexity of the microbiota in the fermented foods used for the practice, the experiment itself involved some extent of uncertainty — not all succeeded in identifying the strains, and frustrations at various steps were visible, but eventually everyone triumphed in their learning. Particularly for those who achieved their first successful DNA sequencing at the training, this marked a big day — that might offer a step-stone for their later career in the field of biotechnology. Who knows what will grow from this tiny seed?

Taking Off with Dreams

The workshop has been a well-anticipated event for the participants since its launch in 2013 when the first food biotech training organized by CoEBio was held, and it is gaining more and more attention due to its competitive nature and the opportunity promised for the winners to develop real products in accordance with their own designs.

This year the workshop recruited as many as 22 proposals, targeting at local problems including spoilage of agricultural products, malnutrition situation of local people or instable quality of some local fermented foods like fish sauce. At the first stage of the workshop, initiators of the proposals were required to present their research designs to the whole class based on analysis of the potential advantages/strengths, disadvantages/weaknesses, prospects on the market, technical/intellectual support needed, and possible problems to solve. On the first day of the training course all the proposals were presented, and from them only five finalists were chosen after a vote attended by all the trainees as well as the lecturers. The five shortlisted proposals were then followed up by five teams formed by the trainees, with the initiator of the idea as the leader for each team. They would have a chance on November 29 to report their progress in a second presentation, and after careful reviewing, a panel

Acclaiming the triumph: Wanna Malaphan from Thailand wins the second prize with her proposal to improve the production of Thai fish sauce. (Photo by SONG J.)

First Prize Winner of the workshop competition, Afolake Atinuke Olanbiwoninu from Nigeria presents her team's work on development of a starter culture for riboflavin bioenriched *Iru*, a fermented product of African locust beans cotyledon popular in rural West Africa as flavoring condiment. (Photo by SONG J.)

of experts would determine the fate/fortune of their ideas — to be or not to be real R&D projects for a new product, based on evaluation of the urgency of the problem to solve, the application of biotechnology, level of innovation, its impact on the society, and feasibility of the project.

By the time the five teams had quite limited time between lectures, lab experiments and tours to industrial sites to develop their ideas, to formulate feasible strategies with detailed step-wise working plans to solve the chosen problems with aid from biotechnology. Taking advantage of the diversity of the participants, they would have the confluence of intellectual support from teammates of different disciplinary background and geographical regions.

The exciting moment eventually came. After the five presentations, Dr. Wilbert Sybesma, mentor of the workshop expressed his appreciation for the great work done by the teams and asked for a "long break" to allow the panel enough time for a thorough discussion.

"You've made it extremely difficult for us, because you made five very very good presentations, well-thought research plans which have their application stepping very nearby," commented Dr. Sybesma when eventually the jury arrived at agreement on the winners.

The winner of the First Prize turned out to be Afolake Atinuke Olanbiwoninu from Nigeria. Her dream was to solve the problem of riboflavin (vitamin B_2) deficiency and malnutrition in local people by developing "*Iru*", a fermentation product based on African locust beans cotyledon and a most popular food flavoring condiment among rural dwellers of West Africa, into a riboflavin bioenriched food and meanwhile a source of protein supplement for poor families. If succeeded, she hoped to replace the traditional starter of *Iru* with a riboflavin-producing equivalent.

To help her with the involved R&D, the organizers offered a financial aid to support her future stay at ETH-Zurich and coaching from Prof. Dr. Ing Christophe Lacroix for necessary research. On top of this, all trainees with her team would receive a special one-year free license offered by the *Biotechnology Journal* for access to its online resources. The winner of the second prize would get an opportunity to work at the Nestlé Research Center in Singapore, and the winner of the third prize would be coached by Prof. Hugenholtz in the Netherlands for the future pursuit of his dream.

Continuous Effort

The workshop closed in a brief celebration spontaneously joined by the winners and their fellow trainees. People acclaimed for what they had learnt during the past days, and celebrated new friendship established.

The training did not close yet.

The next morning, three more lectures integrated for the topic "Choice of organisms and their improvement/selection for desired metabolic activities in food fermentations" given by Dr. Herwig Bachman concluded the first part of the training. However this topic naturally directed the participants to the next part, a top-notched conference in the field of biotechnology co-organized by CoEBio, the Metabolic Engineering Summit held from November 30 to December 2. As the Summit invited almost all the leading researchers in the area to give lectures, the organizers made special efforts to combine the two events together, believing this would offer a great opportunity for the trainees to get informed with the latest developments in metabolic engineering.

"It is important for the Chinese Academy of Sciences to support the development of science in developing countries," said Prof. LI Yin, head of CoEBio when addressing the opening ceremony of the Summit. "Over the last few days we just organized the third training course on food biotechnology for developing countries. We had more than 50 trainees from developing countries. I just want to remind that although science has been advancing very rapidly in developed countries, there are still many

After announcement of the workshop winners, some trainees cannot help joining the lecturers for a group photo. (Photo by SONG J.)

science-lagging countries in the world. Many trainees from there had never sequenced any PCR product, nor had they seen a PCR band on the gel," he continued explaining his intention: "That's why we connected the two events together, hoping to provide the trainees with more opportunities to learn advanced techniques and emerging theories. Science can help us to better understand nature, science can also help us to create innovations for better life. By working together, I hope CoEBio can help people in developing countries to achieve a better future."

Prof. LI Yin, head of CoEBio addresses the opening of the Metabolic Engineering Summit. (Photo by SONG J.)