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A circular inset showing a microscopic view of various rod-shaped bacteria, likely lactic acid bacteria, against a dark background. The bacteria are highlighted with a light blue or white outline, showing their individual shapes and some chain-like arrangements.

Fermented foods

products of science
and craftsmanship

Prof. dr Eddy J. Smid

Inaugural lecture upon taking up the post of Personal Professor
of Food Microbiology at Wageningen University on 11 June 2015



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Rector Magnificus, esteemed colleagues, dear family and friends,

Fermentation is one of the oldest food processing technologies on Earth. The technology to produce a variety of food products and beverages has been shaped by craftsmanship. So why do we need research in this field? What can science bring for such established processes? In this inaugural speech, I will address this specific question, and as part of the scientific method, raise new questions and show directions for future research. I will start drafting a short historical and cultural perspective of the practice of food fermentation, followed by providing definitions and explanations of the essence of food fermentation processes and in particular, describe the key role of microorganisms in this process. Next, the impact of new developments in life sciences and analytical and information technology on research in this field will be discussed. This will pave the way for sharing some thoughts with you about promising and relevant directions for research in the field of food fermentation and the consequences for consumers and food producers. I will try to provide sufficient arguments to underpin my main conclusion that knowledge of microbiology of food fermentation processes can leverage the sustainable development of healthy, good tasting and affordable food products tailored for specific consumer groups.

For this lecture, some flexibility in mind is required because I will travel back in time by millennia to highlight the historical roots of fermentation processes and by centuries to mark the key scientific developments that brought us understanding of such processes. Furthermore, I will switch between processes occurring at molecular level – genes, enzymes and metabolites – and processes occurring at the level of individual cells and microbial communities. Finally, the consequences of all this for the quality of the food products will be discussed.

Historical perspective

Let me start with drafting the historical perspective of the artisanal practice of making fermented foods, followed by the history of scientific discoveries revealing the nature of such processes. The first simple question – “when were food fermentation processes invented?” – is already difficult to answer. Mastering processes that lead to fermented foods has been crucial for the development of mankind. Does the invention of fermented foods deserve a place on the list of most important inventions of mankind?

This list features various inventions that radically changed our lifestyle as well as the face of the Earth. An example is the invention of agriculture, marking the transition from a life style of hunter-gatherers to agricultural societies. Identifying the exact origin of agriculture is difficult because this transition began in pre-historical times, meaning thousands of years before the invention of writing. Estimations to mark the onset of agriculture range from 9000 to 12000 years ago. A sedentary lifestyle as by-product of agriculture, leads to an increase in food production. Suddenly, food was not only produced for today’s needs, but also for the days and months to come. This demanded technology to preserve the surplus of produced food stuffs. Nature most likely provided the first solutions leading to food preserved by fermentation. For instance, fresh milk from cows and sheep, turns sour at ambient temperature within one or two days by what we now know is the action of lactic acid bacteria converting lactose into lactic acid and other organic acids, thereby turning the milk into a harsh environment for spoilage and pathogenic microorganisms. In times of shortage of food supply, these spoiled products were consumed and eventually even appreciated, perhaps initially not for their taste and texture but mainly for their extended shelf life. Once turned sour, the milk could be stored at ambient temperature for days. Turning milk into cheese delivered a product with an even longer shelf life of months to years. Similar events may have occurred for stored cereals, vegetables, fruits and meat, leading to processes for the production of bread, beer, pickled vegetables, wine and sausages.

By mastering food preservation methods such as fermentation, smoking, drying and brining, ancient communities expanded trade with neighbouring communities which in the end provided wealth and prosperity. This development of technology created one of the basic requirements for founding urban communities and trade. By recognizing all this, food fermentation can be seen as one of the oldest food preservation technologies, invented probably multiple times during the history of mankind, in several different cultures and civilisations. For that reason, I see more than enough arguments to grant “fermentation” a place in the top 10 of most important inventions of mankind.

Many fermentation processes we know today are operationally complex, reflecting their long history of use. The skills required for producing good products were passed on for countless generations from mother to daughter and/or father to son, leading to gradual improvements: a textbook example of incremental innovation. One could argue that these processes are indeed the product of cultural evolution initially based on oral tradition. Especially for that reason, fermented products are often regarded as part of the cultural heritage and identity.

A good example of a complicated process is cheese making, which involves a complex sequence of different process steps such as (i) renneting, (ii) whey/curd separation, (iii) brining and (iv) ripening under defined conditions. Evidence for cheese making has been found at several sites in northern Europe as early as the 6000 year BC and was common in Egypt and Mesopotamia some 4000 years ago (1).

Last year, Yang and co-workers (2) reported in the *Journal of Archaeological Science* an advanced proteomics analysis of well-preserved specimens of organic material from cheeses associated with mummies found on an Early Bronze Age cemetery in Xinjiang, China. They delivered direct evidence for the involvement of lactic acid bacteria such as *Lactobacillus kefiranofaciens* and *Lactobacillus helveticus* and the yeast species *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* in the conversion of ruminant milk into a fermented dairy product. Mentioning for the first time in this lecture the Latin names of microorganisms provides for me an ideal bridge to the microbiology in general and in relation to fermentation processes in particular.

The founding fathers of general microbiology are Antoni van Leeuwenhoek (who discovered microscopic organisms), Robert Koch (who demonstrated bacteria as causal agents of diseases), Louis Pasteur (who proved industrially relevant and beneficial activities of microorganisms), Martinus Beijerinck (who discovered viruses), and Albert Jan Kluyver (who was the first to demonstrate that at a biochemical level, all organisms are unified) (3). These pioneers, laid down the foundations for modern microbiology and provided the framework of knowledge that we still use today, for instance for describing, understanding and controlling the microbial processes involved in the transformation of food raw materials into fermented food products with desirable characteristics. Only recently, with the advent of (meta)genomics and the associated technological revolution in genome analysis, medical doctors and biologists started appreciating the key role of microorganism inhabiting our gut and other parts of our body in sustaining health and well-being.

In the entire chain of events starting at food production, via food processing to food digestion, we can see food fermentation as a means of *ex vivo* pre-processing followed by *in vivo* digestion. Both processes are governed by complex communities of microorganisms and therefore studied in the domain of microbial ecology, using similar research approaches. I will come back to this point later on in this lecture.

Prominent role for microbes

Having established that microorganisms are driving fermentation processes, it is now time to introduce the three main groups of microorganisms involved: these are (i) lactic acid bacteria, (ii) yeast and (iii) filamentous fungi (Fig. 1). Although phylogenetically distantly related, the microorganisms belonging to these three main groups share a number of characteristics which make them ideal workhorses for the job. They all are heterotrophs and thrive in an environment with abundant nutrients. They all consume free sugars and some of them are capable of releasing sugars from carbohydrate polymers such as starch. This consumption of free sugars is usually relatively fast and energetically inefficient. In addition, they all produce and secrete an array of primary and secondary metabolites thereby influencing dramatically the chemical composition of their environment (ie the food raw material). Finally, all these organisms have features that provides them a sharp competitive edge in nutrient rich environments. In other words, they occupy their specific niche in relative short periods of time, thereby leaving little space and resources for competitors, which are usually microorganisms that consume nutrients at a slower pace but in an energetically more efficient way. Many pathogenic microorganisms fall into the latter category.



Figure 1. Microbial workhorses in food fermentation: lactic acid bacteria (a), yeast (b) and filamentous fungi (c).

The process of making fermented foods

Having introduced the main players in the arena of fermented foods, let me now move to the different definitions of fermentation. In strict biochemical terms, fermentation is defined as a “biological process that derives metabolic energy from the oxidation of organic compounds (mostly carbohydrates) and uses an endogenous electron acceptor (which is an organic compound). The latter point sets fermentation apart from a process we call “respiration” where the electron acceptor comes from outside being for instance oxygen (for oxidative respiration) or nitrate (in the case of anaerobic respiration). Louis Pasteur defined fermentation in more simple terms as “life in the absence of air” or to use his mother tongue: “*La fermentation c’est la vie sans l’air*”. Neither of these two definitions capture all processes that are generally regarded as food fermentation processes. The most practical definition reads as the “conversion of food raw materials by the action of microorganisms and enzymes into food products with desirable characteristics”. In this definition, the enzymes originate either from the microorganisms or from the fermentable raw material.

Conceptually, fermentation is completely different from all other food processing technologies because it is the only technology that can lead to *de novo* synthesis of nutritional compounds in the food matrix. For instance, the lactic acid bacterium *Streptococcus thermophilus*, which is present in yoghurt starter cultures, not only produces lactic acid thereby acidifying the bovine milk, but also synthesizes and secretes the vitamin folic acid, thus boosting the nutritional value of the yoghurt (4). No other food processing technology leads to this form of natural fortification of food. Another nice example in this context is the use of a vitamin B12 producing lactic acid bacterium: *Lactobacillus reuteri* (5). Santos and co-workers demonstrated that *in situ* microbial B12 production by *Lactobacillus reuteri* is a convenient strategy to achieve natural enrichment of fermented foods, especially from vegetable sources. Important to note here is that the producing organism has a long history of safe use by the food industry. It is not difficult to envision the importance of such natural vitamin B12 enriched food products for people living on a vegetarian diet. Depending on the type of raw materials used, being either fruits, vegetables, cereals, beans, milk, meat or fish and also depending on the types and blends of microbes used in the fermentation, different combinations of functionalities will be delivered to the final fermented product (Fig. 2). In addition to the already mentioned increased nutritional value, the shelf-life of the finished product can be extended, the level of microbial food safety can be increased, the texture can be changed – think of increased viscosity of fermented milk – and finally, taste, aroma and appearance can be improved.

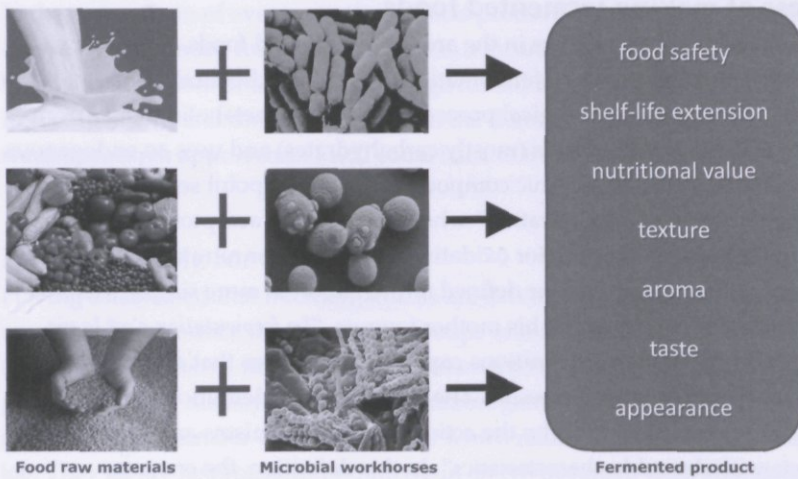


Figure 2. Fundamental scheme of food fermentation processes. Food raw materials are converted into fermented food products possessing a diverse range of desired properties delivered by the microbial workhorses.

At first glance, texture and aroma seem to be a luxury problem and this may be true for some parts of the world or for particular age groups. However, keeping in mind the growing population of elderly people in the western world and some parts in Asia, not only eyesight and hearing gradually deteriorate with aging, also the senses of taste and smell decline. These two senses exert a huge impact on health of elderly people. As we grow older, our olfactory function declines (6). Not only do we lose our sense of smell, we also lose our ability to discriminate between smells. It has been reported that more than 75% of people older than 80 years, suffer major olfactory impairment (6). Decreased smell and taste results in reduced appetite leading to weight loss, malnutrition, impaired immunity, and deterioration in various medical conditions. In conclusion, nutritional problems in elderly can be a direct consequence of smell and taste disorders (7). How is all of this linked to fermented foods? Actually, food products in this category stand out for their characteristic and sometimes strong taste and aroma, mostly the result of the microbial production of primary and secondary metabolites (8) (Fig. 3). Using sensitive gas chromatography coupled with mass spectrometry detection (GC-MS) for the analysis of plain yoghurt, as much as 49 volatile aroma compounds can be detected in plain yoghurt, representing a range 5 chemical classes (9). This degree of richness in aroma compounds (and even higher) is characteristic for fermented food products and reflects the activity of a complex network of metabolic reactions occurring in the microbes and in the food matrix.

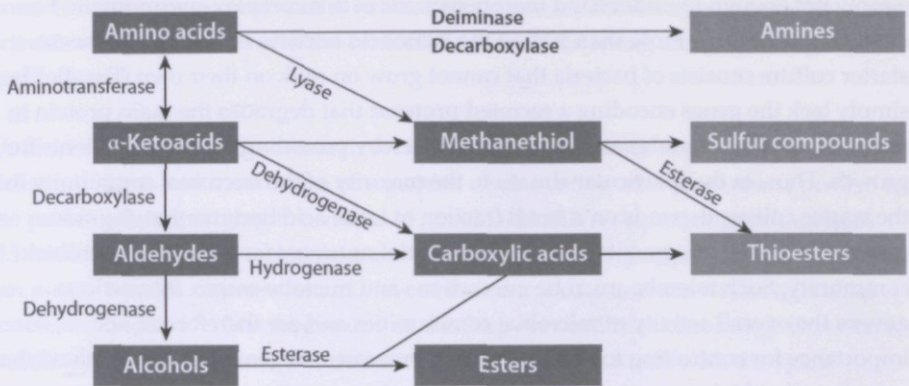


Figure 3. Schematic representation of the most important amino acid conversion pathways for flavour formation in cheese manufacturing. The chemical compounds and corresponding interconversion enzyme classes are indicated. From: Smid and Kleerebezem 2014.

From this it becomes evident that, for a complete description and understanding of fermentation processes, we need integration of multiple scientific disciplines, in particular: (i) microbiology, (ii) food chemistry, (iii) enzymology, (iv) food physics and (iv) food processing. These disciplines provide the pillars for the current research activities in the Laboratory of Food Microbiology at our university. Obviously, this multidisciplinary approach also provides the framework for the educational activities and in particular for the two courses on food fermentation which we deliver to our master students taking specialisations in Food Biotechnology, Food Technology and Food Safety. I will come back to this later on in my lecture.

Linking microbial genes to product functionality

Microbes deliver the special characteristics of fermented food products not only through their metabolic activity but also through secretion of enzymes in the food matrix and by their growth and survival behaviour. Only a few industrial food fermentation processes are driven by microbial cultures consisting of a single strain. Most food fermentation processes depend on mixtures of microbes which act in concert to produce the desired product characteristics. In order to understand the action of these mixtures of microbes or microbial communities, we need to understand the interactions between the microbes in the community and their interactions with

the food matrix. Detailed knowledge of the individual players in the consortium is simply not enough to understand their behaviour in this complex environment. For example, on average more than 90% of the lactic acid bacteria in traditional cheese starter culture consists of bacteria that cannot grow on milk on their own (Fig. 4). They simply lack the genes encoding a secreted protease that degrades the main protein in milk (casein) into peptides and amino acids, thereby providing essential nutrients for growth. Thus, in that particular situation, the majority of the microbial community in the starter culture depends on a small fraction of lactic acid bacteria that degrades casein into smaller fragments, supplying essential nutrients for the entire microbial community. Such microbe-microbe interactions and microbe-matrix interactions govern the overall activity of microbial communities and are therefore of key importance for controlling food fermentation processes. In general it can be stated that complex microbial consortia perform more complex activities (named with the term versatility) and tolerate more variation in the environment (also referred to as robustness) as compared to pure cultures. The versatility and robustness can be explained by two features (10). First, members of the consortium communicate with one another by trading metabolites or by exchanging molecular signals. As a result, each individual cell in the mixture responds to the presence of others in the consortium (11). The second key feature is the division of labour between the members of the consortium leading to an overall output that can be explained only by combining tasks performed by constituent individuals or subpopulations (12).

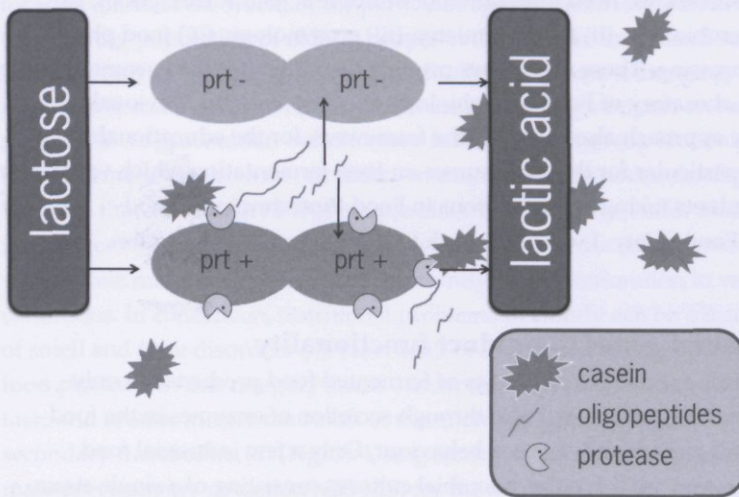


Figure 4. During fermentation of milk, caseinolytic variants of *Lactococcus lactis* (designated as prt+) supply a surplus of peptides and amino acids to support growth of non-proteolytic variants (designated as prt-) of *L. lactis*. Based on Smid and Lacroix, 2013.

The question now arises which combination of experimental approaches is suited for unravelling the gene-content of microbial communities, the metabolic and enzymatic activities of the microbes and their interactions with the living and non-living environment.

Until recently, most of the research on fermentation processes was descriptive and delivered at the best inventories of consortia of co-existing microorganisms present in the process. By combining the results of novel DNA/RNA sequencing technologies (13) with metabolomics techniques and metabolic modelling approaches (14, 15), we can now obtain an unprecedented view of the complexity and activity of microbial communities (16, 17). The magnitude of this leap forward in microbiology is perhaps comparable to the new insights delivered by observations made with the space telescope Hubble, some two decades ago.

Complexity of a dairy starter culture – current lines of research

After this introduction, starting with extensive painting of the historical context of the topic and going all the way to the microbiological level and molecular level of fermentation processes, I will now try to give a flavour of the current research activities at the Laboratory of Food Microbiology at this university. In one of our studies, we are investigating the structure and complexity of an industrially relevant undefined cheese starter culture. Although not well documented, such starter cultures have their roots in artisanal cheese making at farm level (18). As such, the current microbial composition of these cultures is most likely shaped by population dynamics and evolutionary forces, driving the community towards optimal utilisation of raw milk. Although milk is, in terms of nutritional value, one of richest growth media on Earth, it is remarkable that the naturally evolved complex cultures in general only contain two species of lactic acid bacteria (LAB), with dominance in abundance of *Lactococcus lactis* and smaller communities of *Leuconostoc mesenteroides*. Apparently, the fast lactose fermenting LAB's create a selective environment that prevents the establishment of other species of microbes in the community (17).

One of our previous PhD students, Oylum Erkus, designed a research-blueprint for in-depth structural and functional analysis of microbial communities and applied this to a typical complex cheese starter culture named "Ur" (16). Erkus initially focussed on the analysis of genetic diversity among the community members beyond the level of subspecies. In total, 8 distinct genetic lineages of LAB were identified in the undefined starter culture (Fig. 5). Interestingly, each of these distinct lineages could be coupled to a specific functionality in the fermentation process. For example, one lineage could be linked to fast acidification, one to the delivery of enzymes in the

cheese matrix due to lysis, three were linked to citrate degradation and the production of volatile aroma compounds and three to casein degradation. A nice example of division of labour!

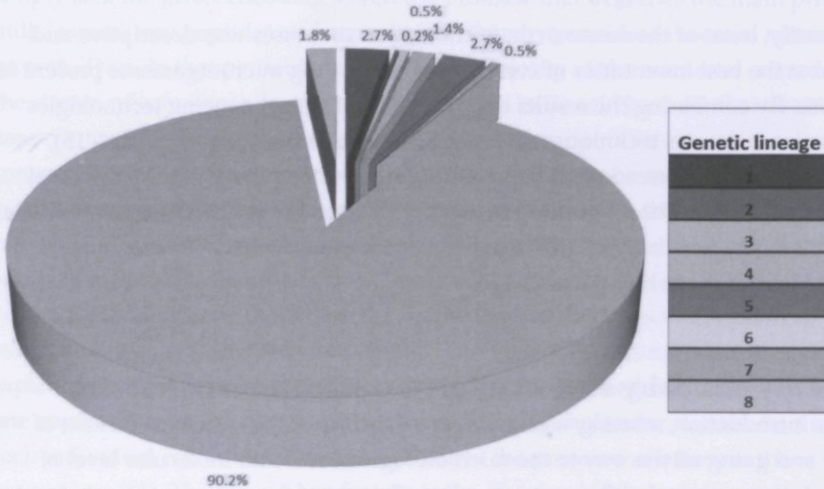


Figure 5 . Composition of undefined starter Ur. The culture consists of *Lactococcus lactis* (lineages 1 to 7) and *Leuconostoc mesenteroides* (lineage 8). Calculation of the composition was based on data taken from Erkus et al. 2013 and Smid et al. 2014.

Analysis of the composition and different properties of the complex starter culture revealed even more layers of diversity which seem to have functional implications for the performance of the culture as a complex system. For example, within each lineage different plasmid profiles could be identified although not all plasmid profiles were detected in each lineage. This observation implies that extensive, but probably also to some extent lineage-restricted exchange of plasmid DNA takes place within the starter culture community. The diversity in plasmid profiles also implies an additional level of diversity added to the microbial culture. Finally, the presence and activity of lytic bacteriophages – these are viruses that predate on bacteria – were found to play a rather unexpected role in the complex microbial community of the starter culture. Interestingly, the degree of sensitivity for these lytic phages was found to be extremely diverse between single colony isolates of a particular genetic lineage and between the lineages.

This highly diverse phage resistance profile of the community members provides an explanation for the observed compositional and functional stability despite the presence of lytic phages. The line of argumentation is that the consistent presence of resistant variants representing one particular lineage potentially prevents the eradication of an entire lineage from the culture by phage predation.

To come back on the previously mentioned analogy with the Hubble telescope, the resolution of the overall picture of an undefined dairy starter culture has tremendously improved.

These observations and their interpretation very much contrast with the general idea that the presence of lytic bacteriophages in a starter culture is synonymous for “bad news”. Bacteriophage activity potentially leads to cell lysis and consequently reduces the acidification potential of the starter which finally leads to failure of the entire fermentation process. At industrial scale, this leads to huge economical losses. This notion has been driving most of the bacteriophage related research in the field of lactic acid bacteria towards a strong focus on prevention of distribution and propagation of the bacteriophages (19).

To demonstrate the functional consequences of the stratified structure of complex starter cultures, one of our current PhD students, Maciek Spus, decided to study the – beneficial - role of lytic bacteriophages in preserving the compositional stability of complex dairy starter cultures. He demonstrated in an elegant experiment that a phage challenged, sequentially propagated reconstituted starter culture, retained full diversity at the level of genetic lineages while a non-challenged control culture lost diversity upon propagation for over 500 generations. The observed persistence of closely related strains within each genetic lineage is in agreement with the density-dependent phage predation model postulated by Rodriguez-Valera and co-workers (20). Our studies show that structural complexity of the culture at the level of genetic lineages provides an explanation for the compositional stability (robustness) of the culture, which displays substantial resilience even under phage predation pressure.

Although this all may sound extremely theoretical, the knowledge can be readily capitalized by starter culture producers and fermentation industry through the development and implementation of rationally designed starter cultures which possess intrinsic stability that leads to reliable starter performance during manufacturing of fermented food products.

New directions for research on fermented foods

Let me now shift gears and move towards new directions for research on fermented foods. Choosing new research directions depends on a several interrelated factors such as: societal trends, industrial relevance, experimental feasibility and of course the scientific interest of the researchers. To start with the societal context, we can observe a growing interest of the general public for fermented food products. This is illustrated by numerous publications in popular magazines and books and at the internet about home brewing, bread making and kitchen-scale production of sauerkraut and kefir etc. Also food professionals such as top-chefs from all over Europe regained interest in exotic fermented products like kimchi or kombucha, specifically because these products deliver new flavours to their creations. In master classes about “Fermentation & Haute Cuisine”, the world of science and gastronomy – one could also say craftsmanship – literally joined forces to show an audience of over 100 interested food professionals the secrets, limitations and opportunities of fermented food products.

If we consider the industrial relevance, fermentation is clearly a key-technology for many European food and food ingredient companies represented by multinationals as well as innovative SME's. The Laboratory of Food Microbiology collaborates extensively in public-private-partnerships such as the Top Institute Food & Nutrition, bringing together major international players in the field of food fermentation to formulate and execute projects on “need-to-know” topics. Starter culture industry and their customers look for possibilities to steer and control fermentation processes with the objective to obtain food products with desired properties. Here, consumer demands and issues in society dictate the wish list, thereby challenging science to come with breakthrough innovations and solutions.

I will illustrate the contribution of our fermentation research to such topics. For example, high salt intake is associated with increased risk of cardiovascular diseases and the actual average daily intake of sodium chloride exceeds the recommended intake. Particular fermentation processes (i.e. sauerkraut, kimchi, cheese, soy sauce) require the addition of salt – called brining – for safeguarding proper process conditions. Simply reducing salt will not lead to the desired end-product and may even benefit undesirable spoilage and pathogenic microorganisms. To be able to steer the development of the community of desirable microorganisms, in-depth knowledge of the microbe-microbe and microbe-matrix interactions is required. This example clearly links public health issues to our main research-themes.

Especially for complex mixed starter cultures, adaptive laboratory evolution (ALE) approaches (21) offer new opportunities for the improvement of culture performance

and the development of new starter functionalities. The rationale for this statement is based on the notion that industrially successful complex starter cultures can be considered as a product of evolution by domestication. The traditional/artisanal method of inoculation by back-slopping, provides the right set of conditions for evolution of individual microbes as well as communities towards fast utilisation of fermentable raw materials. Genomic signatures were found in genomes of established dairy lactic acid bacteria supporting the view of domestication of industrial strains (22, 23). The novelty in our current approach lies in the adaptive laboratory evolution of microbial communities rather than single strain cultures. The evolution of communities of microbes is governed by an interplay of population dynamics, driven by microbe-microbe and microbe-matrix interactions at shorter time lines and evolutionary processes at longer time-lines. In a currently – in our laboratory - executed long-term evolution experiment using a traditional complex dairy starter culture originating from a back-slopping regime, we demonstrated not only qualitative compositional stability (i.e. none of the original genetic lineages of lactic acid bacteria was lost during 2000 generations of evolution), but also observed dramatic changes in fitness of initially low abundant species of lactic acid bacteria. Such evolved cultures kept their compositional stability but also showed new functionalities: the latter being a consequence of dramatic changes in relative abundance of the interacting players in the community. Here, evolutionary adaptations are most likely associated with mechanisms of interaction between community members. This approach not only opens up a variety of opportunities for development of novel industrial starter cultures, but also provides generic and fundamental insight in the functioning of microbial communities. New concepts describing for instance the role of bacteriophages in relatively simple microbial communities such as those found on fermenting foods are also extremely important for understanding more complex microbial communities such as those found in the human gastrointestinal tract.

Academic research and education

At successful universities, research and education are engaged in a tight mutualistic relationship: they strengthen each other! I have experienced that the design and execution of research-embedded courses for master students, is particularly facilitated by cooperation between the different chair-groups in the department of Agrotechnology and Food Sciences. Especially for a radically multidisciplinary topic as food fermentation, collaborations with colleagues from the chair-groups Food Chemistry, Food Process Engineering, Food Quality and Design, Nutrition, Bioprocess Engineering and Microbiology is essential. The standing practice is that the contents of our advanced level course on Food Fermentation is updated annually

with topics and ideas coming directly from running PhD-projects. These “close encounters” with the actual scientific labour is inspiring for master students and therefore very much appreciated as evidenced by positive student evaluations of the course. At the same time it provides a unique and natural training ground for our PhD students as academic teachers. It also works in the opposite direction. For instance, students in the MSc course Advanced Fermentation Science in 2012 delivered data for writing a research proposal on the role of non-conventional yeast species in food fermentation processes. The proposal was granted in 2013 and our PhD student on this project, Irma van Rijswijck, is currently involved in many new and attractive projects for MSc students.

This shows that my statement on the intimate relationship between research and education is not just a marketing phrase but very much the reality in our daily practice. Where teachers provide the engine, obviously the stimulating fresh minds of MSc and PhD students provide the fuel for the interactive process of academic teaching and research. While new developments in teaching such as MOOC's and distant learning modules are changing the academic world and bring new opportunities, I am convinced that we still need teachers to inspire new generations of students through personal interaction.

Words of gratitude

This statement about teachers & inspiration seamlessly merges with my final words of gratitude. Let me start here with my personal sources of inspiration. For me, it started with Charles Darwin. His book “On the Origin of Species” ignited my general interest in biology. His work provided a framework for understanding the incredibly fascinating phenomenon of living entities on our planet. In line with Darwin, I discovered Dawkins who inspired in the late seventies a new generation of biologists with his book “The Selfish Gene”. For me, his vision uncovered the elemental level at which evolution is operational. It proceeded with my own teachers in microbiology: Prof. Hans Veldkamp, for introducing me to the fascinating world of bacteria; Prof. Wil Konings, my promotor, mentor in scientific research and leader of the King's Society. Sadly, Wil Konings passed away last year. He would have been a guest of honour at this ceremony today. Furthermore, I would like to mention my inspiring and supportive colleagues and in particular Prof. Jeroen Hugenholtz, Prof. Tjakko Abee, Prof. Michiel Kleerebezem, Prof. Bas Teusink, Dr. Rob Nout and Prof. Marcel Zwietering. Their support at various stages and in different ways has been very important for my career in science. I also want to thank the Board of Wageningen University and the management of the Agrotechnology and Food Sciences Group, in particular Prof. Bino, for giving me the confidence and supporting my appointment.

A special word of appreciation is for all my colleagues at the Laboratory of Food Microbiology. Each of you have a share in today's event. A special word of appreciation is for Judith Wolkers-Rooijackers. Without her support, commitment and capacity to anticipate, it would have much harder to execute and complete many of our educational and research projects.

For expressing my final words of gratitude, I will now switch to Dutch. Mijn ouders hebben mij, met onvoorwaardelijke steun en vertrouwen, de mogelijkheid geboden om – in vrijheid – mijn weg te vinden. Dit voorbeeld probeer is mee te geven aan mijn eigen kinderen. Mijn lieve Anita, vanaf deze plaats wil ik je uit de grond van m'n hart danken voor jouw onvoorwaardelijke liefde, jouw steun bij het maken van keuzes in mijn loopbaan en het gezamenlijk zoeken naar een juiste balans tussen thuis en werk. Door jouw inbreng bleef het altijd zonneklaar dat een mens van uitsluitend werken niet gelukkig wordt of blijft. Daardoor bleef ons gezin centraal staan in mijn leven en kon ik mijn energie op de best mogelijke manier verdelen tussen werk en privé. Ik hoop dat we samen nog heel lang onze zoektocht naar de juiste balans in het leven kunnen voortzetten. Lieve Eva en Koen, jullie geduld en begrip voor "Vatie" die van tijd tot tijd zo nodig "weer dingen van z'n werk moest afmaken" en "nu even geen tijd heeft", is bewonderenswaardig, dank voor jullie geduld!

Tot slot wil ik alle aanwezigen van harte danken voor de getoonde belangstelling en voor uw komst naar Wageningen.

Ik heb gezegd.

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'Microbial cultures deliver the typical characteristics of fermented food products through their metabolic activity, by secretion of enzymes in the food matrix and by their particular growth and survival behaviour. Knowledge of the structural complexity and population dynamics provides explanations for compositional stability and overall performance of such cultures. Especially, insight into microbial interactions delivers new design rules for robust composite starter cultures with predictable and desirable industrial performance.'