Understanding microbial behavior within and outside the host to improve food functionality and safety

Editorial overview

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Understanding microbial behavior may contribute to enhanced control of food spoilage and pathogenic bacteria on the one hand, and at the same time provide a basis for improvement of functionality and robustness of microorganisms used as starters in fermentations. Since food is a basic need, quality and safety issues are recurrent themes involving a variety of contributors, including consumer organizations, applied research groups and academic research groups, food producers and government agencies. Knowledge sharing and contributions of expert panels in national and international organizations have provided vision papers and strategies to enhance food quality and safety, addressing specific pathogens, food products and processing in various countries in a range of social, economic and industrial settings. The study of relevant food spoilage bacteria and food-borne pathogens and (biological) control measures aims to provide understanding of their eco-physiology and pathogenic potential [1], with a concomitant effort in related areas aimed at starter organisms and optimization of quality, safety and functionality of fermented products.

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Tjakkko Abee is a professor of Food Microbiology at Wageningen University. His research is aimed at understanding the mechanisms underlying the survival and development of spoilage and pathogenic bacteria in the food production chain. Current research themes include the occurrence of genotypic and phenotypic heterogeneity during growth, sporulation, and germination, development of antimicrobial resistance in single and mixed species biofilms, and stress-induced evolution and pathogenic potential in food-borne pathogens.

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Oscar Kuipers is a full professor at the University of Groningen, heading the group of Molecular Genetics since 1999. He is studying various gram-positive bacteria of biotechnological importance, such as lactic acid bacteria for dairy and Bacilli as cell factories or as spoilage microorganisms. His current activities are in making novel antimicrobials by Synthetic Biology, analyzing gene-regulatory networks and phenotypic heterogeneity at the single cell level and developing methods to improve food safety.

Industrial fermentation and microbial stress

Industrial fermentation using microorganisms, ranging from lactic acid bacteria to yeast and fungi, provides means for the production of a wide array of fermented foods. During fermentation the microbes undergo various stresses to which they respond variably. These responses have a major impact on the final food product characteristics and food safety. Van der Veen and Abee describe how bacterial SOS-responses can result in stress resistance and mutagenesis. This can affect the survival of spoilage and pathogenic bacteria and this may have implications for food quality and safety. An important characteristic of industrial fermentations is microbial growth behavior and the effect of the growth conditions used. In a comprehensive overview, Gruss and colleagues show how the fermentation of...
lactic acid bacteria can be enhanced by inducing aerobic respiration, for example by adding heme to the medium. Aerobic growth causes less oxidative stress and acid stress, which offers a major growth advantage in the production of starter cultures. In other food production processes, the yeast *Saccharomyces cerevisiae* is widely used. Also here, cells have to cope with various stresses that eventually affect the food quality and preservation characteristics. *Sa-Correia* and coworkers describe how post-genomics approaches can unveil general and adaptive responses of yeast to exposure of various stresses, for example temperature variation, exposure to elevated ethanol levels, osmotic pressure changes or nutrient limitation. These studies identified various specific genes and pathways that can be engineered to yield improved and more robust strains for food application.

**Biocontrol and food spoilage**

A major challenge in food biotechnology is to develop reliable methods for preservation to prevent food spoilage. Biocontrol methods have been developed for a wide variety of industrially relevant organisms, ranging from bacteria to plants and animals. Development, implementation and validation of so-called hurdle technologies have received much attention, and these involve a combination of two or more preservation methods including cooling, acidification, use of antimicrobial compounds and enzymes, high hydrostatic pressure, vacuum and modified atmosphere packaging. Various approaches are emerging that are not yet widely applied but show great promise. *Van Sinderen* and coworkers highlight phage-mediated methods to fight food pathogens. With the increase in available pathogen sequences and phage sequences and the first approval for phage applications in food protection, there are exciting new opportunities to combat species like *Campylobacter*, *Listeria*, *Streptococcus* and *Salmonella* with phages or their encoded lysins. Another interesting property of phages is that they usually also encode lysins and holins that enable the degradation of bacterial cell walls. In a nice overview, *Callewaert* and colleagues describe first the characteristics of bacterial cell wall hydrolases and phage cell wall hydrolases and then discuss their applications to fight specific pathogens or food spoilage bacteria. One obstacle for application is that genetically modified foods with effective cell wall hydrolases still raise ethical and regulatory concerns. However, it is expected that these problems can be circumvented or taken away, so that these technologies may be implemented in the (near) future. A novel application area is to use phage lysins with different specificities fused to fluorescent proteins for detection and serotype assessments, as was recently shown for *Listeria* [4].

A large problem in fermented and non-fermented food products is spoilage by undesirable microorganisms, like bacteria and fungi. Especially spores and biofilms are hard to get rid of, mostly because brute-force physical methods, such as elevating temperature or pressure, and cleaning and disinfection protocols used, respectively, do not always solve the problem. Bacterial spores are very well protected against heat and desiccation, while biofilms are sometimes hard to penetrate with lethal compounds and can also tolerate desiccation. *Abec* and colleagues describe bacterial biofilm architecture and matrix compounds to explain the properties that make biofilms inaccessible to antimicrobials and other compounds and that hamper treatments to fully eradicate them. The roles of quorum sensing and the involvement of flagella are further discussed, as is the occurrence of spore formation within biofilms that all put a further barrier to fight them. Novel ways of disturbing biofilm formation may include the use of specific fatty acids and D-amino acids that can stimulate dispersal thus facilitating second phase treatments for full eradication.

*Clostridia* and *Bacillus* spores are perhaps the greatest challenge in the maintenance of the sometimes delicate balance between quality and safety, because they can withstand severe processing steps, and reside for prolonged periods in food products and can germinate after extended periods of dormancy, in a heterogenous process that is hard to control. *Eijlander et al.* point out that a major problem in fighting spores in food is the phenotypic heterogeneity that is present most spore crops. The history of the sporulation process already determines this heterogeneity and causes in turn the germination process to be heterogeneous. Although a lot is known about the spore make-up and biosynthesis, including the gene-regulatory processes, little is known about the actual differences between spores and their germination at the single cell (or single spore) level. In their paper they explain the current approaches to fight spores and their germinated offspring, but also look forward how one can study spore heterogeneity and germination heterogeneity, for example using flow cytometry and time lapse microscopy, and how one can make use of that knowledge to develop more efficient ways of preventing and eradicating spores in food.

As plants are an important source for food products, the optimization of growth and the protection against pests are very relevant issues. In this context, *Perez-Garcia* and colleagues describe current approaches for biocontrol by bacteria producing effective antimicrobial agents against fungi and other unwanted invaders. Moreover, live bacteria or spores can be used as biofertilizers and in some cases, for example using Bacilli, various beneficial activities can be combined in one species to optimize the effectiveness.

**Microbe–host interactions**

Many different approaches can be used to study not only the complexity of host–microbe interactions including a wide range of pathogens, but also the highly diverse...
address the performance of *Listeria monocytogenes* microbiota in the GI-tract. In this issue, two papers address the performance of *Listeria monocytogenes* microbiota in the GI-tract. In this issue, two papers provide an overview of the role of iron in the environment including foods, and in the pathogenesis of *L. monocytogenes*. As iron is vital for all cells, host sequestration of iron provides a significant barrier to bacterial infection. The absolute requirement for iron has driven the evolution of refined systems by which pathogenic bacteria such as *L. monocytogenes* can competitively acquire this element during host infection. The role of the Ferric Uptake Regulator (Fur) and recently identified loci within the listerial Fur-regulon and specific systems involved in iron uptake from various sources are discussed, together with the possible implications from food safety and public-health perspectives. The information obtained can be combined with recent data published by the group of Cossart [6]. Using tiling arrays and RNAs from *L. monocytogenes* wild-type and mutant bacteria grown *in vitro*, *ex vivo* and *in vivo*, the transcription of its entire genome was analyzed. Upon reaching the host intestinal lumen or blood, extensive transcriptional reshaping occurs with activation of virulence factors mediated by different transcriptional regulators. These data revealed successive and coordinated global transcriptional changes during infection and pointed to previously unknown regulatory mechanisms in bacteria.

Hinton and co-workers focus on transcriptional responses of another notorious food-borne pathogen, that is, *Salmonella*, upon interaction with the host. The integration of *in vitro* generated transcriptomic data with global gene expression of *S. typhimurium* during infection is discussed, yielding information about coordinated regulation of *Salmonella* gene expression, a key process for survival, adaptation and virulence capacities of the pathogen. Obviously, insight into pathogen–host interactions may further contribute to prevention and/or control of infection processes of food-borne pathogens.

Notably, consumer health is one of the main objectives in research and development of food fermentations targeting enhanced quality and functionality. Hylckama-Vlieg and colleagues discuss fermentation of food components by microbes in food production processes and in the gastro-intestinal tract, with specific compounds produced that can affect the health of the consumer. Recent advances are presented that highlight the potential to improve the nutritional status of a fermented food by a rational choice of food fermenting microbes. In addition, the impact of the gut microbiota on production of health-active molecules is discussed. The authors highlight the need for a multidisciplinary approach in order to increase our understanding of the molecular mechanisms underlying health beneficial effects that arise from the interaction of diet, microbes and the host.

Overall, these 11 papers give a representative and timely overview of the state of the art and new developments in Food Biotechnology and Safety and provide relevant knowledge and tools for developing effective methods to improve and safeguard our daily food.

References