The Impact of Omic Technologies on the Study of Food Microbes

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Abstract

The advent of the molecular biology era in the 1950s and the subsequent emergence of new technologies positively impacted on all areas of biology. New discoveries in molecular biology and experimental tools were developed over the next 60 years that have revolutionized the study of food microbiology. Previously, food microbiology relied on classic microbiology techniques, which had remained relatively unchanged since the discoveries of Louis Pasteur in the 1800s. More recently, new advances resulting in "omic" technologies have exploded the areas of genomics, transcriptomics, and proteomics and revealed many fundamental processes driven by both pathogens and commensals. This review outlines advances in omic technologies and how these have impacted food microbiology through providing examples of recently published landmark work.

INTRODUCTION

Superorganism: humans can be classed as superorganisms because of their symbiotic relationships with associated microbiota

Probiotic: live microorganisms that, when administered in adequate amounts, confer a health benefit on the host

More than thirty years after the introduction of Sanger DNA sequencing, omic technologies have accelerated our knowledge and understanding of food microbes. Over the past five years, there has been an explosive realization of the microbial world around and within us. In fact, the human is now considered a superorganism because of its intimate associations with its massive microbiota (Gill et al. 2006). Because foods provide both nutrients and often a favorable environment for growth, contamination by pathogenic and spoilage microbes continues to be a major concern. Many food groups, notably fermented foods including cheese, yogurt, sausage, beer, and wine, would not exist without the microbes that are responsible for their preservation. Other food microbes, such as probiotic bacteria, are intentionally added for their beneficial attributes. Additionally, the possibility of the delivery of therapeutics through food using beneficial microbes is an exciting development. These recent advances in particular have been due to the developments in omic technologies.

The discoveries of polymerase chain reaction (PCR) and DNA sequencing were pinnacle advancements that impacted biology and hence food microbiology (**Figure 1**). The importance and relevance of PCR in particular are in part due to subsequent improvements and modifications to adapt the method to important aspects of study, from whole genome sequencing to rapid detection of pathogens to unequivocal phylogenetic identification. Since the genome sequencing of the free living organism *Haemophilus influenzae* in 1995 (Fleischmann et al. 1995), the human genome has been sequenced numerous times as well as the genomes of other animals and thousands of microbes (**http://www.ncbi.nlm.nih.gov**). These advances in genome sequencing coupled with other omic technologies such as proteomics and transcriptomics have revolutionized food microbiology, helping us to understand our desirable and undesirable microbial worlds. Omic technologies have been reviewed extensively elsewhere, but herein we highlight recent developments that have exploited omic technologies to significantly advance our knowledge of food microbes.

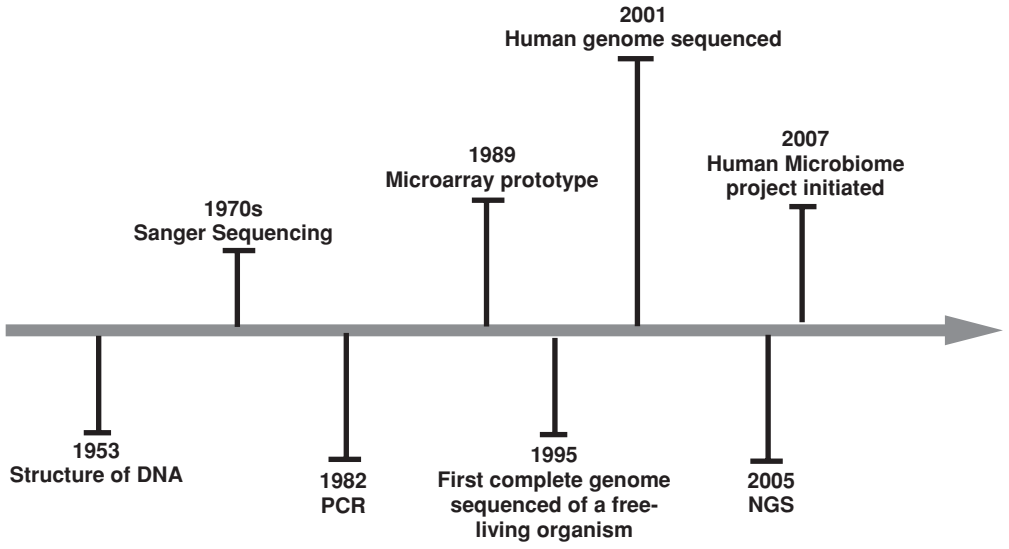

Figure 1

Timeline of important advances in omic-related technologies that impact food microbes.

IMPACT OF WHOLE GENOME AND NEXT GENERATION SEQUENCING TECHNOLOGIES ON FOOD MICROBES

NGS: next generation sequencing

Sanger sequencing, first described in the mid-1970s and long the method of choice for sequencing (Sanger et al. 1977), was improved by the emergence of new technologies that facilitated automated DNA sequencers (Edwards et al. 1990).

Less than 25 years after Sanger sequencing was introduced, the human genome was determined (Venter et al. 2001). Following the human genome sequencing project, it was evident that advances in sequencing technology were required to handle larger sequence output. This realization led to new novel sequencing techniques and ultimately the era of next generation sequencing (NGS). Presently, there are three main NGS technologies (**Table 1**; for details, see Ansorge 2009 and Pettersson et al. 2009). Unlike Sanger sequencing, NGS technologies do not require the cloning of template DNA into vectors but rather the DNA template is fragmented, amplified by PCR, and subsequently sequenced. Additionally, in some platforms millions of reactions take place simultaneously, thereby providing high throughput and large datasets. These large datasets, particularly of short reads, required the development of algorithms for sequence assembly. Sequencing both ends from the DNA fragment, termed pair-end sequencing, also aids in sequence assembly and is utilized by most of the commercial NSG technologies. The Roche 454 Genome Sequencer technology is based on sequencing by synthesis via pyrophosphate detection. Illumina acquired the Solexa sequencing technology in 2007, which is based on sequencing by synthesis chemistry with reversible terminator nucleotides each labeled with a different fluorescent dye. The third system is the ABI SOLiD sequencing platform, which is based on sequential ligation with dye-labeled oligonucleotides (Ansorge 2009, Pettersson et al. 2009). All three companies are constantly improving their technology to result in higher throughputs, longer read lengths, and lower costs.

More recently, advances in nanotechnology have enabled sequencing of a single molecule without the need for PCR amplification. Benefits of this system include reduction of reagents required and the omission of possible sequence bias introduced by PCR amplification. One such system is the Helicos platform, which does not require amplification of the DNA template but sequences single template molecules (Milos 2008). Additional technology in the pipeline is real-time DNA sequencing using single DNA polymerase molecules theoretically resulting in read lengths greater than 1,000 bp (Eid et al. 2009) and DNA nanoarray sequencing (Drmanac et al. 2010). These initiatives will further continue to benefit the study of food microbes, as they will allow for lower costs, larger datasets, and analyses that are not currently available.

Table 1 Details of current commercially available next generation sequencing (NGS) technologies

Manufacturer	Roche Applied Science	Illumina	Applied Biosystems
Next generation sequencer	454 GenomeSequencer	Genome Analyzer	ABI SOLiD System
Year of commercialization	2005	2006	2007
Platform	Pyrosequencing	Reversible terminator chemistry	Ligation chemistry
Latest model	Genome Sequencer FLX Titanium	Genome Analyzer IIx	5500xl SOLiD
Read length	~400–500 bp	~35–150 bp	~35–75 bp
Web site	**http://www.454.com**	**http://www.illumina.com**	**http://www.appliedbiosystems.com**

High-Throughput Sequencing of the 16S rRNA Gene and Metagenomics

IBD: inflammatory bowel disease

Sequencing the 16S ribosomal RNA (16S rRNA) gene as a means to distinguish between species is a powerful tool when coupled with the development of high-throughput sequencing methods. This allows for the assessment of bacterial diversity in a wide range of communities and environmental niches within a short period of time. In fact, omic technology, through high-throughput sequencing of 16S rRNA, has enabled the identification of new candidate beneficial microbes that, after further study and analysis, may be potential probiotic bacteria. One example is the description of *Faecalibacterium prausnitzii*, a member of the *Clostridium leptum* phylogenetic group. It was noted in the ileocolonic mucosa-associated microbiota of inflammatory bowel disease (IBD) patients by 16S rRNA sequencing (Frank et al. 2007, Manichanh et al. 2006) and PCR-denaturing gradient gel electrophoresis (PCR-DGGE) of 16S rRNA gene fragments that the numbers of *F. prausnitzii* were reduced in IBD patients (Martinez-Medina et al. 2006). Sokol et al. (2008) subsequently observed that the proportion of *F. prausnitzii* was lower in patients that showed endoscopic recurrence six months after surgical resection for active Crohns disease (CD). Following these observations, this group hypothesized that administrating this bacterium could be beneficial as a probiotic to ameliorate intestinal inflammation in CD. In vitro work demonstrated antiinflammatory effects by *F. prausnitzii* in peripheral blood mononuclear cells and *F. prausnitzii* supernatant-reduced secretion of the proinflammatory cytokine interleukin-8 (IL-8) by Caco-2 cells (Sokol et al. 2008). Additionally, both *F. prausnitzii* cells and culture supernatant-increased secretion of the antiinflammatory cytokine IL-10 decreased secretion of the proinflammatory tumor necrosis factor-alpha (TNF-α) and more importantly reduced the severity of trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice (Sokol et al. 2008). The identification of this microbe as a candidate probiotic may not have been realized without high-throughput sequencing of the 16S rRNA gene of the mucosa-associated microbiota of Crohn's patients.

There is a growing body of evidence that probiotic bacteria may play a role in amelioration of inflammatory diseases (Grangette et al. 2005, Sartor 2005). The effects of probiotic and pathogenic bacteria on the gut microbiota can be facilitated by high-throughput sequencing. As a consequence of omic technologies and the ability to sequence vast amounts of 16S rRNA, a high density array termed the PhyloChip was created, which identifies approximately 8,500 bacterial taxa in a single experiment (Brodie et al. 2006). This chip has been utilized for numerous applications, such as the determination of bacterial diversity in air (Brodie et al. 2007) and within aspirates from intubated patients colonized by *Pseudomonas aeruginosa* (Flanagan et al. 2007). The technology was also applied to a recent study to analyze the effect of the probiotic bacterium *Lactobacillus casei* subsp. *rhamnosus* GG (LGG) on the composition of the infant microbiome (Cox et al. 2010). Previous studies with LGG administration during infancy of children at high risk of atopic disease resulted in reduced rates (Kalliomaki et al. 2001, 2003). This study determined bacterial diversity in infants at six months that had either high or low abundance of LGG (Cox et al. 2010). The infants were part of a larger study and had received LGG or a placebo daily from birth (Cabana et al. 2007). The results indicated that samples with a high LGG abundance demonstrated a distinct microbial community structure that included phylogenetically clustered and closely related taxa such as other probiotic bacteria (Cox et al. 2010). It was suggested that the beneficial effect of this probiotic bacterium was not only due to its presence in high numbers but also to the changes in the community structure when it was present in high abundance (Cox et al. 2010). In addition, high abundance of LGG supported the presence of other species like itself, which may also contribute toward beneficial effects of probiotic bacteria administered continuously at high numbers.

There are limits to 16S rRNA sequencing. The 16S rRNA gene is highly conserved, therefore it can be difficult to distinguish between closely related species and strains within species using 16S

rRNA sequencing. Metagenomics is one method that overcomes this by sequencing all the nucleic acids directly from a sample and is, therefore, not restricted to the 16S rRNA gene. In addition to sequencing individual strains, one of the main outcomes of high-throughput sequencing has been the emergence of metagenomics. Although this technology was originally applied to environmental biology, in the past five years new information has been established in regard to our microbiome and relationship of the human microbiome and diet. By using NGS technology, the microbiome has been determined for humans from different ethnicities (Li et al. 2008), geographical areas (Kurokawa et al. 2007, Qin et al. 2010), and ages (Claesson et al. 2010). However, additional studies like those described above by Cox et al. (2010) relating to the effect of food microbes and food on the microbiome are needed.

Metagenomics: the global genetic content of microbes within a niche such as the soil or the gastrointestinal tract

Comparative genomics: comparison of the genetic repertoire of two or more genome sequences

Impact of Whole Genome Sequencing and Comparative Genomics

Sequencing technologies have had a profound effect on food microbiology from the detection of pathogens, identification of beneficial microbes, and whole genome sequencing. Advances in sequencing technology are evident by the number of strains that are currently sequenced or in progress (**Table 2**), demonstrating the lower cost and fast turnaround time of sequencing with current technologies. Interestingly, but not surprisingly, is the fact that the number of strains with sequencing "in progress" related to foodborne pathogens in particular outnumbers the amount of currently available finished genome sequences (**Figure 2**).

Comparison of one or more complete genomes termed comparative genomics has also revealed relationships between important industrial food microbes. It was known before genome sequencing of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* that these microbes have a cooperative relationship termed protocooperation in milk fermentations. Whole genome sequencing of *S. thermophilus* and *L. bulgaricus* revealed insights into their cooperation in milk fermentation through in silico analysis of their genomes (Bolotin et al. 2004, van de Guchte et al. 2006). Analysis of the *L. bulgaricus* genome sequence revealed the gene for the extracellular cell wall–bound proteinase, PrtB, (which was known before whole genome sequencing) and genes for the biosynthesis of folate. *S. thermophilus* also encodes genes necessary for the biosynthesis of folate but additionally encodes the genes necessary for *p*-aminobenzoic acid, which feeds into the folate pathway. It is believed that when cocultured with *S. thermophilus* in yogurt fermentation, *L. bulgaricus* benefits from *p*-aminobenzoic acid synthesis (van de Guchte et al. 2006). A recent study using comparative genomics of both genomes predicted genes acquired by horizontal gene transfer for both species, which included the transfer of genes for the metabolism of sulfur-containing amino acids from *L. bulgaricus* (or *Lactobacillus helveticus*) to *S. thermophilus* and the transfer of an exopolysaccaride biosynthesis gene cassette from *S. thermophilus* to *L. bulgaricus* (Liu et al. 2009b). Analysis also indicated the evolution of *L. bulgaricus* from a plant-associated environment to the milk environment through protocooperation with *S. thermophilus* and loss of genes related to a plant-associated niche (Liu et al. 2009b).

Lactic acid bacteria in particular have evolved to nutritionally rich niches, e.g., milk and the gastrointestinal tract. Genome sequencing has revealed that the majority of sequenced lactobacilli isolated from nutrient-rich habitats have gained genes encoding transporters for acquisition of exogenous nutrient sources as they lack complete biosynthetic pathways (Makarova et al. 2006). Although many probiotic lactobacilli have lost certain biosynthetic capabilities, they encode for crucial features that allow transit through, and survival in, the gastrointestinal tract, such as acid and bile tolerance. Adaptation of probiotic lactobacilli to life in the gastrointestinal tract is further evident when genome sequences are compared between the probiotic species *Lactobacillus acidophilus* and the cheese starter species *L. helveticus*. Although *L. acidophilus* and *L. helveticus* share 75%

Table 2 Details of food microbes with complete or in progress genome sequencing projects (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi, compiled August 2010)

Bacteria	Source	Number of strains sequenced (in progress)
Industrial/beneficial food microbes		
Bifidobacterium animalis subsp. *lactis*	Probiotic	3 (1)
Bifidobacterium longum	Probiotic	2 (2)
Lactobacillus acidophilus	Probiotic	1 (1)
Lactobacillus brevis	Starter culture	1
Lactobacillus casei	Probiotic/dairy starter culture	3
Lactobacillus delbrueckii subsp. *bulgaricus*	Dairy starter culture	2 (1)
Lactobacillus fermentum	Probiotic	1 (2)
Lactobacillus gasseri	Probiotic	1 (4)
Lactobacillus helveticus	Dairy starter culture	1 (1)
Lactobacillus johnsonii	Probiotic	1 (1)
Lactobacillus plantarum	Probiotic/vegetable starter culture	2 (1)
Lactobacillus reuteri	Probiotic	2 (5)
Lactobacillus rhamnosus	Probiotic	2 (2)
Lactobacillus sakei subsp. *sakei*	Meat starter culture	1
Lactobacillus salivarius	Probiotic	1 (1)
Lactococcus lactis subsp. *cremoris*	Dairy starter culture	2
Lactococcus lactis subsp. *lactis*	Dairy starter culture	2
Leuconostoc mesenteroides subsp. *mesenteroides*	Vegetable fermentation	1 (1)
Oenococcus oeni	Wine fermentation	1 (2)
Streptococcus thermophilus	Dairy starter culture	3
Foodborne pathogens		
Bacillus cereus	Meats, milk, cheese, vegetables, fish	9 (35)
Campylobacter jejuni	Raw beef, poultry, raw milk, eggs	5 (10)
Clostridium botulinum	Meats, fish, canned foods	10 (5)
Clostridium perfringens	Meats	3 (6)
Escherichia coli O157:H7	Ground beef, raw milk, vegetables	4 (15)
Listeria monocytogenes	Meats, poultry, milk, cheese, vegetables	6 (20)
Salmonella enterica subsp. *enterica*	Meats, poultry, eggs, milk	16 (23)
Shigella spp.	Vegetables, meat, poultry, milk, water	8 (38)
Vibrio cholerae	Shellfish and fish	8 (24)

of their ORFeome, there are important differences reflecting their adaptation to different niches. *L. acidophilus* encodes for numerous mucin-binding proteins and bile salt hydrolases, whereas none are encoded by *L. helveticus*. *L. helveticus* has additional genes for fatty acid biosynthesis and specific amino acid metabolism but notably fewer cell surface factors and transporters for utilization of multiple sugars beyond lactose (Altermann et al. 2005, Callanan et al. 2008).

ORFeome: the total number of open reading frames predicted in a genome

Comparative genomics between the genome sequences of the probiotic bacteria LGG and the dairy starter culture *L. rhamnosus* LC705 showed important differences in genome content relating to their environmental niche. These differences were suggested to explain longer colonization properties of LGG compared with *L. rhamnosus* LC705 in the human gastrointestinal

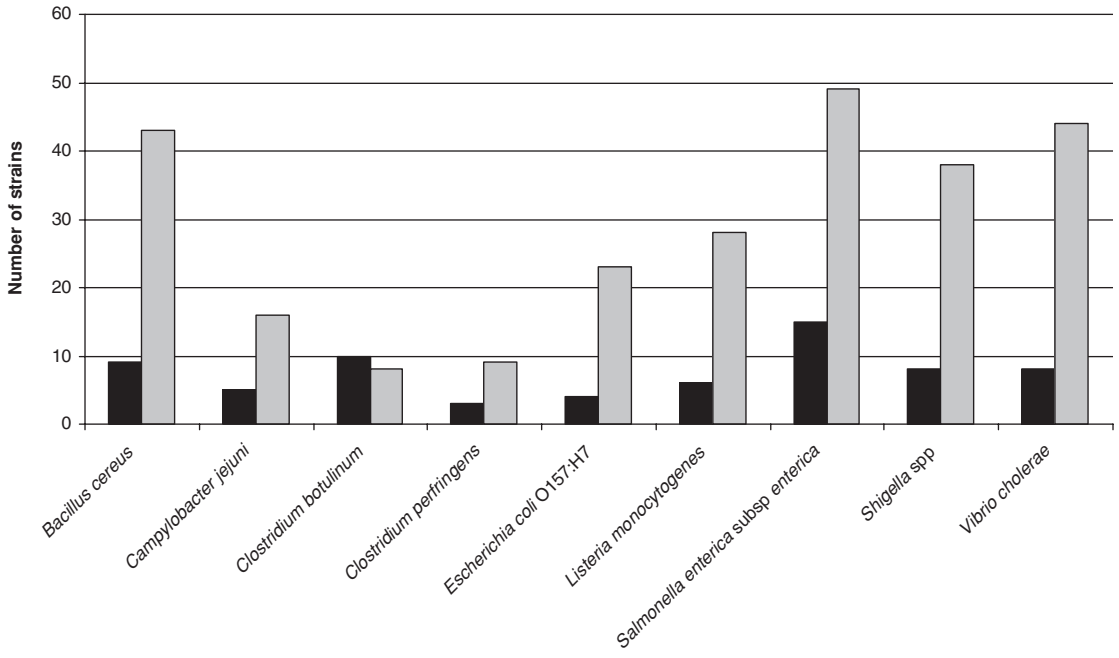

Figure 2

The impact of genome sequencing is demonstrated by the number of genome projects in progress (*gray bars*) and completed (*black bars*).

tract (Kankainen et al. 2009) and higher adherence rates to mucus (Tuomola et al. 2000) and epithelial cell lines (Jacobsen et al. 1999). These included the presence of pili on the cell wall of LGG and in silico analysis confirmed metabolic differences between the two strains that had been demonstrated experimentally. For example, compared with *L. rhamnosus* LC705, LGG is unable to ferment lactose, and in silico analysis revealed this was due to frameshifts in the antiterminator and 6-phospho-β-galactosidase genes (Kankainen et al. 2009). Sequencing of industrially important and beneficial food microbes has expanded our understanding of genome evolution and has also revealed the domestication of certain species toward specific environmental niches through genome reduction, gene decay, and acquisition of key capabilities through horizontal gene transfer (Douglas & Klaenhammer 2010).

Whole genome sequencing of food pathogens has revealed vital information on mechanisms of virulence and survival to better understand and ultimately control these microbes in food. New developments include the identification of virulence factors, mechanisms of survival in both foods and within host tissues, DNA sequences for rapid detection and identification, and antigenic and bacterial components that may serve as vaccines. For example, *Listeria monocytogenes* can survive and replicate in harsh environments such as low pH, high salt, and low temperature, which facilitates its survival in foods as these hurdles are often implemented to control food pathogens (Freitag et al. 2009). The first complete genome sequence of the foodborne pathogen *L. monocytogenes* was determined in 2001 (Glaser et al. 2001). Since then, five more strains have been sequenced and 28 more are in progress (**Table 2** and **Figure 2**). Comparative genomics of *L. monocytogenes* (Nelson et al. 2004) and the nonpathogenic *Listeria innocua* (Glaser et al. 2001), and four subsequently sequenced genomes of *L. monocytogenes* in 2004 revealed that genome organization is highly conserved among these strains. However, bile salt hydrolases, which contribute to survival of microbes

in the gastrointestinal tract, were present in *L. monocytogenes*, but absent in *L. innocua* (Dussurget et al. 2002, Glaser et al. 2001). Genomic, proteomic, and transcriptomic comparisons of strains of different levels of pathogenicity will most certainly help determine the mechanisms by which pathogens survive in food and subsequently cause disease.

Pan genome: the total gene repertoire (core and dispensable genome) of a bacterial species

Genome sequencing and subsequent comparative genomics of pathogenic bacteria led to the description of the pan genome, which represents the total repertoire of genes for a bacterial species. The pan genome also encompasses accessory or dispensable genes unique to single strains and genes present in more than one strain (Medini et al. 2005). The pan genome is dynamic with the dispensable genome, in particular, increasing as more genomes are sequenced. Therefore, the pan genome can be orders of magnitude larger than the genome of a sequenced strain. The description of a pan genome has only become available through advances in omic technologies through sequencing of large numbers of genomes. Pan genomes are also described as open or closed (Medini et al. 2005). *Escherichia coli* and *Salmonella* are examples of food microbes with open pan genomes as they have opportunities for genetic exchange between species because of their environmental niche. The determination of pan genomes contributes to the genetic repertoire of a species or group and can identify important targets for vaccination, detection, and biotherapeutics.

MOLECULAR METHODS FOR IDENTIFYING FOOD MICROBES

As pathogens are undesirable food microbes, the ability to detect and identify them is an important area of research in food microbiology. Subsequently, with omic genetic techniques it is possible to rapidly detect strains and also determine evolutionary and virulence factors. In particular, the foodborne pathogens *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella enterica* subsp. *enterica* have received much attention in this regard (Jasson et al. 2010). Molecular methods of bacterial identification have evolved over a relatively short period of time. DNA-DNA hybridization (Sibley & Ahlquist 1984) was initially used to differentiate between bacterial species. The introduction of PCR in the 1980s (Bartlett & Stirling 2003) revolutionized biology. Subsequently, numerous detection techniques have been developed that use PCR as a foundation, e.g., denaturing gradient gel electrophoresis, real-time PCR, terminal restriction fragment length polymorphism, and restriction fragment length polymorphism (described in Juste et al. 2008).

16S rRNA sequencing is one of the main methods of choice for species identification as the 16S RNA gene is ubiquitous in bacterial genomes and initial results correlated well with DNA-DNA hybridization (Keswani & Whitman 2001). Additionally, sequencing the 16S rRNA gene is a culture-independent method and can identify both live and dead cells. As 16S rRNA sequencing does not distinguish within species, multilocus enzyme electrophoresis (MLEE) was adapted from eukaryotic population genetics to distinguish subpopulations within species (Selander et al. 1986). Subsequently, multilocus sequence typing (MLST) was also utilized because of rapid advances in sequencing technologies (Maiden et al. 1998). Although sequencing large numbers of genomes within species revealed the limitations of these identification methods, they are still valued as a molecular tool in food microbiology.

Whole genome sequencing and microarrays have also been exploited for the identification of many food microbes. Large-scale whole genome sequencing through omic advances permitted the development of microarrays to detect 11 major foodborne pathogens based on probes that were identified through comparative genomics of sequenced genomes (Kim et al. 2008). The results demonstrated that pathogens hybridized to their respective probes, and the array could distinguish between nonpathogenic and pathogenic species. This combination of omic techniques and future advances will provide precise and fast identification of food pathogens, which is essential for their control.

A newer approach also adapted from use in humans and exploited as a consequence of advances in sequencing technology is examining single-nucleotide polymorphisms (SNPs) (Weissman et al. 2003). This method analyzes gene sequences for single nucleotide changes. In the case of *L. monocytogenes*, a successful SNP-based multilocus genotyping assay was developed for subtyping lineage I isolates, which are the main cause of listeriosis (Ducey et al. 2007). Recent studies demonstrated the power of SNP technology to identify clusters of *Salmonella enterica* subsp. *enterica* serovar Typhi, which causes typhoid in Nepalese children (Holt et al. 2010), and to differentiate closely related strains of bifidobacteria (Briczinski et al. 2009).

SNP: single-nucleotide polymorphism

Functional genomics: the determination of gene function using molecular methods

FUNCTIONAL GENOMICS AND BIOENGINEERING

The complete genome sequences of many food microbes classified as beneficial or harmful microbes have greatly facilitated functional genomic efforts to determine the function of one gene in a genome and the contribution of that gene product to the organism as a whole. In particular, methods are available to mutate, delete, or disrupt genes that potentially contribute to a particular attribute such as virulence (Sleator et al. 2005) or acid and bile tolerance (Begley et al. 2005, Klaenhammer et al. 2005). Additionally, once the sequence of a gene is known, it can be cloned and overexpressed in plasmid vectors, which is another method to confirm its phenotype.

Impact of Gene Inactivation and Deletion Systems on Food Microbes

Examples of gene deletions or insertions for food microbes have facilitated increased knowledge in a wide area of research, from pathogenesis to industrial microbes. For example, in the case of protocooperation between *S. thermophilus* and *L. bulgaricus*, deletion of the *prtS* gene from *S. thermophilus* demonstrated the necessity of this gene in cocultures and milk fermentations (Courtin et al. 2002). Functional genomics of probiotic bacteria have demonstrated the importance of numerous gene products in attributes such as bile tolerance, acid tolerance, adherence, and interactions with cells of the immune system, and in treatment of IBD (O'Flaherty & Klaenhammer 2010).

Functional genomics was instrumental in a study by Corr et al. (2007), which protected mice with the probiotic *Lactobacillus salivarius* UCC118 against infection with *L. monocytogenes*, in vivo. Protection was demonstrated as a result of a bacteroicin produced by *L. salivarius*. An isogenic mutant of *L. salivarius* UCC118 was constructed that could not produce the bacteriocin and failed to protect mice from *L. monocytogenes* infection (Corr et al. 2007). Notably, the bacteroicin-negative *L. salivarius* also protected mice from *Salmonella typhimurium*, indicating another mechanism of probiotic action for *L. salivarius* in addition to the production of a bacteriocin lethal to *L. monocytogenes*.

A recent example utilizing comparative and functional genomics was the discovery of a human mucus-binding pili protein in *L. rhamnosus* (Kankainen et al. 2009). Pili are found in many Gram-positive and Gram-negative pathogens (Kline et al. 2010), and their description in LGG indicated that probiotic and commensal microbes can employ shared strategies for survival in the gastrointestinal tract. A cluster of pilus-encoding genes (*spaCBA*) was discovered in LGG after comparative genomic analysis of the probiotic LGG genome and the closely related starter culture *L. rhamnosus* LC705 genome (Kankainen et al. 2009). Pilin proteins in Gram-positive bacteria contribute to adhesion to other bacteria and host cells (Kline et al. 2010). The presence of pili on the cell surface of LGG with the majority clustered at the cell poles was confirmed by immunogold electron microscopy (**Figure 3**). Subsequently, the *spaC* gene, which was predicted to encode for the large-sized minor pilin subunit, was inactivated using functional genomic methods. The *spaC*

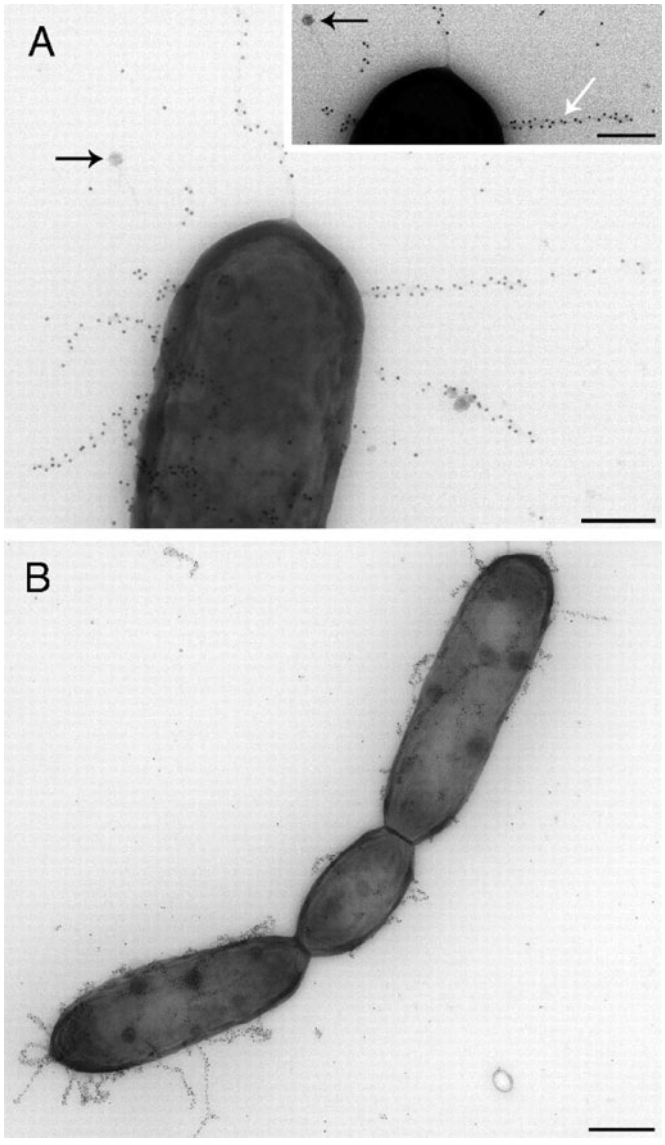

Figure 3

Identification of pili in *Lactobacillus rhamnosus* GG by immunogold electron microscopy. *L. rhamnosus* GG was grown to stationary phase, treated with anti-SpaC serum, labeled with protein A–conjugated gold particles (10 nm), negatively stained, and examined by transmission electron microscopy. (*a*) High-resolution electron micrograph showing multiple pili and an isometric bacteriophage (*black arrow*). Also included is a panel inset adjusted for heightened contrast and darkness to highlight the pilus ultrastructure (*white arrow*). (*b*) Electron micrograph showing pili clustered at the cell poles. (Bars: *a*, 200-nm; *b*, 500-nm.) Reproduced with permission from Kankainen et al. (2009).

mutant exhibited a significant reduction in binding to intestinal mucus (Kankainen et al. 2009). This study utilized comparative and functional genomics and proteomics to demonstrate the role of SpaC in mucin binding and potentially its importance to the survival of some lactobacilli in the gastrointestinal tract.

Using Food Microbes as Delivery Systems and Vaccines

Study of food pathogens such as *L. monocytogenes* has been used to investigate immune responses in antigen-presenting cells and T cells. Traditionally attenuated strains have been used for vaccination purposes. Both beneficial and pathogenic food-related bacteria are being harnessed as carriers of biotherapeutics and vaccines. *Salmonella* and *Listeria* are now being considered as candidates for delivery of antigens for cancer immunotherapy (Paterson et al. 2010). Moreover, the use of beneficial food microbes as delivery systems for different therapeutics and vaccine-type molecules was first pioneered by Wells & Mercenier (2008). One of the original systems employed for heterologous gene expression was the nisin-controlled gene expression system from *Lactococcus lactis* (Zhou et al. 2006). Numerous expression vectors were constructed for expression of the hepatitis B virus surface antigen (Zhang et al. 2010), the anti-*Listeria* agent pediocin (Renye & Somkuti 2010), and genes involved in stress tolerance (Abdullah Al et al. 2010).

The ability to express human IL-10 by *L. lactis* through omic techniques was a landmark development leading to a successful phase I clinical trial evaluating disease activity in CD patients (Braat et al. 2006). A genetically modified *L. lactis* strain was constructed that expressed the regulatory chemokine IL-10 in place of the thymidylate synthase gene *thyA* (Steidler et al. 2003). IL-10 is involved in suppressing inflammation at the gut mucosa by inhibiting proinflammatory cytokine synthesis and hence is a potential treatment for IBD (Li & He 2004). Thymidylate synthase catalyzes the conversion of deoxyuridylate to the nucleotide thymidylate. Replacing the *thyA* gene with *il10* removes the ability of the genetically modified *L. lactis* strain to synthesize thymidylate, thereby halting DNA synthesis and hence acting as an effective biological containment strategy. The use of *L. lactis* to administer therapeutic proteins termed ActoBiotics™ was developed and commercialized by the biopharmaceutical company ActoGeniX. A phase IIa clinical proof-of-concept in an ulcerative colitis model was recently completed (**http://www.actogenix.com**). Results showed that the product was safe, tolerable, and environmentally contained. However, there was not a statistically significant difference between the treatment and placebo in mucosal healing. Additional trials in humans are underway to assess a second product for the treatment of oral mucositis in cancer patients (**http://www.actogenix.com**).

More recently, oral vaccine delivery via *Lactobacillus*-based vectors has been markedly successful by fusion of a C-terminal (DC)-targeting peptide (12 mer) to the protective antigen (PA) for anthrax (Mohamadzadeh et al. 2009). Expression of this PA-DC cassette on a high-copy vector in *Lactobacillus gasseri* provided 100% protection of mice from challenge with anthrax (**Figure 4**) (Mohamadzadeh et al. 2010). Omic technologies have facilitated the expression of DNA, enzymes, allergens, cytokines, antigens, and peptides by lactic acid bacteria (Wells & Mercenier 2008, Mohamadzadeh et al. 2008). The use of modified strains that can be safely ingested at high numbers as delivery agents for therapeutics, vaccines, and in the treatment of disease is an exciting prospect and a significant advancement in the study of food microbes.

TRANSCRIPTOMICS OF FOOD MICROBES

Microarrays have contributed significantly to our knowledge of food microbes, e.g., transcriptomics of food microbes during growth in stressful environments and various foods such as milk, cheese, and yogurt. Recently, gene expression studies from the duodenum of humans demonstrated that administration of *L. plantarum* can elicit immunomodulatory responses (van Baarlen et al. 2009). With regard to pathogens, transcriptomics has revealed important and invaluable insights into their pathogenicity, adaptation, and survival in food. Transcriptomics has been used to investigate intracellular expression of genes in *L. monocytogenes* (Chatterjee et al. 2006,

Joseph et al. 2006) during infection and host adaptation (Camejo et al. 2009) and those genes activated by the transcriptional regulars PrfA (Milohanic et al. 2003), VirR (Mandin et al. 2005), and the sigma B regulon (Hain et al. 2008, Raengpradub et al. 2008). Microarray technology has also expanded to include tiling arrays, which are designed to cover the complete genome and more than one genome (Mockler et al. 2005) rather than covering just the annotated ORFeome of a single genome. These arrays have facilitated a deeper view of transcription responses in food microbes such as *Bacillus subtilis* (Rasmussen et al. 2009), *L. monocytogenes* (Toledo-Arana et al. 2009), and *E. coli* O157:H7 (Jackson et al. 2007). Another technique that was originally applied to eukaryotes and has been adapted to prokaryotes is ChIP (chromatin immunoprecipitation), which in tandem with microarrays (termed ChIP-chip), studies protein-DNA interactions (Wade et al. 2007). ChIP-chip was first used in bacteria in 2002 and has since been utilized to study transcription factors in *B. subtilis*, *E. coli*, and *Helicobacter pylori* (Wade et al. 2007).

Next generation sequencing technologies are fast emerging as valuable tools in transcriptomics and in understanding regulatory processes. Recently, NGS technologies have been applied to sequencing of the transcriptome and are termed as RNA-seq or RNA deep sequencing (Wang et al. 2009). To date, NGS has mostly been utilized to sequence the transcriptome of eukaryotes, including eukaryotic microorganisms. This is in part due to the fact that unlike most bacterial mRNA, eukaryotic RNA contain a poly-A-tail, which is utilized in the amplification procedure to create cDNA. However, recently there have been some publications with food pathogens that have their transcriptome sequenced via RNA-seq (Liu et al. 2009a, Oliver et al. 2009, Perkins et al. 2009). Direct RNA sequencing for transcriptomics has the advantage over DNA microarrays, as transcripts are only detected on DNA microarrays if there is a corresponding probe on the array and DNA microarrays often do not cover the complete transcriptome but rather just annotated open reading frames. cDNA derived from RNA depleted of 16S and 23S rRNA from *S.* Typhi was sequenced using Illumina sequencing technology. The main outcome of this study from RNA-seq was the correction of the original genome annotation, the identification of transcriptionally active prophage genes, 40 new noncoding RNA sequences, and members of the OmpR regulon (Perkins et al. 2009). The OmpR regulon in *S.* Typhi regulates transcription of numerous genes, including those associated with Vi polysaccharide synthesis (Pickard et al. 1994), two component regulatory systems (Feng et al. 2003), and outer membrane porins (Fernandez-Mora et al. 2004). In the case of *Vibrio cholerae* sequencing of noncoding or small RNA (sRNA) was specifically targeted using 454 sequencing (Liu et al. 2009a). Twenty known *V. cholerae* sRNAs, 500 new putative intergenic sRNAs, and 127 putative antisense sRNAs from 407,039 sequence reads were identified. Additionally, a novel sRNA regulator of carbon metabolism was discovered. Illumina sequencing technology was also used to sequence the stationary phase stress response transcriptome of *Listeria* (Oliver et al. 2009). This study compared the transcriptomes of a *L. monocytogenes* strain with an isogenic mutant of the sigma B regulon, which was identified through microarray transcriptome studies as an important regulator of genes involved in virulence, stress response, transcriptional regulation, and carbohydrate metabolism and transport (Raengpradub et al. 2008). The study by Oliver et al. (2009) using RNA-seq demonstrated that 83% of all *L. monocytogenes* genes were transcribed in stationary phase and identified 96 genes with significantly higher transcript levels in the parent strain compared with the isogenic mutant indicating sigma B control of these genes. Importantly, RNA-seq also facilitated the identification of 67 (including 7 novel) noncoding RNA molecules (ncRNAs) transcribed in stationary phase *L. monocytogenes* and 65 putative sigma B promoters upstream of 82 of the 96 sigma B–regulated genes (Oliver et al. 2009). This latter study demonstrates the wealth of information obtained from RNA-seq, which can ultimately help the scientific community better understand the pathogenicity of *L. monocytogenes*. This technology will no doubt be applied to additional food microbes. DNA microarrays are still the method of

choice due to the high cost of RNA-seq, but in the foreseeable future direct sequencing of RNA transcript will be commonplace with advances in technologies, improved bioinformatic software, and lower costs. Indeed, single molecule sequencing such as the Helicos system and other methods under development will also be applicable to RNA sequencing (Ozsolak et al. 2009). Direct RNA sequencing will bypass the need for cloning and/or amplification and reverse transcription of RNA to generate cDNA. Omission of these experimental steps will reduce time and cost and also omit experimental bias during the amplification of the original RNA template.

Metabolomics: global analysis of metabolites

ADVANCES IN PROTEOMICS

Proteomic tools allow the study of protein identification, expression, function, interaction, and structure. In particular, classical proteomic approaches have been used to study important food microbes used in dairy processing (Gagnaire et al. 2008). These techniques include two dimensional gel electrophoresis in which proteins are separated on a gel by their mass and isoelectric point. Protein spots are excised from the gel, proteins are digested within the spot, and then proteins are identified by mass spectrometry (MS). Different proteome profiles or reference maps are determined that allows the resolution of total protein abundance for many beneficial and pathogenic food microbes and also the comparison of protein expression under different stress or growth conditions such as in milk and synthetic media. However, this technique is limited to approximately one third of the ORFeome as not all proteins are represented on the electrophoresis gels, in part because of their hydrophobicity (Gagnaire et al. 2008).

Additional proteomic technologies are based on nuclear magnetic resonance (NMR) spectroscopy and MS, which allow the analysis of a large sample quantity and hundreds or more metabolites in a single run of samples. In MS, components within samples are separated using either gas or liquid chromatography (GC and LC, respectively). Fragmentation of samples isolated in the mass spectrometer and subsequent determination of these partial amino acid sequences, termed MS/MS, improved the system as related to available genetic information. However, current limitations include the low level of detailed molecular identification and the need for improved bioinformatic software suites to deal with the large dataset outputs. Examples of recent proteome studies that advanced our knowledge of food microbes include changes in the proteome of *E. coli* when grown in milk compared with synthetic media (Lippolis et al. 2009) and identification of cell surface–associated proteins from the probiotic bacteria *L. plantarum* (Beck et al. 2009). A major advancement in proteomics is the development of metabolomics, which has in particular been used to study gut microbe metabolites (Nicholson et al. 2005). Metabolomics aims to analyze all metabolites in a given sample or condition. This technology can be used to identify biomarkers that are related to a specific trait or disease, such as IBD. It is difficult to develop a metabolomic platform that could provide analyses of all microbial metabolites, as the number of metabolites varies depending on the type and number of bacterial species. Considering that the estimation of the gut microbiota is greater than one thousand taxa, the number of possible metabolites to be analyzed is a daunting task. However, proteomic techniques can establish a dataset or blueprint that can be linked to a specific trait, e.g., the identification of biomarkers that can be measured after administration of a probiotic bacterium to treat intestinal inflammation. The challenge is to determine those sets of metabolites or biomarkers relevant to the particular study.

CONCLUSIONS

The use of omic tools has unquestionably accelerated our knowledge and comprehension of the complexities of food microbes and their adaptation to specialized niches.

However, even with these advances, contamination of the food chain remains a problem that is a cost for the consumer in terms of health and disease. These costs also impact industry through recalls and lowered consumer confidence in their products. Although significant effort has traditionally been associated with the study of host-pathogen interactions, the use of omic technologies has driven our knowledge of beneficial microbes and how to better exploit them in industrial applications, fermentations, or as dietary probiotic supplements.

As omic technologies are further improved and applied to food microbes, there are a plethora of uses in food microbiology, from detection of pathogens, thereby improving food safety, to understanding the beneficial aspects of industrial strains. Food microbes can be modified by omic tools for production of vaccines and delivery of therapeutics. Omic technologies have advanced the prospect of personalized medicine, which impacts the food industry. Knowledge gained from genomics and proteomics of food microbes and their effects on the gut microbiota will contribute to the future study of personalized diets. Hence, the challenge for the food industry and scientific community is to determine the effect of food and food microbes on the gut microbiota and impact on human health, while ensuring food quality, nutrition, and pathogen-free products.

SUMMARY POINTS

1. Next generation sequencing technologies have greatly impacted the study of food microbes, including detection systems, construction of global microarry platforms, increased numbers of sequenced whole genomes, and identification of microbes with industrial potential.
2. Although there are a limited number of genome sequences currently available for food pathogens, advances in sequencing technology have rapidly increased whole genome sequencing, which will prompt better understanding of pathogen survival in food and pathogenesis in the host.
3. It is evident from omic technologies that studies are transitioning from the study of a single bacterium to groups of strains within a species or across species for a greater understanding of food microbe communities.
4. Functional genomics of beneficial food microbes have resulted in genetic engineering of specific species for the delivery of therapeutic molecules. Human clinical trials with these strains have been initiated, and there exists exciting possibilities in the near future in regard to delivery of therapeutic molecules and vaccines.
5. Advances in proteomics and metabolomics may establish metabolic blueprints that can be utilized as diagnostic tools, particularly for studying the effect of beneficial microbes used as delivery vehicles and in disease management.

FUTURE ISSUES

1. Advances in omic technologies have resulted in large datasets resulting in the requirement of giga- and terabytes of storage. A future issue is the open access and storage of these large datasets.
2. The constant development and improvement of bioinformatic tools are required to keep pace with advances in omic platforms.

3. Datasets from omic technologies must translate to biological meanings that are relevant for the scientific community and food industry, especially for control of pathogens and the treatment or prevention of disease.
4. As additional whole genome sequences become available, global tools will have to be updated to incorporate the new sequence information, e.g., expansion of the pan genome and modified microarray platforms.
5. Despite advances in omic technologies and knowledge on pathogens, food contamination remains a major issue. This is in part due to the rapid globalization of the food market and consumer requirements of the food industry. A challenge is harnessing knowledge from omic technologies for the control of food pathogens and ensuring safety throughout the food chain.

DISCLOSURE STATEMENT

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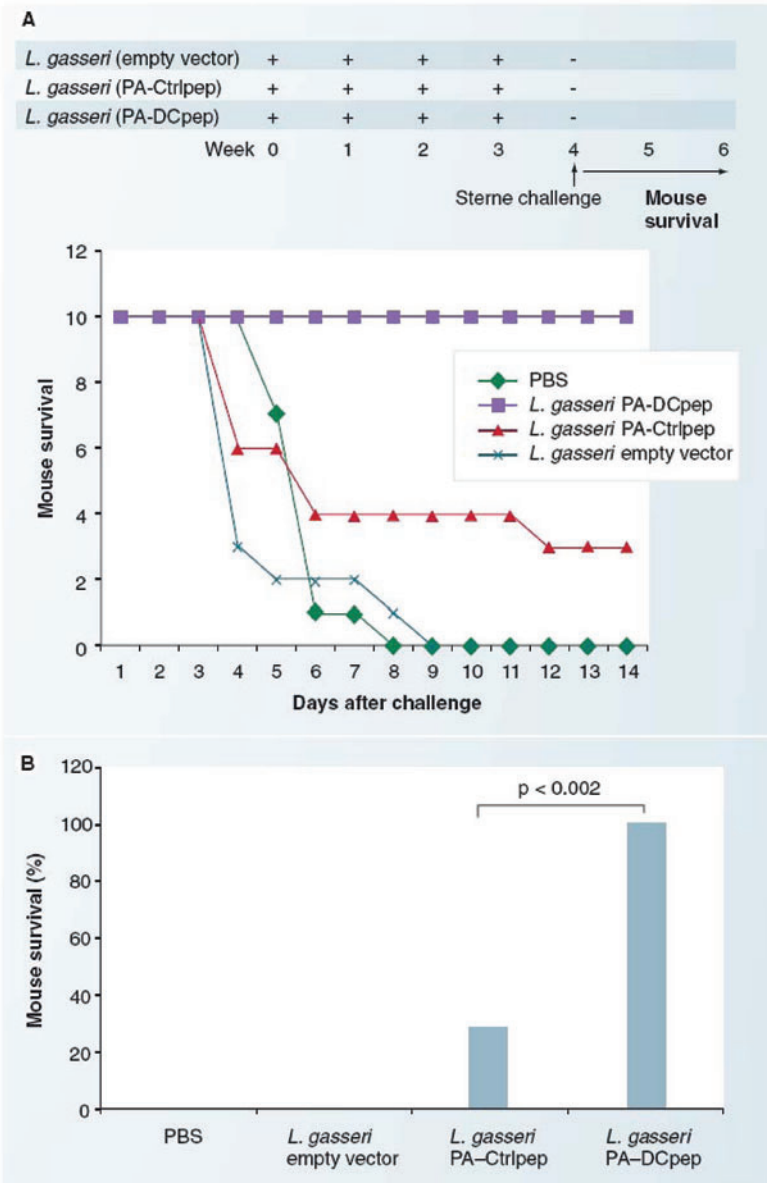

Figure 4

Induction of protective immunity against *Bacillus anthracis*. (*a*,*b*) Feeding and challenge regimen and mouse survival. Groups of mice (n = 10 per group) were orally vaccinated with *Lactobacillus gasseri* expressing PA–DCpep, PA–Ctrlpep, or empty vector (10^8 CFU/100 μl). Oral vaccination was repeated for four consecutive weeks. Seven days after the last vaccination, mice were challenged with Sterne, and mouse survival was monitored until day 14. Abbreviations: Ctrlpep, control peptide; DCpep, dendritic cell–targeting peptide; PA, protective antigen; PBS, phosphate buffered saline. Reproduced with permission from Mohamadzadeh et al. (2010).

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Errata

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