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CRISPR-Based Technologies and the Future of Food Science

Kurt Selle and Rodolphe Barrangou

Abstract: The on-going CRISPR craze is focused on the use of Cas9-based technologies for genome editing applications in eukaryotes, with high potential for translational medicine and next-generation gene therapy. Nevertheless, CRISPR-Cas systems actually provide adaptive immunity in bacteria, and have much promise for various applications in food bacteria that include high-resolution typing of pathogens, vaccination of starter cultures against phages, and the genesis of programmable and specific antibiotics that can selectively modulate bacterial population composition. Indeed, the molecular machinery from these DNA-encoded, RNA-mediated, DNA-targeting systems can be harnessed in native hosts, or repurposed in engineered systems for a plethora of applications that can be implemented in all organisms relevant to the food chain, including agricultural crops trait-enhancement, livestock breeding, and fermentation-based manufacturing, and for the genesis of next-generation food products with enhanced quality and health-promoting functionalities. CRISPR-based applications are now poised to revolutionize many fields within food science, from farm to fork. In this review, we describe CRISPR-Cas systems and highlight their potential for the development of enhanced foods.

Keywords: CRISPR, genome, microbiology

Introduction

Food science may be generally defined as the application of many scientific fields and disciplines to food products and processing. Although many distinct sciences have historically been applied to the genesis, formulation, processing, storage, enhancement, and enjoyment of food products over time, food science is arguably in a *renaissance* stage, which is fueled by the availability of key technologies that allow food scientists and engineers to develop health-promoting products. Notwithstanding the many advances we have witnessed over the last century, the field of genetics has contributed critical advances in the recent past. In particular, the use of recombinant genetic technologies has profoundly impacted food science, agriculture, ecology, animal husbandry, and medicine. Tangible and impactful improvements in the human condition, such as the industrial biosynthesis of vitamins, enzymes, pharmaceuticals, antibiotics, and bioactive peptides, have been enabled by advances in genetic methodologies. Forthcoming DNA technologies have accelerated the rate of molecular biology research in diverse backgrounds, where few technologies previously existed or were suboptimal. Specifically, clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated sequences (Cas) are an adaptive immune system against invasive genetic elements in bacteria (Barrangou and Marraffini 2015), which has been co-opted for genome editing in a diverse set of organisms ranging from scientific models to industrial workhorses (Doudna and Charpentier 2014). The ability to cleave and edit DNA with CRISPR-Cas systems has reinvigorated reductionist biology during a scientific era defined by sequencing, and the rate of functional genomics has accelerated to new limits as a result. However, early investigation of CRISPR-Cas systems developed applications outside of genome editing, including manipulation of microbial consortia (Gomaa and others 2014), designed vaccination of microorganisms against invasive genetic elements (Barrangou and others 2013), and typing of bacterial strains (Barrangou and Horvath 2012). Many of the early applications of CRISPR-Cas systems actually arose from food science-driven research during characterization of industrial starter culture bacteria for improving milk fermentation processes (Barrangou and others 2007). Food science is a growing field investigating all biological, chemical, and physical processes to improve production of safe and sustainable food for a growing world population. Overall, given the pace at which CRISPR-Cas technology is being developed, one can already envision how applications of CRISPR-Cas systems may further be harnessed or engineered to address challenges related to the food and agriculture industries at every level of food manufacturing, from farm to fork.

MS 20151303 Submitted 7/31/2015, Accepted 9/3/2015. *Authors are with Dept. of Food, Bioprocessing and Nutrition Sciences, Functional Genomics Graduate Program, North Carolina State Univ., Raleigh, NC 27695, U.S.A. Direct inquires to author Barrangou (E-mail: rbarran@ncsu.edu).*

CRISPR basics and background

Two genetic elements constitute the adaptive immune system in bacteria: CRISPR arrays which confer immunological memory and surveillance, and *cas* genes, which encode effector proteins in all stages of immunity (Figure 1). CRISPR-Cas mediated immunity is categorized into 3 temporally overlapping but mechanistically distinct molecular processes: acquisition, expression, and interference (Barrangou 2013; Barrangou and Marraffini 2015). Acquisition occurs via sampling of foreign genetic elements by the universal Cas1/Cas2 surveillance complex, from which short sequences, termed spacers, are integrated in a polarized fashion into the CRISPR array (Barrangou and others 2007). New spacer sequences are added at the leader end of the array, resulting in an

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ordinal record of foreign DNA exposures that spans from most recent at the 5' end to most ancient at the 3' end. Expression of CRISPR arrays is constitutive under standard conditions, but is also inducible during phage infection (Young and others 2012). The array is transcribed as a long pre-CRISPR RNA (crRNA), and is typically further processed via Cas proteins and host ribonucleases into mature, small interfering crRNAs (Brouns and others 2008). Mature crRNAs guide Cas proteins to target DNA via sequence-specific complementarity for recognition and cleavage of target nucleic acids, causing direct interference of exogenous DNA elements (Marraffini and Sontheimer 2008; Garneau and others 2010). Various aspects of CRISPR biology, genetics and applications have been extensively covered in comprehensive and focused reviews (Makarova and others 2011; Barrangou and Horvath 2012; Doudna and Charpentier 2014; Barrangou and Marraffini 2015; Barrangou and May 2014; Selle and Barrangou 2015)

CRISPR arrays consist of highly conserved, partially palindromic DNA repeats that alternate with variable short spacer sequences. Repeat sequences, the compelling and defining feature of CRISPR-Cas systems, function in the formation of hairpins necessary for structure-dependent RNA processing during biogenesis of crRNAs. CRISPR repeat sequences also exhibit partial complementarity to *trans*-activating CRISPR RNAs (tracr RNA) and leader sequences, suggesting roles in acquisition and in Cas protein recognition (Deltcheva and others 2011). Specific to certain systems, tracr RNAs are noncoding RNAs that elicit processing of crRNAs and target DNA recognition and cleavage by Cas proteins (Deltcheva and others 2011; Karvelis and others 2013). By contrast, the origin and function of most spacer sequences is mostly unknown, as only a small percentage exhibit reliable identity to foreign invasive elements or chromosomal sequences (Horvath and others 2009). This may be because of extinction of genetic elements or the lack of environmental surveillance (sequencing) of the elements to which spacers correspond. Most of the known spacer targets correspond to plasmids and bacteriophages, but many also appear to target either self or foreign chromosomal sequences (Horvath and others 2009). Although the intuitive function of an adaptive immune system is targeting invasive genetic elements, many spacer sequences exhibit self-complementarity, and many do not appear to be transcribed, suggesting that CRISPR-Cas systems may play additional roles in microbial physiology beyond targeting of genetic elements (Barrangou 2015). An essential feature of type I and type II systems (Makarova and others 2011) is the protospacer adjacent motif (PAM), a short conserved sequence proximate to the spacer sequence in the target DNA (Deveau and others 2008; Horvath and others 2008; Mojica and others 2009). The PAM governs both the acquisition and interference processes, as it determines viable protospacers in the target sequence, and functions in differentiation of the target from the CRISPR array. PAM sequences vary between CRISPR types and orthogonal systems within CRISPR types, but must be characterized in order to fully exploit functional systems.

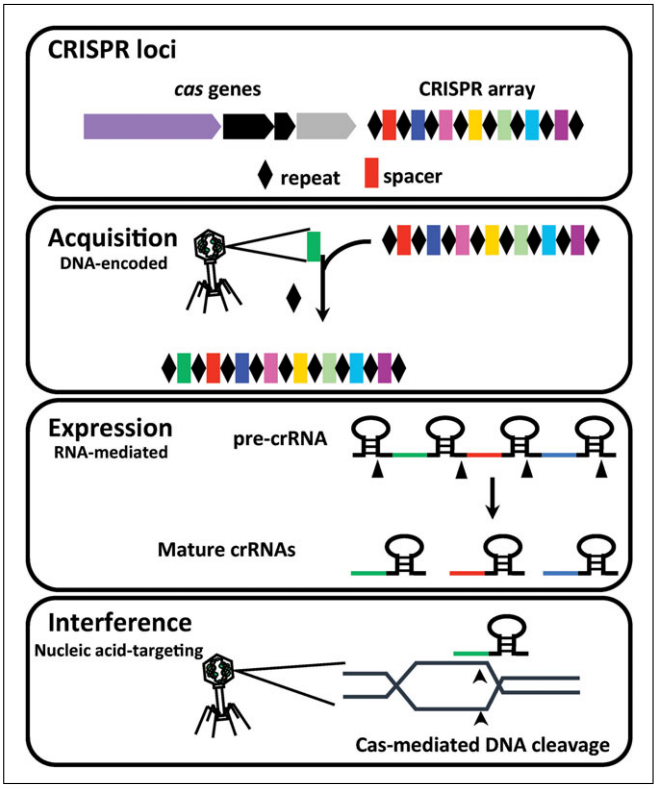

Figure 1–CRISPR-based adaptive immunity. An overview of composition and processes of CRISPR-mediated antibacteriophage immunity is shown as an example. Top, CRISPR loci: CRISPR- Cas systems are comprised of cas genes (left) and repeat spacer arrays encoded in tandem (right). Second panel: *acquisition* occurs upon introduction of bacteriophage DNA into the host cell and sampling a novel spacer sequence from its chromosome, by copy-paste processes that yield an additional CRISPR repeat-spacer unit. Third panel: *expression* and RNA biogenesis form mature surveillance CRISPR RNAs (crRNAs) that comprise a portion of the CRISPR spacer sequence, which defines the target. Bottom: *interference* against exogenous phage sequences, driven by Cas nucleases that drive recognition and cleavage of complementary DNA elements, defined by the crRNA guide sequence.

Cas proteins are fundamental to each stage of CRISPR-based immunity, as they are responsible for acquisition of new spacers, processing of crRNAs, and recognition and degradation of sequences complementary to crRNAs. However, convergent evolution has resulted in a myriad of DNA recognition and cleavage mechanisms in keeping with microbial diversity, necessitating categorization of CRISPR-Cas based on gene content, operon organization, and distinct clusters of sequence homology (Makarova and others 2011). The most recent definitive description of CRISPR-Cas systems highlights 2 classes based on the composition of the immune effector complex (single vs. multi-subunit). The systems are further delineated into several main types, specifically depending on the presence of signature *cas* genes (Makarova and others 2011). The hallmark features of type II systems are the large multifunctional endonucleolytic Cas9, the tracrRNA, and ribonuclease III processing of crRNAs (Deltcheva and others 2011). Type I and type III systems encode Cascade, Csy, and Csm proteins that constitute the multi-subunit effector complexes responsible for target nucleic acid recognition (Brouns and others 2008). The signature gene of type I systems is *cas3*, a single stranded nickase with 3'-5' exonuclease activity, which is recruited to the target via the CRISPR-associated complex for antiviral defense (Brouns and others 2008; Sinkunas and others 2011). In contrast to other CRISPR system types, type III systems target either or both DNA and RNA, and the signature gene is *cas10* (Makarova and others 2011) Estimates indicate that 46% of bacterial and 84% of archaeal genomes contain at least one CRISPR-Cas system (Makarova and others 2011). Despite the high distribution of CRISPR-Cas systems in bacteria, the relative youth of the field means that very few of the systems have been characterized for activity in each of the 3 stages of immunity: acquisition, expression and

interference. To date, only 14 systems have demonstrated activity in interference, highlighting the need for investigating orthogonal systems for holistic understanding of CRISPR-Cas mechanisms and applications (Bondy-Denomy and Davidson 2014).

The study of CRISPR-Cas systems had relatively humble origins in food science-related research, with its biological function being discovered during investigation of phage resistance mechanisms of dairy starter cultures (Barrangou and others 2007). In particular, *Streptococcus thermophilus* notoriously undergoes attack by predatory bacteriophage during yogurt and cheese fermentation, and is highly enriched for active CRISPR-Cas systems (Barrangou and others 2013). *S. thermophilus* encodes up to 4 CRISPR-Cas systems, 2 of which are innately active in both acquisition and interference, likely due to the high selective pressure of bacteriophage in dairy processing environments. Accordingly, functional genomic analysis of a food-grade starter culture bacterium and its bacteriophages established the role of CRISPR-Cas systems in phage/DNA protection (Barrangou and others 2007). Several seminal contributions to the field were conducted in *S. thermophilus*, because it harbored highly active CRISPR-Cas systems, the genome sequence was available, and there were characterized lytic phages, all of which was related to its widespread industrial use as a starter culture in food fermentations. Basic food science research therefore led to determination of spacer origin (Barrangou and others 2007), inference of PAM sequences (Deveau and others 2008; Horvath and others 2008), unravelling of phage-host dynamics (Sun and others 2013; Paez-Espino and others 2015), demonstration of Cas9 endonuclease activity (Garneau and others 2010; Sapranauskas and others 2011; Gasiunas and others 2012), characterization of tracrRNA structural motifs governing function and orthogonality (Deltcheva and others 2011; Briner and others 2014), and recently, designed removal of large genomic islands (Selle and others 2015).

Applications across the food bacterial spectrum

Lactobacillus spp. are scientifically, industrially, and medically relevant microorganisms that are propagated at high levels for fermentation processes or to elicit health benefits as probiotic microorganisms. Thus, as constituents of the human microbiome, or as fermentative bacteria, exposure to bacteriophage is highly likely, suggesting that bacterial phage resistance mechanisms would be abundant in these environments. Indeed, *in silico* surveys have revealed that type II systems are disproportionately present in lactobacilli (Horvath and others 2009; Briner and others 2014), making them a reservoir for CRISPR-Cas systems. In this light, the human and food microbiomes are a relatively unexplored trove of new and diverse CRISPR-Cas systems that are potentially suitable for use in food-grade systems. Given that bacteria are ubiquitous throughout the production and consumption of food, CRISPR technologies have to the potential to impact all classes of bacteria across the food spectrum, including pathogenic, commensal, fermentative, probiotic, and spoilage organisms. CRISPR-based technologies with applications in food science include genotyping of bacteria, manipulation of microbial consortia, vaccination against phages, and genome editing (Figure 2).

CRISPR-based genotyping

Identification and typing of bacterial strains is a considerable challenge due to the inherent diversity of microorganisms and their tendency to undergo horizontal gene transfer. Although genome sequencing can be considered the "gold standard" for identification and typing of bacterial strains in terms of resolution, it is a costly, analytically-challenging and time-intensive process not suitable for high-throughput or rapid applications. The same limitations apply to pulsed field gel electrophoresis, although fingerprinting of restriction digestion profiles is still performed for strain typing during foodborne outbreaks. Recently, repetitive-element PCR-based genotyping using high-resolution microfluidics has proven to be rapid and reliable in strain differentiation, but identification of strains requires a database of fingerprint data for comparison. 16S rDNA sequencing, although not a typing tool, is relatively fast, and affordable for rough identification of bacterial genus and species, but can be unreliable even for applications requiring resolution down to the species level. By contrast, CRISPR array genotyping offers a rapid, affordable, and high-resolution means of typing bacterial strains within species that carry them (Barrangou and Horvath 2012). Due to the unique polarized nature of spacer acquisition in CRISPR-Cas systems, the highly ordinal composition of CRISPR arrays provides a means of typing with high resolution at the strain level (Shariat and others 2013), requiring a few PCR amplifications and sequencing of the array to provide a clear comparison of spacer content. Despite this, proof of concept for CRISPR-based typing has only been provided for a limited set of bacteria. In order to effectively be used as a tool for identification and typing, the same CRISPR-array locus must be enriched or ubiquitous within defined taxonomic groups (Genus or species) and the spacer content of the array must be diverse across all strains in a given subset. The presence or absence of a CRISPR array may also be used to differentiate strains, but is only reliable when it correlates with the phylogeny of the organism. In order for adequate spacer comparison to occur, within a given CRISPR locus, the most ancient spacers must share a common origin, which then diverges over the course of the array. In other words, there must be some shared and some disparate spacers in order to effectively type strains based on array content. Thus, the process is largely contingent on having had active spacer acquisition machinery at some point in evolutionary history, although degeneracy of CRISPR arrays can also add to polymorphisms in spacer content. Of course, CRISPR-based typing also depends on the presence of CRISPR-Cas systems in the genomes of genera and species of interest, and these loci have been identified in most archaea and many bacteria, but only documented to occur in approximately 46% of bacterial genomes. To date, CRISPR-based typing schemes have been effectively employed in foodborne pathogens such as *Salmonella* (Shariat and others 2013) and *Escherichia coli* (Toro and others 2013; Yin and others 2013), industrial fermentation starter cultures such as *S. thermophilus* (Horvath and others 2008), probiotics such as *Lactobacillus casei* (Broadbent and others 2013), and spoilage organisms such as *Lactobacillus buchneri* (Briner and Barrangou 2014), illustrating the broad potential of CRISPR-based genotyping across the bacterial spectrum.

Vaccination of industrial microbes

Mobile genetic elements (MGEs) are a class of DNA entities encompassing plasmids, bacteriophages, transposable elements, and integrative and conjugative elements. MGEs exhibit high rates of transfer and hijack bacterial DNA homeostasis pathways, causing continuous challenges to both population and genetic stability of bacteria. To cope with the permanent threat of predatory bacteriophages and selfish genetic elements, bacteria have evolved both innate and adaptive immune systems targeting exogenous genetic elements. Innate immune mechanisms include cell-wall modification, restriction/modification systems, and abortive phage

infection (Labrie and others 2010). In the food industry, predatory bacteriophages constitute a significant threat to efficiency of preservation and continue to be a major source of inconsistent quality or loss in dairy fermentations. Many strategies have thus been developed to combat the ever-present and dynamic phage populations present in processing plant environments. Starter cultures are especially susceptible to lytic phage infection due to a mono-culture population, wherein a single infective phage type can cause the crash of an entire population. Furthermore, the high rate of mutation in phages necessitates the use of multiple resistance mechanisms and control strategies to compensate for their high capacity for adaptation to the host. Specifically, both native biological mechanisms of resistance and environmental control are employed to prevent phage proliferation. Starter culture rotation, growth in the presence of chelators, multistrain starter formulations, and steam sterilization of manufacturing equipment are all means of controlling phage in the dairy processing environment, whereas genetic transfer of plasmids containing native phage resistance mechanisms and/or CRISPR can be used to combat phage in the bacterial population (Horvath and Barrangou 2012). CRISPR provides unique advantages in vaccination of starters against predatory bacteriophage (Barrangou and others 2013). Specifically, the process of adding spacers corresponding to phages is iterative, which means that additional spacers can always be acquired to target-emerging phages. Moreover, resistance is sequence specific, which means that the resistance mechanism can be as broad, or specific as desired, especially if conserved functional sequences are targeted in phage genomes. One spacer may therefore be able to confer resistance to multiple phages if the respective phage genomes contain the same sequence targeted by the spacer. Finally, unlike innate immune mechanisms of phage resistance, the target sequence of the phage must mutate in order to circumvent CRISPR-Cas as a mechanism, which can lead to detrimental mutations in phage machinery (Sun and others 2013; Paez-Espino and others 2015).

Antibiotic resistance of pathogens is an alarming issue in the medical community, leading to extensive efforts in reducing the uptake and dissemination of antibiotic resistance genes in bacteria. To this end, the presence of transmissible antibiotic resistance genes is prohibitive during selection of starter culture and probiotic bacteria. Antibiotic resistance genes can be encoded genomically, or by plasmids, bacteriophages, and transposable elements, all of which can be targeted by CRISPR-Cas systems. Spontaneous mutations conferring antibiotic resistance can also be corrected using template-mediated genome editing. Similarly, limiting transfer of antibiotic resistance genes into food-grade bacteria during fermentation is highly desirable. In strains that contain an active CRISPR-Cas system, it is possible to introduce the antibiotic resistance gene on a plasmid and screen for CRISPR-based loss of the plasmid due to targeting of the antibiotic resistance gene (Garneau and others 2010). Thus, it is a natural means to vaccinate food-grade bacteria against transmissible antibiotic resistance genes, which can be achieved through incorporating a spacer sequence corresponding to that of the coding sequence for antibiotic resistance. Conversely, it is also possible to heterologously introduce an active CRISPR-Cas system into organisms lacking an endogenous system and vaccinate the recipient against the uptake of undesirable genetic content (Sapranauskas and others 2011).

Antimicrobials

Self-targeting events of CRISPR-Cas systems are highly lethal, which has been determined experimentally and observed *in vivo* (Vercoe and others 2013; Beisel and others 2014). The lethality of self-targeting events relates to the nature of DNA destruction induced by CRISPR-Cas interference mechanisms. Type I systems elicit extensive DNA damage through the exonuclease activity of Cas3, introducing deletions that span approximately 40 kb in some experiments (Vercoe and others 2013). DNA damage of this nature is reparable at low frequency (10^{-5}), likely by an alternative end-joining mechanism (Vercoe and others 2013). Type II systems elicit double-stranded DNA breaks via Cas9 activity, for which few DNA repair mechanisms exist in bacteria (Garneau and others 2010; Gasiunas and others 2012; Selle and Barrangou 2015). Bacteria typically use the high fidelity pathway of homologous recombination to repair double-stranded DNA breaks, but restoration of the target locus to the wild-type does not circumvent targeting by CRISPR-Cas systems (Selle and

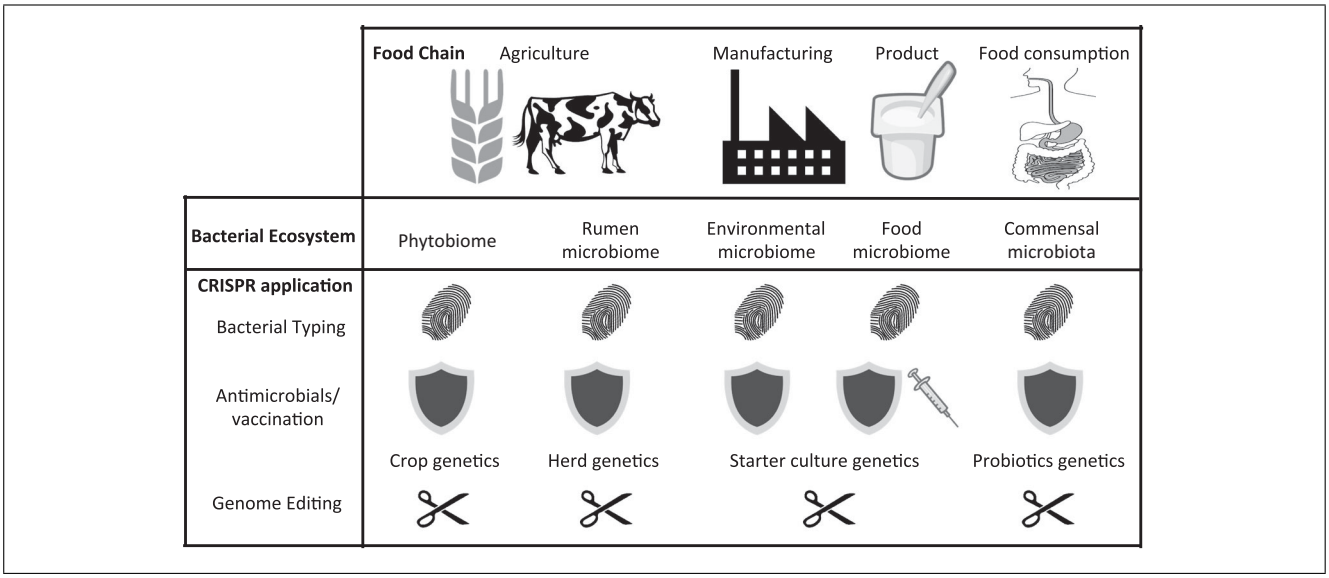

Figure 2–Applications of CRISPR-Cas systems across the food chain. CRISPR-Cas as antimicrobials and typing tools may be applied at every stage of food manufacture, whereas genome editing may be applied to food crops, animal herds, and industrial microbes. Vaccination of bacteria against mobile genetic elements can be used to protect the population and genetic stability of starter cultures.

Barrangou 2015). Pathways of dsDNA break repair in bacteria also include the low-fidelity alternative end-joining, and non-homologous end-joining, although both occur at low frequency, and the latter is generally present in spore-forming bacteria and *Mycobacterium* spp (Aravind and Koonin 2001). Due to a paucity of high-frequency DNA damage repair pathways that can cope with CRISPR-Cas targeting, population-wide depletion of cells exhibiting the target genotype occurs, generally on the order of 3 to 5 logs under experimental conditions. Self-targeting with type I systems is highly efficacious, as lethality is not dependent on chromosomal location, expression level, or strand bias (Gomaa and others 2014). Moreover, it was demonstrated that proper design of self-targeting spacers could lead to differentiation of highly related strains (Gomaa and others 2014). Several experiments repurposing endogenous or delivered CRISPR-Cas systems have been performed in pathogenic organisms related to the food industry (Beisel and others 2014; Citorik and others 2014; Bikard and others 2014; Gomaa and others 2014). Control of microbial consortia in processing facilities and in food products is fundamental to protecting the food supply from contamination or proliferation of foodborne pathogens. Self-targeting CRISPR-Cas systems therefore present a novel and high-potential means to deplete microbial populations in a sequence-specific manner, sparing the innocuous native microbiota present in foods. As CRISPR-based microbiome engineering technologies further evolve, we anticipate several application avenues will be generated across the food supply chain, to optimally manage the composition of various microbial populations associated with soil, plants, livestock, manufacturing environments, and the consumer.

Genome editing and remodeling in bacteria

Bacterial genomes exhibit site-specific plasticity that belies the linearity of their interpretation as straightforward sequences (Darmon and Leach 2014). The ability to reprogram CRISPR-Cas systems to target any sequence in the genome offers promising applications towards defining minimal bacterial genomes, determining essential genes, and characterizing genetically heterogeneous bacterial populations (Jiang and others 2013; Selle and Barrangou 2015). Recently, CRISPR-Cas targeting was used to show that MGEs contribute to genomic plasticity in *Streptococcus thermophilus* (Selle and others 2015). Specifically, recombination between insertion sequences of high identity caused spontaneous deletion of large genomic islands, spanning from 8 to 102 kbp in length. Targeting the genomic islands with an endogenous CRISPR-Cas system enabled selection and recovery of naturally occurring mutants lacking genes necessary for acidification and preservation of milk. The approach also confirmed the nonessentiality of genes encoded on the genomic islands, ultimately resulting in excision of 7% of the genome of *S. thermophilus*. This approach could similarly be applied for removal of genomically encoded MGEs, increasing genome stability. Moreover, CRISPR-based removal of pathogenicity islands and/or virulence factors is an attractive method for neutralizing pathogenic bacteria. Thus, CRISPR-Cas systems facilitate characterization of MGEs and elucidation of bacterial genome plasticity. Similarly, this technology can also be harnessed in combination with single-strand DNA recombineering to drive genome editing in probiotic strains such as *Lactobacillus reuteri* (Oh and van Pijkeren 2014; van Pijkeren and Britton 2014).

The CRISPR revolution as it applies to the food chain

The use of CRISPR-based technologies has revolutionized the field of genetics in general, and genome editing of eukaryotes in particular. To date, this approach has been successfully employed for targeted mutagenesis of a plethora of genomes including *Homo sapiens, Mus musculus*, *Danio rerio*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Oryza sativa*, and *Saccharomyces cerivisiae* (Doudna and Charpentier 2014). The streamlined and multifunctional nature of Cas9 from Type II systems is practical for programmable genome editing through precise and directed targeting of chromosomal loci. The tipping point for genome editing was arguably the provision of a synthetic guide molecule that combines the functions of a native crRNA and tracrRNA (Jinek and others 2012) and the development of a corresponding 2-component sgRNA:Cas9 genome editing system. The programmable specificity of exacted chromosomal cleavage is facilitated by selection and design of a spacer sequence unique to the target allele. Specificity is compounded by the PAM, a short conserved sequence that must be proximate to the protospacer in the target sequence (Deveau and others 2008; Mojica and others 2009). Cas9-induced mutagenesis in eukaryotes occurs subsequent to cleavage and is typically mediated through the imperfect DNA repair mechanism of nonhomologous end joining (NHEJ). Following the genesis of double-stranded breaks, NHEJ yields efficient recovery of insertion and deletion knockout clonal genotypes.

CRISPR-based genome editing has already been applied to organisms of interest across food science, including yeast, corn, rice, and tomatoes. Genome editing of crops has applications for targeted engineering to improve growth under drought conditions, application of insecticide, low nutrition/fertilizer conditions, and also to improve the nutrition potential of food crops. Similarly, genome editing can improve yield in animal breeding through desirable alteration and selection of herd genetics. Moreover, there is the potential to increase the disease-resistance of both crops and cattle, but despite the promising outcomes of genome editing using CRISPR-Cas systems, the practical implications of doing so are yet to be unanimously defined.

One key consideration moving forward is the regulatory status of various CRISPR-derived products. In some cases, the native activity of CRISPR-Cas systems can be harnessed for screening of natural events, such as vaccination against phages, immunization against plasmids, or lethal-self targeting bactericidal activity. These would constitute non-GMO processes that would be readily acceptable to regulatory agencies and, by extension, to the public. In contrast, there are many means to exploit engineered CRISPR-Cas systems in heterologous backgrounds that hinge on recombinant DNA technologies that are typically construed as genetic-engineering methods. On-going efforts are focused on exploiting regulatory frameworks already in place for the genesis of genomically modified variants using other gene editing technologies such as programmable nucleases. Recent advances based on the use of RNA and ribo-nucleoprotein complexes, as opposed to DNA, have open intriguing avenues based on the process, rather than the outcome.

Conclusions and Perspective

While much of the on-going CRISPR craze (Pennisi 2013; Ledford 2015) has been focused on genome editing applications in human cells, and the potential of Cas9-based gene therapies for clinical applications (Barrangou and May 2015), CRISPR-Cas systems and CRISPR-based technologies hold much promise for a broad range of applications across food science. For applications in food bacteria, native CRISPR-Cas systems present opportunities for genotyping of pathogens, for vaccination of cultures against phages, and as next-generation antimicrobials. Furthermore,

engineered systems can be harnessed for genome-editing applications in crops and livestock for trait-enhancement in next-generation breeding approaches. Building off recent advances in their exploitation in agriculture, husbandry, and industrial fermentations, we envision that CRISPR-Cas technologies will drive research and development in many food products, and open new avenues for the future of food science.

Conflicts of Interest

R.B. and K.M.S. are inventors on several patents related to various uses of CRISPR-Cas systems. R.B. is a board member of Caribou Biosciences, and a founder and advisor of Intellia Therapeutics, 2 companies that are involved in commercialization and exploitation of CRISPR applications.

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