Engineered *Akkermansia muciniphila*: A promising agent against diseases (Review)

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**Abstract.** Achieving a harmonious gut microbial ecosystem has been hypothesized to be a successful method for alleviating metabolic disorders. The administration of probiotics, such as *Lactobacillus* and *Bifidobacteria*, is a known traditional and safe pathway to regulate human commensal microbes. With advancements in genetic sequencing and genetic editing tools, more bacteria are able to function as engineered probiotics with multiple therapeutic properties. As one of the next-generation probiotic candidates, *Akkermansia muciniphila* (*A. muciniphila*) has been discovered to enhance the gut barrier function and moderate inflammatory responses, exhibit improved effects with pasteurization and display beneficial probiotic effects in individuals with obesity, type 2 diabetes, atherosclerosis and autism-related gastrointestinal disturbances. In view of this knowledge, the present review aimed to summarize the effects of *A. muciniphila* in the treatment of metabolic disorders and to discuss several mature recombination systems for the genetic modification of *A. muciniphila*. From gaining an enhanced understanding of its genetic background, ingested *A. muciniphila* is expected to be used in various applications, including as a diagnostic tool, and in the site-specific delivery of therapeutic drugs.

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**1. Introduction**

With increasing economic development, chronic non-communicable diseases have emerged as a substantial global concern due to common risk factors, such as unhealthy diets and environmental pollution (1,2). Although a range of pharmacological and surgical interventions are constantly being devised to address the increased numbers of cases of non-communicable diseases, the side effects and contraindications of certain medicines or radiotherapy have limited the number of patients that are able to receive such treatments (3,4). Furthermore, the unavoidable postoperative complications such as surgical site infection, abscess, active bleeding, hematoma and anastomotic leak, resulting from surgery may worsen a patient's state (5). Therefore, researchers have begun to consider other possibilities to cope with this global problem (6,7).

The term dysbiosis refers to the major changes in the gut microbial ecosystems that contribute to a range of metabolic disorders, including obesity, type 1 and type 2 diabetes and inflammatory bowel disease (8‑11). Numerous other types of disease like autism or allergies have also been associated with an imbalance in gut microflora composition (12,13). Several strategies to normalize human gut microbial ecosystems are available to treat different syndromes, including fecal microbiota transplantation, which has demonstrated promising results (14). At present, probiotics are commonly used to improve the intestinal environment (15‑17).

To further determine the relationship between the gut microflora and a healthy human state, the use of metagenomics and metatranscriptomic sequencing techniques has been proven to provide a compositional snapshot of the microbial species in the gut, as well as to sequence their expressed genes (18,19). With the elucidated genetic background of different gut microflora, it also provides a wide range of possibilities to modulate the gut microflora composition genetically for greater therapy. Advances in synthetic biology have extended this therapeutic potential, as selected bacteria can be tailored to deliver drugs or molecules to act directly on the host (20). An increasing abundance of human-associated bacteria have been identified to provide health benefits, including *Lactococcus lactis* (*L. lactis*), *Escherichia coli* (*E. coli*) and *Bifidobacterium*, making them desirable engineering targets for therapeutic application (21‑27). Similar efforts may also be applied to *Akkermansia muciniphila* (*A. muciniphila*), a microbial...
species that has been proposed as a novel candidate for probiotic therapy (28). In the present review paper, the potential of A. muciniphila as an engineered bacterium was discussed by first reviewing other engineered bacteria. The review covered its colonization sites in human intestines, therapeutic effects and probiotic characteristics, prior to identifying potential avenues for modification.

2. Commonly engineered bacteria used for the treatment of diseases

The most commonly engineered bacteria provide a platform to develop probiotics as a novel direction in therapeutic studies. In the following sections, some of these cases are discussed in further detail. By noting similarities in their therapeutic effects, characterization status and the strategies used for their modification, the current review aimed to demonstrate why A. muciniphila is being considered for similar engineering approaches.

E. coli. E. coli is an inhabitant of the human gastrointestinal tract; its well-characterized genome, accessible and versatile plasmid vector, susceptibility to genetic manipulation and high recombinant protein synthesis rates renders it one of the most desirable hosts for the expression of recombinant proteins (29,30). Since recombinant human insulin was first produced in E. coli by Genentech in 1978, numerous genetic engineering strategies have been developed for E. coli, providing a genetic circuit model for the subsequent genetic manipulation of bacteria (31). For example, by deleting the arginine repressor gene of E. coli Nissle (EcN) and integrating a feedback-resistant arginine synthase into the intergenic region controlled by the fnrS promoter, Kurtz et al (24) generated the SYNBI020 clinical candidate for the treatment of hyperammonemia. In addition, Whelan et al (32) ligated a functional nematode gene into the pMu13 plasmid and transformed it into EcN; in the EcN, the expressed nematode cystatin, reported to have anti-inflammatory properties, decreased the inflammatory monocyte/macrophage migration and positively affected the epithelial barrier function in both mice and piglets. The introduced genetic material was able to overcome the defense barrier of the host cell and was stably maintained as a plasmid with the aid of selectable markers and a compatible origin of replication, or by integration into the genome (24).

Lactobacillus (LAB). LAB is a commensal intestinal microbiota species with widespread use in the production of fermented foods (33,34). By virtue of its numerous health-promoting effects in humans and its decoded genetic sequence, LAB has become one of the most convincing engineered probiotics (35-37). In 2015, Yang et al (27) constructed the recombinant strain LAB plantarum (L. plantarum) NC8, which expresses angiotensin-converting enzyme inhibitory peptides (ACEIPs) for prolonging antihypertensive effects; the recombinant expression vector pSIP409-ACEIP was built by replacing the gusA gene in the pSIP409 plasmid with genes encoding ACEIPs. Through incubating its DNA with available methyltransferases in vitro to match the host's DNA methylation patterns, the transformation efficiency of L. plantarum was raised to a level comparable with that of E. coli. This vector was subsequently transformed into L. plantarum NC8. An antihypertensive effect was noted following the oral administration of the engineered strain to spontaneously hypertensive rats, as evidenced by a reduction in abnormal systolic blood pressure and in triglyceride, endothelin and angiotensin II levels. For further consideration, the expression levels of the integrated genes should be monitored and regulated (38).

Different promoter-repressor systems have been constructed for the induction of recombinant protein expression in L. plantarum to evaluate their stability and efficiency (39). An increasing number of systems have emerged, such as the quorum-sensing system, chemical-based induction system and temperature-sensitive system, which enhanced the abilities of microbes to sense, respond to and record their local environment, as well as improving the ability to evaluate and control the expression levels of the desired genes, which are designed to produce the required product (40-42).

Bifidobacterium. Of all the commensal bacteria inhabitants in the mammalian gut, bacteria of the Bifidobacterium genus represent some of the most prevalent probiotic species, which have been used to prevent or treat colorectal cancer, diarrhea, necrotizing enterocolitis and IBD (43-48). In view of these prominent therapeutic characteristics, molecular genetic studies are of crucial importance. Among the Bifidobacterium genus, Bifidobacterium longum (B. longum) was identified to exert more significant positive effects on the gut environment compared with others (49). The complete genome sequence of this strain has been deciphered and it frequently used in genetic manipulation. As it was discovered to selectively grow in the hypoxic regions of solid tumours, genetic modifications to B. longum for cancer therapy have been proposed (45). In a previous study, the tumstatin gene was inserted into a plasmid and electrically transformed into the B. longum NCC2705 strain, which generated an anticancer effect in tumor-bearing mice by inhibiting the apoptotic vascular endothelial cells of the transplanted tumours (50). A similar strategy was employed in other B. longum strains, enabling them to express more anticancer drugs (51-53). These achievements demonstrate the strength and utility of engaging the immune system at the level of the intestinal mucosa using ingested microbes.

Commonly engineered pathogens. Foodborne pathogens, such as Salmonella typhimurium (S. typhimurium) and Listeria monocytogenes (L. monocytogenes), have also been engineered using an attenuation operation for therapeutic purposes (54). Examples of attenuation strategies include interrupting the transport of lipids, purines and/or metabolites (54). A previous study developed an attenuated S. typhimurium strain, VNP20009 DNase I, which contained defective adenine and lipopolysaccharide metabolism genes, and a plasmid with a humanized toxin DNase I sequence inserted; the results indicated that the combination of VNP20009 DNase I and triptolide significantly reduced tumor volume, prolonging the survival of mice (55). A similar strategy has been adopted for modifying the L. monocytogenes strain for use as a vaccine for different types of disease; for instance, the administration of the Lmdd-multiple peptide fusing genes (MPFG) strain, which was based on a vaccine against hepatocellular carcinoma (HCC) (56),
created an antitumor response towards the human leukocyte antigen (HLA) epitopes of MPFG (HLA-A0201), presenting a potentially feasible strategy for the prevention of HCC (57). Biocontainment and biosafety are crucial factors in the clinical application, to avoid the harm that engineered pathogens like S. typhimurium and L. monocytogenes cause, thus the attenuation of these strains to lower the expression levels of pernicious genes is a critical step. At present, to achieve greater control and safety, kill switches and genetic firewalls have been added into genetic circuits (58).

3. Next generation of engineered bacteria: A. muciniphila Akkermansia

Overview of A. muciniphila. A. muciniphila was first isolated from a fecal sample in anaerobic medium containing gastric mucin (its sole energy source) in 2004 by Derrien et al (59). A. muciniphila was discovered to directly bind to enterocytes to enable colonization, while its degradation of mucin was identified to stimulate mucin production and increase mucin thickness, thereby strengthening epithelial integrity (60). In addition, metabolites, mainly short-chain fatty acids, produced by A. muciniphila were found to be absorbed in the colon and serve as an energy source for colonocytes, and they also exhibited potential therapeutic and anti-inflammatory effects in various types of metabolic disorder, such as obesity, IBD, and diabetes (61-63), as illustrated in Fig. 1. Moreover, the effects of some exposed active molecules of A. muciniphila have been demonstrated to remain after pasteurization; for instance, as Amuc_1100 is heat-stable, it is able to replicate almost all of the effects of live A. muciniphila or inactivate the inhibitory compounds for live A. muciniphila (64,65).

A. muciniphila in metabolic disorders and other types of disease.

Obesity. Globally, the prevalence of excess weight between the years 1980 and 2013 has increased to 27.5% in adults and 47% in children, with 2.1 billion people in the world classifying as overweight (BMI ≥25 kg/m²) and over 500 million being classified as obese (BMI >30 kg/m²) (66). Obesity has become a worldwide health concern, with current medical and lifestyle interventions largely failing to offer adequate solutions. Increasing evidence has indicated that probiotics are involved in gut barrier maintenance and inflammation normalization, suggesting that their adoption could eventually result in a long-term treatment for obesity (67,68).

In recent years, A. muciniphila has been proposed as a potential probiotic for the treatment of obesity, as significantly decreased levels of A. muciniphila were observed in obese or overweight individuals (69,70). Everard et al (71) demonstrated that administering a daily dose of live A. muciniphila to mice with diet-induced obesity significantly lowered their body weight and sanguineous lipopolysaccharide levels (71). However, this treatment was reported to increase fat mass development and alter adipose tissue metabolism. Similarly, a study of overweight and obese insulin-resistant volunteers indicated that oral supplements coated with pasteurized A. muciniphila normalized the mean adipocyte diameter and lowered plasma leptin concentrations (72).

Other diseases. In the majority of the studies discussed, when supplied in a viable form, therapeutic effects of A. muciniphila were noted for metabolic disorders. However, such treatment could also extend to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88). The fecal matter of patients with cancer with positive responses to immunotherapy was noted for metabolic disorders. However, such treatment could be extended to other diseases. For example, in cancer treatment, A. muciniphila employment was suggested to enhance the effects of immunotherapy (87,88).
Evidence for the viability of engineered *A. muciniphila*. The advent of next-generation sequencing and whole-genome sequencing has provided additional scope for more bacteria to be genetically modified. Based on this, the prospects for engineering *A. muciniphila* are promising.

The genome of *A. muciniphila* BAA-835 was first sequenced in 2011, from which *A. muciniphila* was predicted to synthesize all 20 canonical amino acids, as well as important cofactors and vitamins (95). In 2015, genes from the *A. muciniphila* strain Urmite were assigned to strain ATCC BAA-835, suggesting that the majority of these genes were involved in metabolic reactions (96). Recently, 39 new *A. muciniphila* strains were sequenced and analyzed, with several gene flow and recombination events being noted, indicating the development of a feasible background for future genetic engineering studies (97).

Moreover, an efficient and scalable workflow for the cultivation and preservation of *A. muciniphila* cells has been developed, resulting in viable *Akkeransia* colonies with high yields and very high stability, as well as up to 97.9±4.5% survival of >1 year when stored in glycerol-amended medium at -80°C (98). The growth of *A. muciniphila* can be monitored and controlled by various quality assessment and control procedures to ensure that viable cells of *A. muciniphila* are available. In addition, although *A. muciniphila* is an anaerobic bacterium, it has demonstrated an ability tolerate and even benefit from nanomolar concentrations of oxygen in liquid medium (99). These properties extend the possibility of *A. muciniphila* to be manipulated for engineering (Fig. 1).
Potential genome editing tools for engineering A. muciniphila.

In general, plasmids are the first tool considered when genome editing is required. Plasmids contain appropriate DNA as the bacterial origin of replication, an antibiotic resistance cassette and the gene of interest, which is transcribed from a prokaryotic promoter (100,101). Adequate expression of the therapeutic gene or genes is ensured by using appropriate promoters and other regulatory elements (100,101). In previous years, the genetic toolbox of plasmids has been greatly expanded by adding sensors, regulators, memory circuits, delivery devices and kill switches (102). Once the recombinant plasmid carrying the desired gene tracks down signal molecules secreted by target cells or tissues, it releases therapeutics locally, and is subsequently self-digested as programmed to avoid any infection (103,104). After construction, plasmids are converted to the hosts by chemical, mechanical or physical techniques, with mammalian cell ‘poration’ systems (electroporation and sonoporation) being the most important and common techniques used (105-107).

In addition, extra genome integration in a chromosome of the host cell has been discovered to support the development of engineered bacteria (108). Normally, a designed homologous single-stranded DNA donor is provided based on the introduction of a site-specific double-strand DNA break (DSB) into the locus of interest (109). Information encoded on this template can be used to repair the DSB, resulting in the addition of the desired gene at the site of the break (109). Recombination systems carried by helper plasmids are crucial during this process (109-112). In the following sections, several mature recombination systems developed in LAB or E. coli are described, which could be applied to A. muciniphila once limitations relating to species differences have been eliminated.

Nisin-controlled gene expression (NICE) system. The NICE system is one of the most widely used tools for chromosomal integration exploited for engineering Lactobacillus. It is constructed for gene expression based on nisA and nisF promoters via a two-component regulatory system consisting of the histidine protein kinase, nisK, and the response regulator, nisR (113-116). When a gene of interest is placed behind the inducible promoter, PnisA, on a plasmid and transformed into a nisRK strain, the expression of the cloned gene can be activated by the addition of nisin (Fig. 2). Using the dual plasmid system, the classic NICE system can be successfully introduced into the majority of bacteria. For example, Mohseni et al (117) genetically engineered L. lactis using a NICE system with pNZ8148 to express the native and codon-optimized recombinant E7 (E7 is a good candidate protein for vaccine development against human papillomavirus (HPV)-related cervical cancer) oncogenes isolated from HPV: the results for the overall production of E7 by L. lactis NZ9000 containing codon-optimized E7 was 2.7-fold higher compared with NZ9000 containing the native E7 strain. The findings also indicated that the amount of recombinant E7 oncoprotein accumulation depended on the concentration of nisin added, with the highest concentration achieved in the presence of 10 ng/ml nisin for both recombinant L. lactis strains. However, the exposed drawback of the system was that its basal expression was leaky; therefore, it may not applicable for production of the desired proteins or for the expression of toxic proteins (118).

λ recombination system. The bacteriophage λ Red homologous recombination system has been studied over the past 50 years as a model system for the transfer of chromosomal DNA from species (119). The λ recombination system, designated 'Red,' consists of two proteins; α, an exonuclease that acts on double-stranded (ds)DNA, and β, a single-stranded (ss)DNA binding protein capable of annealing complementary ssDNA strands (120). Red-mediated recombination is assisted by the γ protein, which increases α and β activity on linear dsDNA by inhibiting E. coli RecBCD exonuclease (121,122). In the past, NICE restricted the integration of molecular weight DNA into the host strain; however, the new lambda Red recombinase-mediated integration strategy was found to transform higher molecular weight DNA of variable lengths into any non-essential locus in the host chromosome (123). Juhas and Ajikoka (124) successfully integrated 15 kB DNA encoding sucrose catabolism and lactose metabolism and transport operons into the flsu locus of the flagellar region 3b in the E. coli K12 MG1655 chromosome; this approach preferred the use of overlapping DNA fragments for integrating the high molecular weight DNA. Elongation of the integrated DNA sequence is facilitated by the alternative use of kan and cat-yfp cassettes tagged in different DNA fragments, which is less time-consuming compared with the standard lambda Red recombinase-mediated integration (124). Under monitoring, this new strategy did not reveal any negative effects on the host strain. However, compared with E. coli, to the best of our knowledge, there are fewer reports regarding the use of this technique on other strains.

CRISPR-Cas system. In the CRISPR-Cas system, the small CRISPR RNAs encoded by CRISPR spacer sequences form a duplex with a trans-activating CRISPR RNA. The duplex with the Cas9 protein subsequently searches the presented DNA for a Cas-specific sequence (Fig. 3) (125-127). Upon recognition of the specific sequence, Cas9 induces the targeted DSB, enabling the modification of a target gene sequence through host bacterial DNA repairing systems (128,129). The presence of a homologous template ensures the insertion of the addition in the region of the DSB. CRISPR-based technologies have been implemented for E. coli, Streptococcus pneumoniae,
L. lactis and probiotic LAB species for the production of pharmaceutical products and precursors of high industrial significance (130-132). Various types of CRISPR are currently used for producing desired strains with therapeutic potentials. Δ-integration CRISPR enables strains to have multiple loci chromosomal integration, whereas CRISPR-based homology-directed repair allows site-specific integration (133). A catalytically inactive form of Cas9 (dCas9), has been developed to direct the promoter or coding regions to prevent transcription rather than cleaving the DNA, known as CRISPR interference (78). This technique has been used to control gene expression in Corynebacterium glutamicum, in which it was employed to downregulate multiple genes by concatenating single guide RNA sequences encoded on one plasmid (134). Genomic sequencing of the A. muciniphila strain determined the CRISPR loci, suggesting that the A. muciniphila system initiates the CRISPR defensive mechanism frequently and can be modified using CRISPR-Cas9 (95). An automated pipeline named CRISPR discovery has since been developed for the identification of CRISPR repeats and Cas genes in genome assemblies, to determine the type and subtype and to describe system completeness (135). With this knowledge, it is hypothesized that an endogenous CRISPR-Cas9 system can be developed for A. muciniphila, allowing it to avoid the host's immune system.

4. Conclusion

As illustrated in Fig. 1, A. muciniphila is a potential probiotic that binds to enterocytes for colonization, which can regulate the host's metabolism and immune response. It has been revealed to be a promising therapeutic for the treatment of obesity, type 2 diabetes, atherosclerosis, autism-related gastrointestinal disturbances and other types of disease (Fig. 1). Due to an increased understanding of how its genetics relate to its pathogenicity, as well as the...
techniques required for effective culturing and preservation, *A. muciniphila* is expected to find use as one of numerous engineered bacteria. The present review described the potential of *A. muciniphila* as an engineered bacterium for the modulation of metabolic pathways and the production of desired proteins of therapeutic value, with high yields (using promoters, enhancers and terminators), and introduced several mature recombination systems that could be used for its genetic modification. Based on the deployment of other strains used in the aforementioned procedures, it was suggested that ingested *A. muciniphila* may be programmed to interact with signals secreted within its environment and respond to information. Thus, it could be applied to treat metabolic imbalances, pathological conditions in tissues and to assist postoperative recovery. Apart from its use as a diagnostic tool, it is feasible that *A. muciniphila* may also be designed for site-specific delivery of therapeutic compounds based on genetic circuit modulation. In addition, given that *A. muciniphila* has the capability to inhibit regulatory pathways that control immune responses, it may also be remodeled for application in vaccinations. Takei et al (136) successfully modified *B. longum* to express full-length antibodies against chronic hepatitis C virus infections in a murine model, demonstrating the ability to engaging the immune system using engineered commensal microbes.

However, despite its encouraging prospects, further studies of engineered *A. muciniphila* are still required. Currently, engineered bacteria are more frequently applied in animal or preclinical models, thus further clinical trials are required to check their efficacy and risk ratio. These clinical trials should include the following: i) Several control groups using the same dosage of normal bacteria for comparison; ii) randomization and double blinding for causality; and iii) statistical analyses for significance (137). However, Lawenius et al (138) discovered that pasteurized *A. muciniphila* did not protect against ovariectomy-induced bone loss. Thus, *A. muciniphila* treatment may not be as good as initially expected.

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TC conceived the idea for the review and designed its framework. YZ conducted the research and wrote the manuscript. Both authors edited the manuscript. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**References**

1. WHO and UNICEF: Health in the post-2015 development agenda report of the Global Thematic Consultation on Health. World We Want 31: 514-526, 2014.
2. Dora C, Haines A, Balbus J, Fletcher E, Adair-Rohani H, Alabaster G, Hossain R, de Onis M, Branca F and Neira M: Indicators linking health and sustainability in the post-2015 development agenda. Lancet 385: 380-391, 2015.
3. Novelli G, Biancolella M, Latini A, Spallone A, Borgiani P and Papaluca M: Precision medicine in non-communicable diseases. High-Throughput 9: 3, 2020.
4. Imoka T, Nishimura M, Dino K, Takabatake M, Moriyama H, Nishimura Y, Morioka T, Shimada Y and Kakinuma S: Risk of second cancer after ion beam radiotherapy: Insights from animal carcinogenesis studies. Int J Radiat Biol 95: 1431-1440, 2019.
5. O’Malley RB and Revels JW: Imaging of abdominal postoperative complications. Radiol Clin North Am 58: 73-91, 2020.
6. Suri S, Kumar V, Kumar S, Goyal A, Tanwar B, Kaur J and Kaur J: DASH dietary pattern: A treatment for non-communicable diseases. Curr Hypertens Rev 16: 108-114, 2020.
7. Zhu J, Yang J and Luo Y: Applications of engineered intestinal bacteria in disease diagnosis and treatment. Sheng Wu Gong Xue Bao 35: 2350-2366, 2019 (In Chinese).
8. Parséus A, Sommer N, Sommer F, Caesar R, Molinaro A, Ståhlman M, Greiner TU, Perkins R and Bäckhed F: Microbiota-induced obesity requires farnesoid X receptor. Gut 66: 429-437, 2017.
9. Kostic AD, Gevers D, Siljander H, Vatanen T, Hystialainen T, Hämäläinen AM, Peet A, Tillmann V, Pöhlö P, Mattila I, et al: The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. Cell Host Microbe 17: 260-273, 2015.
10. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, et al: A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490: 55-60, 2012.
11. Zhang M, Sun K, Wu Y, Yang Y, Tso P and Wu Z: Interactions between intestinal microbiota and host immune response in inflammatory bowel disease. Front Immunol 8: 942, 2017.
12. Lu T, Chen Y, Guo Y, Sun J, Shen W, Yuan M, Zhang S, He P and Jiao X: Altered gut microbiota diversity and composition in chronic urticaria. Dis Markers 2019: 6417471, 2019.
13. Cryan JF, O’Riordan KJ, Sandhu K, Peterson V and Dinan TG: The gut microbiome in neurological disorders. Lancet Neurol 19: 179-194, 2020.
14. Jiménez-Avalos JA, Arrevalilla-Boni G, González-López L, García-Carvajal ZY and González-Avila M: Classical methods and perspectives for manipulating the human gut microbial ecosystem. Crit Rev Food Sci Nutr: Mar 2, 2020 (Epub ahead of print). doi: 10.1080/10408398.2020.1724075.
15. Szajewska H: What are the indications for using probiotics in children? Arch Dis Child 101: 398-403, 2016.
16. Chua KJ, Kwok WC, Aggarwal N, Sun T and Chang MW: Designer probiotics for the prevention and treatment of human diseases. Curr Opin Chem Biol 40: 8-16, 2017.
17. Sanders ME, Akkermans LMA, Haller D, Hammerman C, Heimbach J, Hörmannsperger G, Huys G, Levy DD, Lutgendorff F, Mack D, et al: Safety assessment of probiotics for human use. Gut Microbes 1: 164-185, 2010.
18. Khangwal I and Shukla P: Combinatory biotechnological intervention for gut microbiota. Appl Microbiol Biotechnol 103: 3615-3625, 2019.
19. Yadav M and Shukla P: Recent systems biology approaches for probiotics use in health aspects: A review. 3 Biotech 9: 448, 2019.
20. Kumar M, Yadav AK, Verma V, Singh B, Mal G, Nagpal R and Hemalatha R: Bioengineered probiotics as a new hope for health and diseases: An overview of potential and prospects. Future Microbiol 11: 585-600, 2016.
21. Yadav R, Singh PK and Shukla P: Metabolic engineering for probiotics and their genome-wide expression profiling. Curr Protein Pept Sci 19: 68-74, 2018.

22. Steffler PU, Koch W, Neirynck S, Obermeier F, Falk W, Fiers W and Remaut E: Treatment of murine colitis by Lactococcus lactis secreting interleukin-10. Science 289: 1352-1355, 2000.

23. Braat H, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JF, van Devenier SJ, Neirynck S, Peppelenbosch MP and Steffler PU: Lactococcus lactis expressing interleukin-10 in Crohn's disease. Clin Gastroenterol Hepatol 4: 754-759, 2006.

24. Kurtz CB, Millet YA, Puurunen MK, Perreault M, Charbonneau MR, Isabella VM, Kotula JW, Antipov E, Dagon Y, Denning DW, Wang Y: Engineered Lactococcus plantarum expressing interleukin-10. Microbiol Cell Fact 14: 202, 2015.

25. Cani PD and De Vos WM: Next-generation beneficial microflora: The case of Akkermansia muciniphila. Front Microbiol 8: 1765, 2017.

26. Rodríguez V, Asenjo JA and Andrews BA: Design and implementation of a high yield production system for recombinant expression of peptides. Microb Cell Fact 13: 65, 2014.

27. Saheb S, Khattar SK and Saini KS: Production of active eukaryotic proteins through bacterial expression systems: A review of the existing biotechnology strategies. Mol Cell Biochem 307: 249-264, 2008.

28. Chance RE and Frank BH: Research, development, production, and safety of biosynthetic human insulin. Diabetes Care 16 (Suppl 3): S133-S142, 1993.

29. Whelan RA, Rausch S, Günzel D, Richter JF, Hering NA, Schulzke JD, Kühl AA, Keles A, Janczyk P, Whelan RA, Rausch S, Ebner F, Günzel D, Richter JF, Hering NA, Schulzke JD, Kühl AA, Keles A, Janczyk P, et al.: Recombinant human growth hormone: Safety and efficacy of an engineered commensal bacterium in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine Parkinson disease model by producing glucagon-like peptide-1. PLoS One 11: e0150775, 2016.

30. Saltzman DA, Kasanis E, Heise CP, Hasz DE, Vigdorovich V, Kelly SM, Curtis RR III, Leonard AS and Anderson PM: Effective treatment of hypertension by recombinant Lactobacillus plantarum expressing angiotensin converting enzyme (ACE) inhibitor peptide. Microbiol Cell Fact 14: 202, 2015.

31. Fang X, Tian P, Zhao X, Jiang C and Chen T: Neuroprotective effects of an engineered commensal bacterium in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine Parkinson disease mouse model via producing glucagon-like peptide-1. J Neurochem 160: 441-452, 2019.

32. Yang G, Jiang Y, Yang W, Du F, Yao Y, Shi C and Wang C: Effective treatment of rheumatoid arthritis by Lactobacillus plantarum K-110. J Clin Nutr 85: 488-496, 2007.

33. Le Leu RK, Hu Y, Brown IL, Woodman RJ and Young JP: Synthetic intervention of Bifidobacterium lactis and resistant starch protects against colorectal cancer development in rats. Carcinogenesis 31: 246-251, 2010.

34. Bae EA, Han MJ, Song MJ and Kim DH: Purification of Rotavirus infection-inhibitory protein from Bifidobacterium breve K-110. J Microbiol Biotechnol 12: 553-556, 2002.

35. Patole SK, Rao SC, Keil AD, Nathan EA, Doherty DA and Simmer KN: Beneficial effects of Bifidobacterium breve M-16V supplementation in premature neonates-A retrospective cohort study. PLoS One 11: e0150775, 2016.

36. Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P, Matteuzzi D and Camperi M: Impact on the composition of the faecal flora by a new probiotic preparation: Preliminary data on maintenance of patients with ulcerative colitis. Aliment Pharmacol Ther 13: 1103-1108, 1999.

37. Yadav R, Kumar V, Bawea M and Shukla P: Gene editing and genetic engineering approaches for advanced probiotics: A review. Crit Rev Food Sci Nutr 58: 1735-1746, 2018.

38. Wei C, Xun AY, Wei XX, Yao J, Wang JY, Shi YY, Yang GH, Li XY, Xu ZL, Lai MG, et al.: Bifidobacteria expressing tumstatin protein for anti-tumour therapy in tumor-bearing mice. Technol Cancer Res Treat 15: 498-508, 2015.

39. Sun B: Development of a recombinant Bifidobacterium breve M-16V vaccine against prostate cancer. Cancer Immunol Immunother 57: 103-111, 2008.

40. Hu B, Kou L, Li C, Zhu LP, Fan YR, Wu ZW, Wang JJ and Xu GX: A new expression plasmid in Bifidobacterium longum as a delivery system of endostatin for cancer gene therapy. Cancer Gene Ther 14: 151-157, 2007.

41. Zhu LP, Yin Y, Xing J, Li C, Kou L, Hu B, Wu W, Wang JJ and Xu GX: Therapeutic efficacy of Bifidobacterium longum-mediated human granulocyte colony-stimulating factor and/or endostatin combined with cyclophosphamide in mice transplanted tumors. Cancer Sci 100: 1896-1900, 2009.

42. Bolhassani A and Zahedifard F: Therapeutic live vaccines as a potential anticancer strategy. Int J Cancer 131: 1733-1743, 2012.

43. Chen T, Zhao X, Ren Y, Wang Y, Tang X, Tian P, Wang H and Xin H: Triptolide modulates tumour-colonisation and anti-tumour effect of attenuated Salmonella encoding DEnase I. Appl Microbiol Biotechnol 103: 929-939, 2019.

44. Shahabi V, Reyes-Reyes M, Wallecha A, Rivera S, Paterson Y and Macplag P: Development of a Listeria monocytogenes based vaccine against prostate cancer. Cancer Immunol Immunother 57: 1301-1313, 2008.

45. Chen Y, Yang D, Li S, Gao Y, Jiang R, Deng L, Frankel FR and Sun B: Development of a Listeria monocytogenes-based vaccine against hepatocellular carcinoma. Oncogene 31: 2140-2152, 2012.

46. Chan CT, Lee JW, Cameron DE, Bashor CJ and Collins JJ: Anti-tumour effect of attenuated Salmonella encoding DNase I. Anticancer Res 29: 1352-1355, 2009.

47. Pool-Zobel B, Karlsson PC, Klinder A, O'Riordan M, O'Sullivan GC, Rafter J, Bennett M, Caderni G, Clune Y, Hughes R, et al.: effects of an engineered commensal bacterium in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine Parkinson disease model by producing glucagon-like peptide-1. PLoS One 11: e0150775, 2016.

48. Leung PP and Zou X:earable and resistant starch protects against colorectal cancer development in rats. Carcinogenesis 31: 246-251, 2010.

49. Bae EA, Han MJ, Song MJ and Kim DH: Purification of Rotavirus infection-inhibitory protein from Bifidobacterium breve K-110. J Microbiol Biotechnol 12: 553-556, 2002.

50. Patole SK, Rao SC, Keil AD, Nathan EA, Doherty DA and Simmer KN: Beneficial effects of Bifidobacterium breve M-16V supplementation in premature neonates-A retrospective cohort study. PLoS One 11: e0150775, 2016.

51. Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P, Matteuzzi D and Camperi M: Impact on the composition of the faecal flora by a new probiotic preparation: Preliminary data on maintenance of patients with ulcerative colitis. Aliment Pharmacol Ther 13: 1103-1108, 1999.

52. Yadav R, Kumar V, Bawea M and Shukla P: Gene editing and genetic engineering approaches for advanced probiotics: A review. Crit Rev Food Sci Nutr 58: 1735-1746, 2018.

53. Wei C, Xun AY, Wei XX, Yao J, Wang JY, Shi YY, Yang GH, Li XY, Xu ZL, Lai MG, et al.: Bifidobacteria expressing tumstatin protein for anti-tumour therapy in tumor-bearing mice. Technol Cancer Res Treat 15: 498-508, 2015.

54. Sun B: Development of a recombinant Bifidobacterium breve M-16V vaccine against prostate cancer. Cancer Immunol Immunother 57: 1301-1313, 2008.

55. Chen Y, Yang D, Li S, Gao Y, Jiang R, Deng L, Frankel FR and Sun B: Development of a Listeria monocytogenes-based vaccine against hepatocellular carcinoma. Oncogene 31: 2140-2152, 2012.

56. Chan CT, Lee JW, Cameron DE, Bashor CJ and Collins JJ: Anti-tumour effect of attenuated Salmonella encoding DNase I. Anticancer Res 29: 1352-1355, 2009.

57. Pool-Zobel B, Karlsson PC, Klinder A, O'Riordan M, O'Sullivan GC, Rafter J, Bennett M, Caderni G, Clune Y, Hughes R, et al.: effects of an engineered commensal bacterium in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine Parkinson disease model by producing glucagon-like peptide-1. PLoS One 11: e0150775, 2016.
62. Thibault R, Blachier F, Darcy-Verillon B, De Coppet P, Bourrelle A and Segain JP: Butyrate utilization by the colonic mucosa in inflammatory bowel diseases: A transport deficiency. Gut 50: 1684-695, 2001.

63. Puertollano E, Kolida S and Yaqoob P: Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. Curr Opin Clin Nutr Metab Care 17: 139-144, 2014.

64. Ottman N, Reuman J, Meijerink M, Piétalé T, Kainulainen V, Klievink J, Huusokinen L, Aalvink S, Skurnik M, Boeren S, et al.: Pilocic acid proteins of Akkermansia muciniphila modulate host immune responses and gut barrier function. PLoS One 12: e0173004, 2017.

65. Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, Chillioux L, Ottman N, Duparc T, Lichtenstein L, et al.: A purified membrane protein from Akkermansia muciniphila of the pasteurized bacterium improves metabolism in obese and diabetic mice. Nat Med 23: 107-113, 2017.

66. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullen EC, Biryukov S, Abbafati C, Ahora SF, et al.: Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet 384: 766-781, 2014.

67. Cani PD, Neyrinck AM, Fava F, Knauf C, Curcveloglu NG, Tuohy KM, Gibson GR and Delzenne NM: Selective increases of bifidobacteria in high-fat-diet-induced mice in nicothrom through a mechanism associated with endotoxemia. Diabetologia 50: 2374-2383, 2007.

68. GBD 2015 Obesity Collaborators; Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Ester K, Lee A, Marczak L, Mokdad AH, Mokdad-Lakey K: Health effects of overweight and obesity in 195 countries over 25 years. N Engl J Med 377: 13-27, 2017.

69. Santacruz A, Collado MC, García-Valdés L, Segura MT, Martín-Lagos JA, Anjos T, Martí-Romero M, Lopez RM, Florido J, Campoy C and Sanz Y: Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr 104: 83-92, 2010.

70. Karlsson CL, Önnervält J, Xu J, Molin G, Ahnén S and Thorngren-Jerneck K: The microbiota of the gut in preschool children with normal and excessive body weight. Obesity (Silver Spring) 20: 2257-2261, 2012.

71. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Giudì Y, Derrien M, Muccioli GG, Delzenne NM, et al.: Cross-talk between diet controls diet-induced obesity. Proc Natl Acad Sci USA 110: 9066-9071, 2013.

72. Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, Falony G, Raes J, Delzenne NM, et al.: Supplementation with Akkermansia muciniphila protects against atherosclerosis by preventing metabolic endotoxemia-induced inflammation in ApoE-/- mice. Circ Res 133: 2434-2446, 2017.

73. Campion D, Ponzo P, Alessandria C, Saracco GM and Balzola F: The role of microbiota in autism spectrum disorders. Minerva Gastroenterol Dietol 64: 333-350, 2018.

74. Wang L, Christiansen CT, Sorich MJ, Gerber JP, Angley MT and Conlon MA: Low relative abundances of the mucolytic bacterium Akkermansia muciniphila and Bifidobacterium spp. in feces of children with autism. Appl Environ Microbiol 77: 6718-6721, 2011.

75. Naito Y, Uchiyama K and Takagi T: A next-generation beneficial microbe: Akkermansia muciniphila. J Clin Biochem Nutr 63: 3-35, 2018.

76. Routy B, Le Chatelier E, Derosa L, Quong CP, Alou MT, Daillère R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, et al.: Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 359: 91-97, 2018.

77. Zheng H, Liang H, Wang Y, Miao M, Shi T, Yang F, Liu E, Yuan W, Ji ZS and Li DK: Altered gut microbiota composition associated with eczema in infants. PLoS One 11: e0166026, 2016.

78. Wu W, Lv L, Shi D, Ye J, Fang D, Guo F, Li Y, He X and Li L: Protective effect of Akkermansia muciniphila against immune-mediated liver injury in a mouse model. Front Microbiol 8: 1804, 2017.

79. Peng CW, Lindén SK, Gilshennan KS, Zentodell EG, McSweeney CS, Sly L, McQuin CA and Florin TH: Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. Am J Gastroenterol 105: 2420-2428, 2010.

80. Wang RX, Liu M, Weng SY, Li JJ, Xie C, He HL, Guan W, Yuan YS and Gao J: Immune mechanisms of Concanavalin A stimulation in a model of autoimmune hepatitis. World J Gastroenterol 18: 119-125, 2012.

81. Drell T, Larionova A, Voort T, Simm J, Julge K, Heilman K, Tillmann V, Štšepetova J and Sepp E: Differences in gut microbiota composition and immune responses and gut barrier function. PLoS One 12: e0184314, 2017.

82. van Passel MWJ, Kanti R, Zentodell EG, Pluigge CM, Derrien M, Malfatti SA, Chain PS, Woyke T, Palva A, De Vos WM and Smidt H: The genome of Akkermansia muciniphila, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. PLoS One 6: e16876, 2011.

83. Caputo A, Dubourg G, Croce O, Gupta S, Robert C, Papazian L, Rolain JM and Raoult D: Whole-genome assembly of Akkermansia muciniphila sequenced directly from human stool. Biol Direct 10: 5, 2015.

84. Feng X, Li S, Zhang J, Wu F, Li X, Wu D, Zhang M, Ou Z, Jie Z, Yan Q, et al.: Genome sequence of 39 Akkermansia muciniphila isolates reveals its population structure, genomic and functional diversity, and global distribution in mammalian gut microbiotas. BMC Genomics 18: 800, 2017.

85. Ouwerkerk JP, Aalvink S, Belzer C and De Vos WM: Preparation and preservation of viable Akkermansia muciniphila cells for therapeutic interventions. Benef Microbes 8: 163-169, 2017.

86. Ouwerkerk JP, van der Ark KCH, Davids M, Claassens NJ, Finestra TR, de Vos WM and Belzer C: Adaptation of Akkermansia muciniphila to theoxic-anoxic interface of the mucus layer. Appl Environ Microbiol 82: 6981-6993, 2016.

87. Vectors DP: Suicide gene therapy of cancer. Mol Ther 3: S98-S115, 2001.
101. Baban CK, Cronin M, O’Hanlon D, O’Sullivan GC and Tangney M: Bacteria as vectors for gene therapy of cancer. Bioeng Bugs 1: 385-394, 2010.

102. Pedrolli DB, Ribot MC, Squizano PN, de Jesus VN and Cozetto DA: Team Q&A Unesp at iGEM 2017: Engineering microbial living therapeutics: The synthetic biology toolbox. Trends Biotechnol 37: 100-115, 2019.

103. Waller MC, Bober JR, Nair NU and Beisel CL: Toward a genetic tool development pipeline for host-assOCIated bacteria. Curr Opin Microbiol 38: 156-164, 2017.

104. Riglar DT and Silver PA: Engineering bacteria for diagnostic and therapeutic applications. Nat Rev Microbiol 16: 214-225, 2018.

105. Welker DL, Hughes JE, Steele JL and Broadbent R: High efficiency electrot transformation of Lactobacillus casei. FEBS Microbiol Lett 362: 1-6, 2015.

106. Walsh M, Tangney M, O’Neill MJ, Lakin JO, Soden DM, McKenna SL, Darcy R, O’Sullivan GC and O’Driscoll CM: Evaluation of cellular uptake and gene transfer efficiency of pegylated poly-L-lysine compacted DNA: Implications for cancer gene therapy. Mol Pharm 3: 644-653, 2006.

107. Ahmad S, Casey G, Sweeney P, Tangney M and O’Sullivan GC: Optimised electroporation mediated DNA vaccination for treatment of prostate cancer. Genet Vaccines Ther 8: 1, 2010.

108. Kado CI: Historical events that spawned the field of plasmid biology. Microbiology 152: 2502-2512, 1971.

109. Specificity and the mode of action of lambda exonuclease. J Biol Chem 250: 7377-7387, 1975.

110. Murphy KC: Lambda Gam protein inhibits the helicase and chi-stimulated recombination activities of Escherichia coli RecBCD enzyme. J Bacteriol 173: 5808-5821, 1991.

111. Karu AE, Sakaki Y, Eckert M and Linn S: The gamma protein specified by bacteriophage gamma. Structure and inhibitory activity for the recBC enzyme of Escherichia coli. J Biol Chem 250: 7377-7387, 1975.

112. Murphy KC: λ recombination and recombineering. EcoSal Plus 7, 2016.

113. Conmech and Ajoka JW: Lambda Red recombine-mediated integration of the high molecular weight DNA into the Escherichia coli chromosome. Microb Cell Fact 15: 172, 2016.

114. Delitcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, Pizzada ZA, Eckert MR, Vogel J and Charpentier E: CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature 471: 602-607, 2011.

115. Bolotin A, Quinquis B, Sorokin A and Dusko Ehrlich S: Clustered regularly interspaced short palindromic repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology (Reading) 151: 2551-2561, 2005.

116. Deveau H, Barrangou R, Garneau JE, Labonté J, Freamus C, Boyaval P, Romero DA, Horvath P and Moineau S: Phage response to CRISPR-encoded resistance in Streptococcus thermophilus. J Bacteriol 190: 1390-1400, 2008.

117. Tong Y, Charusatant P, Zhang L, Weber T and Lee SY: CRISPR-Cas9 based engineering of actinomycetal genomes. ACS Synth Biol 4: 1020-1029, 2015.

118. Garneau JE, Dupuis MÉ, Villion M, Romero DA, Barrangou R, Boyaval P, Freamus C, Horvath P, Magadan AH and Moineau S: The CRISPR/Cas bacteriophage immunity system cleaves bacteriophage and plasmid DNA. Nature 468: 67-71, 2010.

119. Jiang W, Bikard D, Cox D, Zhang F and Marraffini LA: RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat Biotechnol 31: 233-239, 2013.

120. One step cloning and chromosomal integration of DNA. ACS Synth Biol 4: 1020-1029, 2015.

121. van der Els S, James JK, Kleerebezem M and Bron PA: Versatile Cas9-driven subpopulation selection toolbox for Lactococcus lactis. Appl Environ Microbiol 84: e02752-17, 2018.

122. Manghwar H, Lindsey K, Zhang X and Jin S: CRISPR/Cas system: Recent advances and future prospects for genome editing. Trends Plant Sci 24: 1102-1125, 2019.

123. Gautham R, Seibold GM, Mueller P, Weit T, Weiß T, Handrick R and Eikmanns BJ: A simple dual-inducible CRISPR interference system for multiple gene targeting in Corynebacterium glutamicum. Plasmid 103: 25-35, 2019.

124. Crawley AB, Henriksen JR and Barrangou R: CRISPRDico: An automated pipeline for the discovery and analysis of CRISPR-Cas systems. CRISPR Cas J 1: 171-181, 2018.

125. Takei S, Oamoto C, Kitagawa K, Morishita N, Katayama T, Shigemura K, Fujisawa M, Kawabata M, Hotta H and Shirakawa T: Oral administration of genetically modified Escherichia coli expressing the gamma protein of phage lambda in genetic recombination. II. Substrate specificity and the mode of action of lambda exonuclease. J Biol Chem 246: 2502-2512, 1971.