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Editorial
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Application of Novel Gene Editing Tools in Bioengineering of Probiotics

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Editorial

The Gastrointestinal (GI) tract acts as a pool for composite and assorted microbiomes that play a significant role in nurturing the homeostasis of GI microenvironment. As per report, approximately 1013-1014 bacteria from >1000 diverse species harbored the gut [1]. These commensal bacteria use the nutrients in the GI tract to generate metabolites to form host-microbiome metabolic axes in order to control nutrient absorption, energy metabolism, and diverse physiochemical processes. Simultaneously, the gut microflora contributes towards providing crucial nutrients to aid in digestion, anti-inflammation and toxin degradation, promotion of hematopoiesis, and modulation of the immune system. When observed from a molecular perspective, enzymes play the fundamental task of regulating cellular metabolism and catalyzing intricate biological operations to sustain life as we know it. The homeostatic state of metabolism hangs on several enzymatic functions which can be arrested by enzyme paucity. Later, these absent or malfunctioning enzymes result in numerous metabolic disorders and complications. Current research suggests that specific enzymes possessed by engineered probiotics and their regulatory role in the metabolic cascade could positively influence relieving metabolic disorders/complications in hosts [2,3].

Therefore, it can be speculated that bioengineering of beneficial microbes could tremendously revolutionize the course of therapeutic intervention if investigated in depth. For example, Danino et al. [4] engineered a diagnostic tool using the probiotic *Escherichia coli* Nissle 1917 for detecting liver metastasis in mice; this tool can produce a signal that can be spectrophotometrically detected in urine. In detail, they co-expressed cassettes luxCDABE (encoding luciferase) and lacZ (encoding β-galactosidase) in *E. coli*, which facilitates them to create a probiotic strain PROP-Z to generate luminescent signal and colorimetric readout. Likewise, there are other beneficial bacteria which have been genetically engineered like *Salmonella typhimurium*, *Listeria monocytogenes*, *Clostridium sporogenes*, *E. coli* BL21DE3, *Lactococcus lactis*, *Streptococcus gordonii*, *Bacillus subtilis*, *Bacteroides ovatus*, etc. that have been proven effective for diagnostic or therapeutic applications [5]. Additionally, due to their effectiveness, some of these bacteria, like *L. lactis*, *E. coli* Nissle 1917, *L. monocytogenes*, *S. typhimurium* and *Streptococcus mutans*, are already involved in clinical trials [5]. Nevertheless, the traditional genome engineering tools applied, although effective for developing engineered lactobacilli and/or bifidobacteria are non-replicative and temperature-sensitive, plasmid-mediated homologous recombination, and involve prophage recombinase assisted double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA) recombineering. Moreover, these tools encompass certain limitations like transformation efficiency dependence, unstable mutations, limited host range, and non-seamless editing [6].

With the advancement of interdisciplinary biological science, novel genome editing tools have emerged like Zinc-Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), mega nucleases, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 nucleases, and most recently, OMEGA (Obligate Mobile Element Guided Activity) [7,8]. Nucleases ZFNs are a class of restriction enzymes or protein chimeras that are artificially designed by combining zinc finger DNA binding proteins/domains with a DNA-cleavage domain. The nuclease TALEN contains TALEs proteins which bind and activate promoters, thus, altering the transcription of genes in host cells. Likewise, mega nucleases are naturally occurring endodeoxyribonucleases that contain a large recognition site of around 18 to 40 base pairs in a double-stranded DNA sequence with which it replaces, deletes or alters target sequences efficiently. Furthermore, although these restriction endonucleases are highly efficient, their utilization in bioengineering is scanty. However, CRISPR-Cas9 nucleases have recently caught the attention of researchers; in simple terms, it is

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a microbial adaptive immune system that exploits RNA-guided nucleases named Cas9 to cleave target-specific sights in DNA.

For further comprehensive investigation, other Class 2 endonucleases, particularly Cas12a (Cpf1), from *Acidaminococcus* and *Lachnospiraceae*, have also been used for genome editing, exploring the CRISPR toolbox. Progressively, CRISPR-based technologies are being exploited for genome editing of bacteria like *E. coli*, *Streptococcus pneumoniae*, and other species of *Clostridium* and *Streptomyces*, *L. lactis*, and probiotic species like *Lactobacillus* [9]. Most recently, researchers at MIT's McGovern Institute for Brain Research, the Broad Institute of MIT, and Harvard, have discovered a novel class of programmable DNA modifying systems called OMEGAs, which inherently involve jumbling small pieces of DNA throughout the bacterial genomes [7]. In detail, they showed two RNA-guided nucleases, namely, IscB protein (encoded by the family of IS200/IS605 transposons) and TnpB protein (IS200/605 transposon-encoded protein). They also demonstrated that the restriction nuclease IscB protein utilizes a single noncoding RNA for RNA-guided cleavage of double-stranded DNA and can be utilized for genome editing in human cells. Moreover, it is also evidenced that these RNA-guided nucleases are small, about 30% the size of Cas9, making them less troublesome to be delivered into the cell in comparisons to larger enzymes [7,10].

In conclusion, there lies immense potential in exploring how these cutting-edge, sophisticated RNA-guided genome editing tools can be applied for the bioengineering of probiotics. As stated above, the application of bioengineered probiotics is safe and unprecedented with a vital role in regulating the major metabolic cascades and ameliorating the severity of heterogeneous bacterial and viral infections. Therefore, more exhaustive research regarding the exploration of OMEGA for the bioengineering of probiotics must be carried out to conceptualize the diagnostic and theranostic possibilities.

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