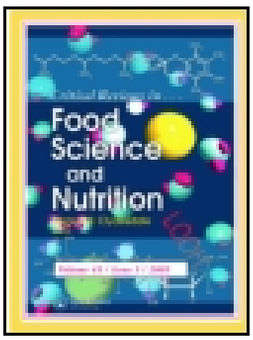

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REVIEW

Artificial intelligence and synthetic biology approaches for human gut microbiome

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ABSTRACT

The gut microbiome comprises a variety of microorganisms whose genes encode proteins to carry out crucial metabolic functions that are responsible for the majority of health-related issues in human beings. The advent of the technological revolution in artificial intelligence (AI) assisted synthetic biology (SB) approaches will play a vital role in the modulating the therapeutic and nutritive potential of probiotics. This can turn human gut as a reservoir of beneficial bacterial colonies having an immense role in immunity, digestion, brain function, and other health benefits. Hence, in the present review, we have discussed the role of several gene editing tools and approaches in synthetic biology that have equipped us with novel tools like Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas) systems to precisely engineer probiotics for diagnostic, therapeutic and nutritive value. A brief discussion over the AI techniques to understand the metagenomic data from the healthy and diseased gut microbiome is also presented. Further, the role of AI in potentially impacting the pace of developments in SB and its current challenges is also discussed. The review also describes the health benefits conferred by engineered microbes through the production of biochemicals, nutraceuticals, drugs or biotherapeutics molecules etc. Finally, the review concludes with the challenges and regulatory concerns in adopting synthetic biology engineered microbes for clinical applications. Thus, the review presents a synergistic approach of AI and SB toward human gut microbiome for better health which will provide interesting clues to researchers working in the area of rapidly evolving food and nutrition science.

KEYWORDS

Artificial intelligence; CRISPR-Cas; gut microbiome; nutraceutical; probiotics; synthetic biology

Introduction

The human gut microbiome consists of diverse groups of commensal microorganisms. Their genes encode different crucial metabolic functions that are responsible for most of the health-related issues in human beings. It is documented that more than 10^{14} microorganisms of over 2000 distinct species of bacteria, virus, and eukaryotic species colonize the gut (Thursby and Juge 2017). In this regard, a human microbiome mapping project was started in 2007 by the National Institute of Health, USA. The mapping highlighted a number of the gut microbiome that is being influenced by both environmental and genetic factors (Neish 2009; Sekirov et al. 2010) and, as a consequence, how it highly varies from a person to person (Grenham et al. 2011). The literature suggests that 2% of the variations in human microbiome is attributed to the genetic makeup while the remaining percentage is influenced by diet and life style of an individual during the course of life (Rothschild et al. 2018). Microbiome is affected intensely by the health of its host, and, in turn, also has a key role in shaping the health of its host, ranging from the metabolism pattern to the development of immune system to the stress response behavior (Arnold, Roach, and Azcarate-Peril 2016; Chu et al. 2017; Elkrief et al. 2019; Ma, Guo, et al. 2018; Huttenhower et al. 2012).This happens because gastrointestinal bacteria produce various metabolites that cross the intestinal epithelial lining and get transported throughout the body by bloodstream. These metabolites upon passing through the various organ systems affect their functioning, thereby affecting human health in various ways (Han et al. 2019; Ma and Ma 2019; Markle et al. 2014).

The diversity of the gut microbiome is highly influenced by dietary intake of plant products, animal foods, medicine and others (McDonald, et al. 2018; Wu et al. 2019). Design of the food intake on the population of gut microbiota has been discussed in a review by Ercolini D et al. (Ercolini and Fogliano 2018). They mentioned that type of food acts as a substrate for the growth of typical microbial population. This in turn leads to the production of biochemicals that might positively or negatively affect human health. For instance, Chi Ma et al. (2018) observed that the use of antibiotics on the mice model caused depletion of the

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Clostridium species, a bile metabolizing bacterium, inhabiting the gut of the mouse. This resulted in an increased bile acid content that promoted an increase in the number of circulating natural killer T (NKT) cells, thereby boosting its cellular immunity (Chi Ma et al. 2018). Studies have indicated that people on the carbohydrate diet show dominance of abdominal bacteria involved in the carbohydrate metabolism and energy production. It is also reported that carbohydrate diet affects both immune response as well as memory (Moeller et al. 2014; Spor, Koren, and Ley 2011). Similarly, changes in the gut microflora of the mouse model fed on lard-derived lipids led to an increased systemic Toll-Like Receptor (TLR) activation; impaired insulin sensitivity and white adipose tissue inflammation as compared to the ones fed on fish derived lipids (Caesar et al. 2015). Further, people on high animal protein intake are more likely to have gut microbial population that increases the levels of trimethylamine-N-oxide (TMAO) and decreases the content of short chain fatty acids (SCFAs), eventually leading to an increased risk of contracting cardiovascular diseases and inflammatory bowel disease conditions (Singh et al. 2017). Thus, food plays an important role in populating the gut with beneficial microbes for health and well-being. It is also reported that dysbacteriosis had been linked to a group of disease states, spanning from diabetes to colitis to even mood disorders due to the changes in gut microbiota (Camp et al. 2014; Markle et al. 2014; Round and Mazmanian 2009). However, the food intake is highly governed by the cultures, geographical locations of a given population, socio-economic conditions and dietary preferences (Kearney 2010). Therefore, intake of engineered microbes as probiotics for maintaining the population of beneficial microbiota, irrespective of the dietary intake, can serve as an alternate approach to conserve the diversity of human gut microbiome.

Maintaining the gut health by populating it with healthy microbiota holds the potential for improving overall human health (Yadav and Shukla 2017). The potential strategy to improve gut health depends on the knowledge about the nature of microbiota, their metabolic pathways involved, type of food substrate and the host-microbe interactions. Understanding these aspects of gut microbiome had attracted the attention of researchers in the field of microbiology, molecular biology, systems biology, synthetic biology, computational biology, biomedical engineering, food science and others. Recently, researchers have attempted to exploit the use of artificial intelligence (AI) and synthetic biology (SB) to enhance the gut microbiome and turn human gut in to a reservoir of beneficial microbes that can promote the traits of normal physiology in humans.

AI, where computers accomplish tasks usually anticipated to require human intelligence, is now being discussed in various fields including those of data science, speech recognition, or natural language processing. Such computational advances open questions on their ability to support or enhance human decision making in health and health care practices. The strength of AI-based techniques to simplify the multi-dimensional, complex metagenomic data and elucidate the peculiar signatures of beneficial microbes based on 16S ribosomal RNA gene profile in the gut microbiome has already been reported and is under significant development (Qu et al. 2019). Thus, AI tools are continuously enriching our understanding of healthy and diseased gut flora based on genomic, proteomic and metabolomic data. Moreover, AI based technologies are also recently being explored in the area of personalized nutrition. An individual's data related to parameters such as food habits, gut microbiome, health, physical activity etc. are analyzed by AI algorithms to deliver nutritional recommendations that are personalized and aimed at better health outcomes. Further, the predictive models developed on several machine learning (ML) algorithms can be used for diagnosis and treatment planning of diseases associated with gut dysbiosis (Zhou and Gallins 2019). For instance, researchers have developed ML algorithm based on multi-dimensional clinical and microbiome data of normal and diseased people to predict glucose response in individuals after their meal. Such results can be used by diabetes patients for regulating their dietary intake and planning their meal (Zeevi et al. 2015).

Similarly, SB approaches are also opening up new frontiers in microbial engineering of probiotics. Novel gene-editing approaches such as recombineering or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9) are proving to be beneficial in genome engineering. These technologies are enabling development of the therapeutic probiotics and modulating the existing metabolic machinery of commensal gut microbiota for the synthesis of metabolites (e.g., short-chain fatty acids, peptides, or quorum-sensing molecules) that can rewire metabolism and immune response in humans. Individually there are several reviews describing the role of AI in gut metagenomics (Nikolski 2016; Zhou and Gallins 2019). However, the synergistic role of AI and SB in the development of food supplements like probiotics for gut health, a key to diagnosis of gut dysbiosis and treatment, is rarely discussed. The complexity associated with SB approaches can be addressed from the strong support by current AI techniques to engineer probiotics. Although significant advancements have happened in the design, manufacturing and use of engineered probiotics for curing several diseases in animal models, a robust AI support with advanced analytics can pave way for exploration of these live engineered probiotics as therapeutic agents in human subjects. In this review, we present a synergistic approach of employing AI and SB for improved gut microbiome health as shown in Figure 1. We have discussed the role of SB in the engineering microbes to maintain a healthy gut. We have also discussed the role of AI in enriching our understanding of the gut microbiome and facilitating the applications of SB in generating a microbial community that can be taken as food supplements (probiotics) for the improvement of human health. Eventually, instances of SB engineered microbes as dietary supplement for diagnosis of gut microenvironment and acting as potential therapeutics is also discussed. Engineering of the human gut microflora will thus play a vital role in modulating the composition of the gut microbiota, ultimately enhancing the

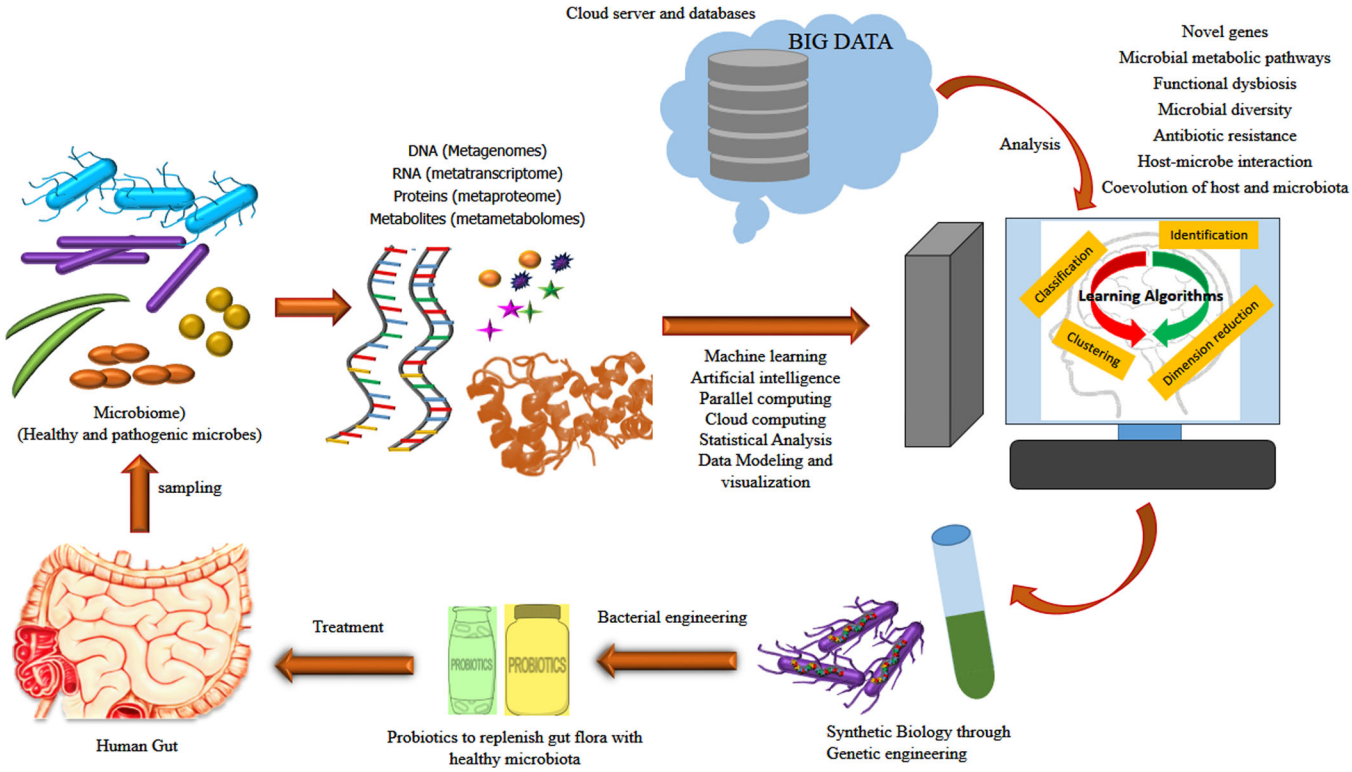

Figure 1. Schematic representation showing the interaction of AI and SB to develop probiotics for improved health of gut microflora.

ability of an individual to preserve beneficial microbial community and fight pathogenic ones.

Synthetic biology approaches for engineering microbes for improved gut health

The gut microbiome is responsible for directing a host of metabolic pathways in the human body, and therefore can be altered to positively impact human health by changing their population, composition or functional output of metabolic pathways. As such, they become potential targets of microbial engineering for improved therapeutic applications. Prebiotics, probiotics, engineered probiotics, or bacteriophages have been used to directly manipulate the gut microbiome (Lee et al. 2018). Synthetic biology is a rapidly developing field that involves the engineering of new functionalities into bacteria or attaining programmed cellular behavior by using natural and synthetic biological components (Figure 2). It merges biology and engineering approaches for design and synthesis of biological entities such as enzymes, cells, genetic circuits etc. (Gupta and Shukla 2016). Engineering of the gut microbiome through SB is evolving as a new approach toward the improvement of human health (Dou and Bennett 2018). Advances in SB toward engineering of commensal and probiotic bacteria, along with its future potential have been comprehensively discussed (Bober, Beisel, and Nair 2018). Some of the key developments in this space include CRISPR based gene editing, synthetically induced communication between cells, and development of novel genetic circuits. Table 1 shows various synthetic biology approaches and tools for modification of gut microorganisms.

CRISPR technology in synthetic biology

New gene-editing tools like CRISPR-Cas, Transcription-activator like effector nucleases (TALEN) and Zinc finger nucleases (ZFNs) have opened new avenues in synthetic biology (Dangi et al. 2018; Sander and Joung 2014). Of these, the most efficient and straightforward approach is that of CRISPR-Cas based gene editing (Kanchiswamy et al. 2016). In fact, it has been said to revolutionize gene editing because of their ease of reprogramming to target defined DNA sequences with a single guide RNA (Mali et al. 2013). CRISPR-Cas works on a system of natural defense mechanism in bacteria. One of the most popular CRISPR-Cas systems, is the Type II CRISPR-Cas system that makes use of the Cas9 endonuclease from *Streptococcus pyogenes*. Here, we discuss the use of CRISPR-Cas9 based genetic engineering for modification of gut microorganisms and bacteriophages. Several recent reviews have discussed the CRISPR-Cas based engineering of bacteria in the gut microbiome (Cañez et al. 2019; Hidalgo-Cantabrana et al. 2017; Ramachandran and Bikard 2019). *Saccharomyces boulardii,* probiotic yeast, is known for its ability to compete with gut pathogens causing diarrhea and therefore effectively prevent or treat diarrhea. The lack of auxotrophic mutants and necessary gene-editing tools has limited genetic alterations of this yeast. Liu et al. (2016) used CRISPR-Cas9 to construct marker-free auxotrophic mutants of *S. boulardii*, which would enable further metabolic engineering of the probiotic yeast (Liu et al. 2016). Following this, the human lysozyme gene (cHLY) containing a chicken lysozyme signal sequence was constructed and introduced into the *S. boulardii* genome by CRISPR-Cas9 technology for expression of

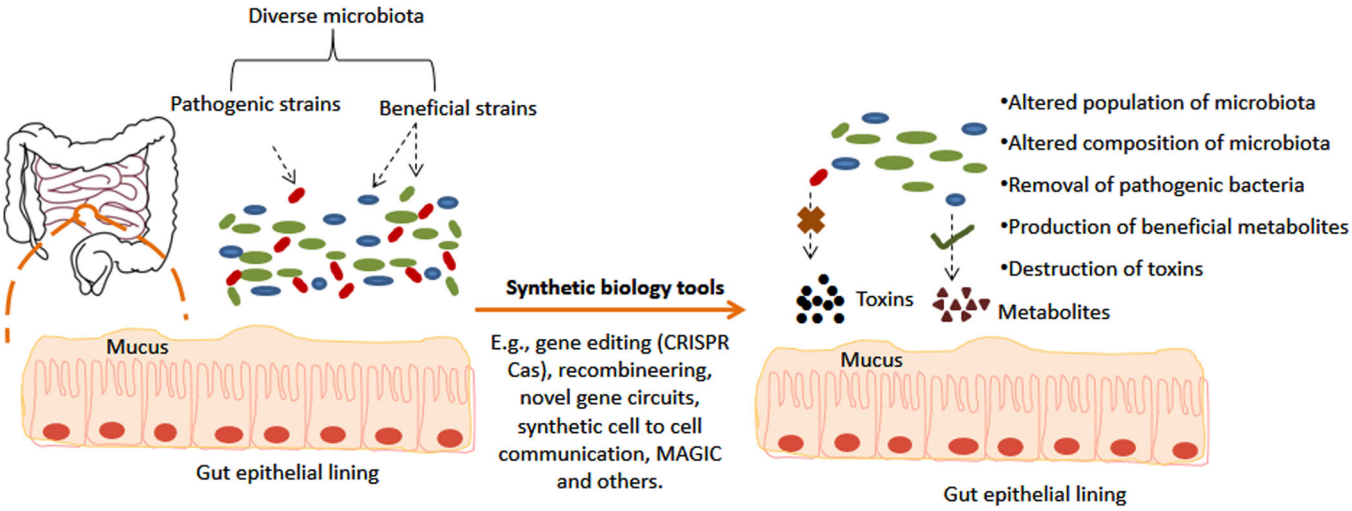

Figure 2. Schematic application of synthetic biology tools in gut microbes engineering.

lysozyme. By preferentially hydrolyzing 1,4 glycosidic linkages between the *N*-acetylmuramic acid and *N*-acetylglucosamine groups of the cell wall in Gram-positive bacteria, lysozymes can not only provide for host defense, but also help in developing a healthy gut environment, particularly in case of infants. It was found that the engineered *S. boulardii* was able to secrete human lysozyme in comparison to the wild type, thereby demonstrating its potential to continuously provide lysozyme in the gut and also to become a better probiotic for human use.

Not just yeast, the role of CRISPR-Cas9 systems has also been reported for bacteria, mostly for those belonging to *Lactobacillus* sp. CRISPR-Cas9 genome editing has also been applied in combination with single-stranded DNA recombineering (SSDR) in lactic acid bacteria *L. reuteri* 6475 (Oh and van Pijkeren 2014). The combination of recombineering and gene editing allowed fine base changes in the chromosome and identification of mutations that occur at low frequencies with 100% efficiency. In the process, Cas9 was directed to the wild-type sequence of the genome, thereby destroying those bacterial cells which have not been edited by recombineering, and hence eliminating the need for mutant screening. While SSDR has been used for genome editing in several *Lactobacillus* sp., the combination of SSDR with CRISPR–Cas9 further widens the scope of fine-tuned genome editing in gut microbiota for an improved health. In another interesting study, wild-type Cas9 was replaced with $Cas9^{D10A}$ (nickase) resulting in a CRISPR-$Cas9^{D10A}$nickase-based plasmid, pLCNICK, for genome engineering of *Lactobacillus casei,* an important microorganism in the food industry (Song et al. 2017). This was performed to address the issue of *L. casei* inability to repair the double stranded break (DSB) induced by the wild type Cas9/sgRNA complex. The plasmid pLCNICK showed an increased the genome engineering efficiency. In-frame deletions of four independent genes and heterologous expression of enhanced green fluorescence protein (eGFP) was achieved using this pLCNICK system.

In addition to CRISPR-Cas based systems, CRISPR interference (CRISPRi) has also been used to manipulate cellular metabolic processes in the commensal microorganism. In this case, a catalytically inactive version of Cas9, referred to as dCas9, is used to regulate gene expression at the target DNA sequences by blocking transcription of RNA polymerase. The use of CRISPRi in gut bacterium *Bacteroides thetaiotaomicron* achieved a regulated knockdown of recombinant and endogenous gene expression that alters its metabolic capacity and resistance to antimicrobial peptides (AMPs)(Mimee et al. 2015). An isopropyl b-D-1-thiogalactopyranoside (IPTG)-dependent implementation of CRISPRi was used to bring about suppression of BT1854 gene expression (required for resistance to AMPs like polymyxin B) and BT1754 gene (required for regulation of growth on fructose as a sole source of carbon). Induction of $dCas9_{BT1854}$ (CRISPRi targeted against BT1854) led to sensitization of the *B. thetaiotaomicron* cells to polymyxin B treatment while wild-type and nonspecific control cells containing BT1854 gene showed high polymyxin B resistance in the presence or absence of dCas9 induction with IPTG. Similarly, induction of $dCas9_{BT1754}$ drastically decreased cell growth in minimal media-fructose, while wild type and control cells showed similar growth of *B. thetaiotaomicron* in minimal media-glucose. Authors suggest that with such kind of CRISPRi intervention, *B. thetaiotaomicron* could be a useful platform for understanding host-microbe interactions in the human gut.

Others tools and techniques in synthetic biology for engineering microbes

Several other and novel SB approaches have also been explored for their potential in aiding the improvement of gut microbiota, and eventually the overall gut health. A new approach that could be employed for in situ engineering of the mammalian microbiota is the Metagenomic Alteration of the Gut microbiome by In situ Conjugation (MAGIC) (Ronda et al. 2019). Here, the genetic modification of gut microbiota is carried out in their natural environment by engineering the mobile genetic elements in the gut microbiome. This approach enables infiltration of "genetic payloads" into a native microbiome, and further allows the

Table 1. Some key synthetic biology approaches for modification of gut microorganisms.

Synthetic biology approach	Mode of action	Application or potential in modification of gut microorganism [Reference]
CRISPR-Cas9	A gene editing system that involves a Cas9 endonuclease and a guiding RNA (sgRNA). Guided by RNA, the Cas9 endonuclease breaks DNA at a specified target sequence	Development of marker-free auxotrophic mutants of *S. boulardii*; integration of lysozyme gene with chicken secretion signal. Engineered strain *S. boulardii* was able to secrete human lysozyme (Liu et al. 2016)
CRISPR-Cas9 and ssDNA Recombineering	Recombineering is an in-vivo genetic engineering technique facilitated by phage-derived recombination proteins such as Red system, RecET. It allows construction of DNA molecules with precise junctions without relying on restriction enzymes. Merging ssDNA recombineering with CRISPR/Cas9 can offer a number of genomic edits	Targeted codon saturation mutagenesis in *L. reuteri* chromosome; elimination of unedited cells by introduction of CRISPR-Cas plasmid into ssDNA-recombineered *L. reuteri* ATCC PTA 6475(Oh and van Pijkeren 2014)
CRISPR-Cas9^{D10A} nickase based genome editing plasmid (pLCNICK)	Wild-type Cas9 replaced with Cas9^{D10A} (nickase) to address the issue of the poor ability of bacteria under consideration to repair the double stranded break induced by the wild type Cas9/sgRNA complex	Chromosomal engineering of *Lactobacillus casei* carried out by deletion (in-frame) of 4 independent genes and insertion of the enhanced green fluorescent protein expression cassette into the chromosome (Song et al. 2017)
CRISPR interference	RNA based genetic modification technique that allows for sequence-specific and targeted repression of gene expression in prokaryotic and eukaryotic cells. Cas9-sgRNA complex binds to DNA elements complementary to the sgRNA and causes a steric block that halts transcript elongation by RNA polymerase, resulting in gene repression	Regulated knockdown of recombinant and endogenous gene expression in *Bacteroides thetaiotaomicron* that alters metabolic capacity and resistance to AMPs; inducible CRISPRi and recombinase systems also shown to function in bacteria colonizing mice gut (Mimee et al. 2015)
Metagenomic Alteration of the Gut microbiome by In situ Conjugation	Involves (a) Construction of an *E. coli* donor that delivers a genetic payload into target recipients using the IncPα-family-RP4 conjugation system (b) Development of mobile plasmids or pGT vectors (replicative or integrative) including the RP4 transfer origin, a selectable marker and required genetic payload. The vectors can be transferred from the donor strain to the recipient strain in the microbiome Transconjugant bacteria are identified based on the expression of green fluorescent protein gene and an antibiotic-resistance gene engineered into the vectors	Potential strategy to manipulate the horizontal gene pool and facilitate implementation of novel genetic circuits in variety of microbial communities
Bacteriophages	Genetic engineering of phages to secrete enzymes or deliver genes that can adversely affect the survival of harmful bacteria in the gut. CRISPR-Cas engineered of bacteriophage can be used to target or remove a specific or population of pathogenic bacteria	Emergent strategies for modulating gut microbiota
Manipulation of quorum sensing	Manipulating the microbiome using engineered bacteria which can in turn manipulate cell communication processes within the gut	*E. coli* Nissle 1917 was engineered to detect the pathogen-associated quorum-signal molecule excreted by *P. aeruginosa* through the quorum-sensing pathway. The engineered probiotic also exerted prophylactic and therapeutic effects in vivo, against *P. aeruginosa* during gut infection in *Caenorhabditis elegans* and mice (Hwang et al. 2017)
Use of conjugative plasmids (midbiotics)	Introduction of modified and mobilizable CRISPR-Cas9 editing components into conjugative plasmids. This plasmid system (midbiotic) is delivered from bacterial vector into target bacteria via conjugation	A Midbiotic system was developed using a conjugative IncP plasmid RP4 and a mobilizable pCas9 plasmid containing *Streptococcus pyogenes*–derived CRISPR/Cas9 that targets conserved sites in two different beta-lactamase genes via plasmid-encoded CRISPR RNA (crRNA). The conjugative plasmid in association with a mobilizable antibiotic resistance gene targeting CRISPR-plasmid caused ESBL-positive transconjugants to lose their resistance. Can be explored for beneficial changes in the gut flora (Ruotsalainen et al. 2019)

isolation of strains which are genetically modifiable, from their diverse communities. An *E. coli* donor strain is constructed which can deliver a genetic payload into target recipients using the IncPα-family-RP4 conjugation system. This conjugation system can efficiently conjugate into Gram-positive and Gram-negative cells, and deliver plasmids

into the both type of cells. The approach also involves the development mobile plasmids or pGT vectors (replicative or integrative) including the RP4 transfer origin, a selectable marker and required genetic payload. Replicative vectors contained the origin of replication (oriR), while integrative vectors contained a transposable Himar cassette and transposase (Tnase). Through MAGIC, the replicative or integrative pGT vectors can be transferred from the donor strain to the recipient strain microbiome. Transconjugant bacteria can be detected based on the expression of green fluorescent protein gene and an antibiotic-resistance gene which have been engineered into the vectors. Unlike the CRISPR-Cas9 system, MAGIC relies on bacteria's natural ability to facilitate DNA exchange. The advantage of MAGIC is that it allows playing around with bacterial community that is already adapted to the mammalian gut environment. This approach, therefore, can be employed as a potential strategy to manipulate the horizontal gene pool and facilitate implementation of novel genetic circuits in the variety of microbial communities.

The role of bacteriophages in manipulation of gut microbiome through in-situ microbiome engineering is also gaining attention. Genetic engineering approaches have been used to engineer phages to secrete enzymes or deliver genes that can adversely affect the survival of harmful bacteria in the gut (Voorhees et al. 2020). Phages have also been designed to transduce plasmids into pathogenic bacteria, so as to enable a non-lytic bacterial death, and reduce adverse effects caused by the release of harmful endotoxins upon bacterial lysis which happens as a result of lytic phage therapy (Krom et al. 2015). CRISPR-Cas systems can be used to modify the genomes of bacteriophages as well, which dominates the viral component of the gut microbiome. A bacteriophage engineered using CRISPR-Cas can be used to target or selectively remove a specific pathogenic bacteria or a population of pathogenic bacteria, thereby re-adjusting the balance of beneficial bacteria in the gut (Lee et al. 2018). (Ramachandran and Bikard (2019) have discussed that such "subtractive therapies" or elimination of target can be achieved either through engineering of lytic bacteriophages, or through the delivery of CRISPR-Cas systems themselves as antimicrobials [35]. A recently published review has highlighted phagotherapy (i.e., phages linked with the CRISPR/Cas9 system) as one of the most promising emergent strategies for modulating gut microbiome (Jiménez-Avalos et al. 2020). Phages designed by molecular biology could serve as promising options for treatment of dysbiosis. However, despite promising future applications, phage therapy-CRISPR Cas9 combination systems will, need greater research and experimentation before ascertaining their efficiency in human subjects.

Naturally prevalent quorum sensing in bacteria involves a process of communication between cells that enables bacteria to respond to the changes in the surrounding through modifications in their behavior. Manipulation of quorum sensing processes in natural microbiome using SB could have positive health outcomes (Duan and March 2010; Thompson et al. 2015). Synthetic biology strategies for manipulating quorum sensing processes in microbial consortia, has been recently reviewed (Stephens and Bentley 2020). Recently, an information transfer system was engineered to probe whether the acyl-homoserine lactone (acyl-HSL)-mediated Gram-negative bacterial quorum sensing, can be repurposed into the mammalian gut. The information transfer system demonstrated the potential for artificial communication among gut consortia in the murine gut (Kim et al. 2018). The study provides an understanding of inter-bacterial interactions and the potential of inter-cellular signaling molecules of the gut consortia. In another study, a probiotic strain *E. coli* Nissle 1917 was engineered to detect the pathogen-associated quorum-signal molecule excreted by *P. aeruginosa* through the quorum-sensing pathway (Hwang et al. 2017). The engineered probiotic also exerted prophylactic and therapeutic effects in vivo, against *P. aeruginosa* during gut infection in *Caenorhabditis elegans* and mice. In an interesting finding, the concept of midbiotics has been introduced (Ruotsalainen et al. 2019). Midbiotics are conjugative plasmids that are used for genome engineering of natural gut microbiota. These plasmids are circular genetic elements which are antagonistic and can bring about their transfer from one bacterium to another. Midbiotics, therefore, represent an alternative form of biotic substances which can be used to acquire beneficial changes in the gut microflora.

Functional metagenomic mining using SB is said to expand the potential for new biological discoveries such as molecules that modulate the gut microbiota (van der Helm, Genee, and Sommer 2018). This evolving interface between SB and functional metagenomics could therefore be exploited for the synthesis of molecules that modulate the gut microbiome-human host system, which would otherwise be likely missed by sequence homology-based searches. Moreover, SB-functional metagenomics approach is also evolving as a new area with potential of novel biological interventions and can be explored (van der Helm, Genee, and Sommer 2018). Pathway engineering or gene circuit engineering has been explored when genome scale mutation is required. Chromosomal manipulation in lactic acid bacteria has been done by using the pathway engineering vehicle for lactic acid bacteria (PEVLAB) system (Mays and Nair 2018; Yadav and Shukla 2020). One essential feature of the PEVLAB system is its copy number controllability, which enables efficient engineering of both small DNA fragments, through ssDNA-based recombineering, and large building blocks, using the RED/ET-based recombineering (Kong, Kapuganti, and Lu 2016). The PEVLAB system has shown high-copy numbers in *E. coli* but single-copy availability in *L. lactis*.

From the above, it becomes apparent that engineering of the gut microbiome forms an essential avenue of research. Novel SB approaches are only recently being explored for their potential in microbiome manipulation. This, on one hand, will be important for generating a better understanding of host-microbiome interactions in the gut, determination of complex structure-function relationships among gut microflora or even deciphering artificial communication among gut consortia. On the other, it can help modify

bacterial populations, composition, or metabolic activities thereby reflecting changes in the overall human health. For example, SB can be employed to remove a population of bacteria that aids pathogenic bacterial growth, thereby reducing chances of infection caused by the bacteria and at the same time fine-tuning and restoring the balance of beneficial bacteria in the gut microbiome. At the same time, there is scope for addition of modified species of bacteria as well. Manipulation of gut microbiome can also lead to the microbiome itself producing beneficial drugs that have therapeutic potential. Strains can also be engineered for efficiency-altering pathways within the gut that may lead to increased production of secondary compounds such as vitamins. The re-sensitization of resistant bacteria to antibiotics or their immunization to determinants of antibiotic resistance is also another area that could be explored. The success of SB depends upon the availability of genetic engineering tools. Currently, this toolbox is restricted to the few host-associated species and model laboratory strains. With growing advances in genetic tools, doors for new and emerging applications for engineered gut microbiome will be opened. However, the challenges and potential risks associated with SB also need to be taken into consideration. Some such limitations linked to manipulation of the gut microbiome, such as difficulty in introducing external DNA into the cellular machinery or limited understanding of the complex interactions between microbe and host immune system has been discussed recently (Ramachandran and Bikard 2019).

Computational tools can increase the efficiency of existing SB workflow, which so far has primarily dependent on manual efforts, laboratory based outcomes and domain area expertise in handling large volumes of data. In addition, computational tools can enhance the ongoing genomic research through an accelerated data analysis. AI-based approaches have been explored for their application is healthcare (Vashistha, Chhabra, and Shukla 2018). Along similar lines, the right integration of SB and AI can have a positive impact on the manipulation of the gut microbial community for improved overall health.

Role of AI in gut microbiome and health

In a diseased condition like dysbiosis, there is perturbation in the equilibrium of pathogenic microbial population and beneficial resident microbes. This affects the balanced dynamism between the native gut flora and human intestinal cells, thereby leading to diseases like inflammatory bowel diseases (IBD) irritable bowel syndrome (IBS), cholera and others (Wei-Lin Wang 2015). Thus, understanding the nature of gut microbiome in healthy and diseased individuals to predict the health status of a given gut is quite clinically relevant. In the absence of culturing methodologies for the several of human gut microbial species, the whole genome sequencing techniques like gene amplicon/marker sequencing and shotgun sequencing have dispensed a large volume of genomic data from an entire gut population (Bharti and Grimm 2019). These data have been populated in the databases like Ribosomal Database Project (Cole et al. 2014), Greengenes (DeSantis et al. 2006), Silva (Quast et al. 2013) and many more. Further, curation of the data from databanks can facilitate tracking of the compositional changes in the microbial community of gut flora under different health and diseased conditions. While deciphering the species variation in gut microbiota might be possible but it is challenging to figure out the strain variations among species responsible for several diseases. The genomic markers like 16 s ribosomal RNA of bacteria, and internal transcribed spacer regions of fungi are the universally accepted markers to characterize the microbial community from the metagenomic data. Based on these markers, approaches like marker gene analysis, whole metagenome analysis, functional profile, taxonomic classifications, and transcriptome analysis is being performed to understand the microbes that are actively transcribing, to differentiate between active live bacteria and dormant/dead bacteria and the relative abundance of microbial functional genes (Bharti and Grimm 2019; Knight et al. 2018). However, there are numerous computational challenges owing to the complexity of biological data, incompleteness of the metadata information, lack of standard data format and scarcity of computational tools and resources to handle these large volumes of data (Fricke and Rasko 2014; Treangen and Salzberg 2011). Although traditional bioinformatics approaches have been used for multi-dimensional genome data analysis, above computational challenges can be taken care of by newer tools and techniques like AI/ML for an accelerated analysis of such big data of genome, proteome, transcriptome, metabolome and others (Qu et al. 2019)

AI/ML for understanding multi-dimensional gut microbiome data

The advancements in AI and ML have proven to provide better tools for storage and management, identification, categorization, pattern recognition, optimization, and others in such a broad set of multi-dimensional data. There are some most common methods of ML for metagenomics, metabolomics and metaproteomics studies, which might play a crucial role in understanding gut microbiota, is represented in the Figure 3. These include understanding diverse nature of human microbiome, discovery of novel genes, novel metabolic markers and metabolic pathways in commensal microbes, investigating microbial activity kinetics, functional dysbiosis, bacterial population, correlation studies, metagenomic taxonomy and functional assignment, multi-omics studies, resistome analysis and others. Before moving onto any analysis of multi-featured microbiome data, they need to be classified through robust models based on criteria like filter methods, embedded methods and wrapper methods (Knights, Costello, and Knight 2011).

The genomic sequences similarity is used for binning and organized into operational taxonomic units (OTUs). However, major challenge arises when 16s RNA genes of number of bacterial species have only one or two variable regions. This results in biases in diversity metrics and poses challenges while comparing results obtained with amplicons

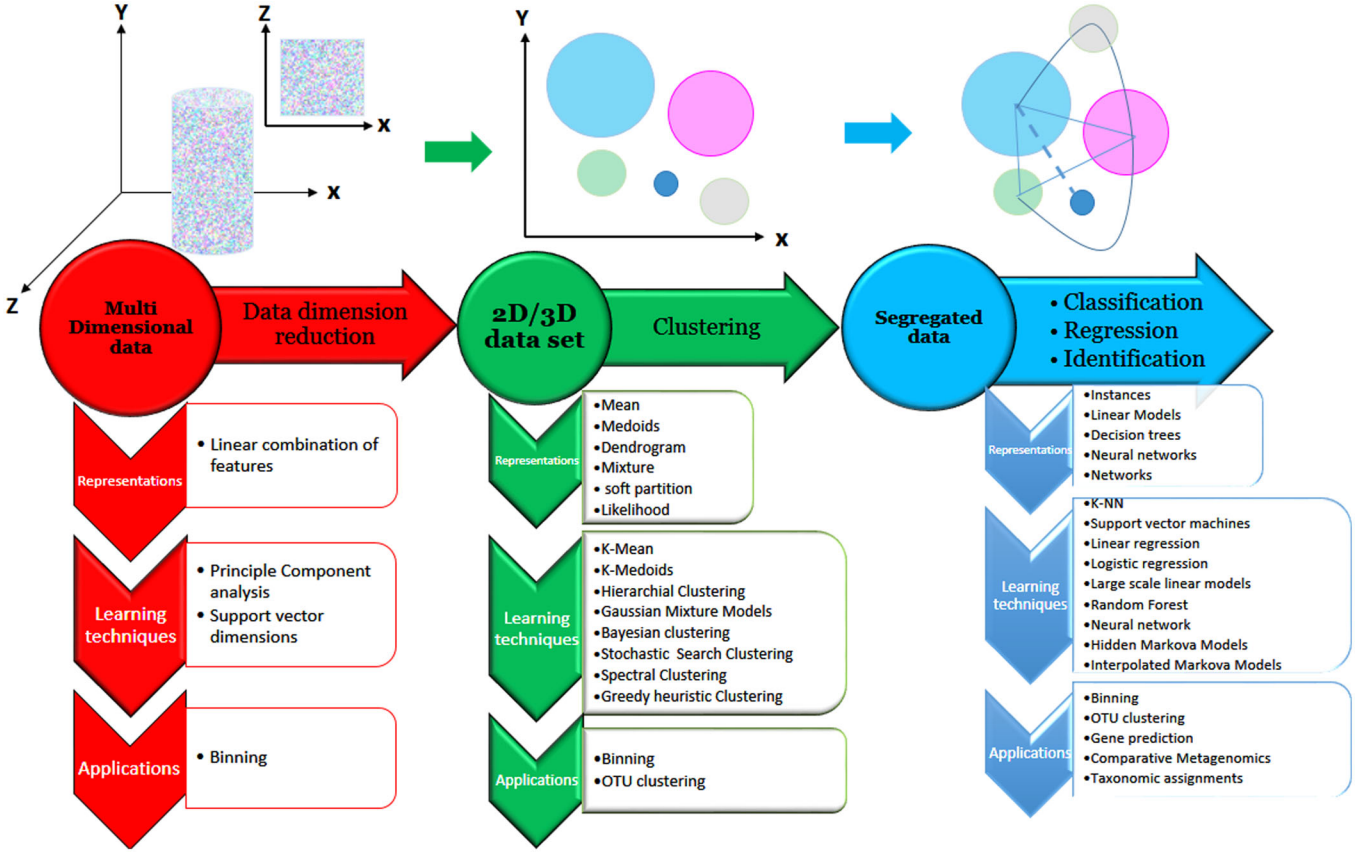

Figure 3. Machine learning approaches to understand metagenomics.

derived with different primer sets. The method therefore fails to address the classification of bacterial species through their metagenome sequence. Thus, it might be advisable to consider the conserved regions for development of OTUs (Mysara et al. 2017). Further, this also results in inconsistency in microbiome classification results reported across various labs. The genomic data obtained from Next generation Sequencing (NGS) might be incomplete due to inherent limitation of the adopted sample preparation and sequencing methods (Thomas, Gilbert, and Meyer 2012). Therefore, while developing an algorithm for clustering, it is left to the judicious decision of a researcher to opt for the tools from an armor of ML that models the data in the best possible way. It has been observed that missing data set leads to the problem of over-fitting if the choice happens to be random forest or neural network for modeling in a large data set. However, provided the data set is small, naïve Bayesian approach is a better approach but its limitation is that it does not allow complex decision function generation (Nikolski 2016). The comparison of 18 ML methods for useful classification was carried out by (Alexander Statnikov 2013) and they suggested that random forest, support vector machine, kernel ridge regression and Bayesian logistic regression with Laplace prior are the most useful classification algorithms (Alexander Statnikov 2013). After clustering, it is imperative to determine the genes of the resident microbes responsible for gut dysbiosis. One of the best tools for gene prediction is the Hidden Markov Model (HMM). However, the considering the fragmented genome of microbiome with several missing data during NGS, the accuracy of HMM has been highly compromised (<70%) (Trimble et al. 2012). Extracting the information of genes through GC content and di-codon frequency used logistic regression model to predict gene in a metagenomic data (Noguchi, Park, and Takagi 2006). Further, the modified HMM model by incorporating explicit modeling of the sequencing error have yielded much better results in gene prediction (Yoon 2009). The identification of microbiome features through genetic programming, linear logistics, random forest, genetic and evolutionary feature selection has been implemented with reasonable success to determine the factors for bacterial vaginosis (Beck and Foster 2014).

Understanding the functional genomics to track the metabolic pathways that might be responsible for the production of metabolites in gut will help in genome engineering through systems biology. ML can be employed in this to map genomics, proteomics or metabolomics data in multilayered network theory in order to study their interactions. Researchers have employed the support vector machine recursive feature elimination (SVM-RFE) approach of ML to extract the essential features of genome scale metabolic network present in *E. coli* and label reaction-gene combination based on experimental data set. They have used flux coupling analysis (FCA) as one of the 64 features that describes each reaction–gene combination to overcome limitations associated with metabolic network flux distribution analysis dependence on the environment (Nandi, Subramanian, and Sarkar 2017). The application of logistic regression

prediction algorithm on 300 biomarker genes from the microbiome of 806 Chinese patients helped in understanding the correlation between the biomarker genes and the clinically observed phenotypes (Wu et al. 2018). ML has also been useful in correlating the blood-borne infection complications associated with antibiotic abuse in immune compromised cancer patients through analysis of their dynamic microbiota (Espinoza 2018).

DeepARG: based ML techniques have been utilized for predicting antibiotic resistance in 30 patients. The study suggested that the prediction was having high precision and recall (>90%) (Arango-Argoty et al. 2018). The genome resolved metagenomics coupled with ML was used to investigate the processes of enrichment of antibiotic genes in the microbiota of formula-fed infants and their population-level perturbation in response to different antibiotics (Rahman et al. 2018). The relative abundance of orthologous proteins in the gut microbiome from (Kyoto Encyclopedia of Genes and Genomes) KEGG database in healthy and IBD patients revealed the metabolome pattern of the microbial colony. Owing to the complexity and volume of the data, ML and natural language processing tools were implemented to decipher patterns in gene expression and associate it with their functional description, respectively (Yazdani et al. 2016). The predictive performance of a ML classifier can be improved by adopting ensemble learning methods. This method built a series of dynamic models from the training set data obtained from experiments. These dynamic models avoid over-fitting of data when model is used for individual predictions. Further, as the method is data driven, it does not require prior knowledge either about the dynamics of reaction or the nature of the network (Henriques et al. 2017). Such unprecedented growth of multi-dimensional data fueled through ever decreasing cost of gene sequencing has pushed the boundaries of computational techniques (AI, ML) to handle these enormous genomic data and extract valuable knowledge related to human microbiota.

AI for microbe reengineering through synthetic biology approaches

The knowledge rendered through the curation of human microbiome data have a potential to be translated into solutions through the efforts of synthetic biologists for treating gut dysbiosis. Considering the systematic approach of SB in developing engineered living systems, it requires building an optimized genetic circuit architecture to achieve dynamic gene expression, re-wired metabolic pathways to produce meaningful expression of desired metabolites, control over the population of engineered cells, performing Boolean logic functions and sensing the outside environment, automation and control in robotics for handling SB experiments (Beal, Adler, and Yaman 2016; Yaman, Adler, and Beal 2018). The mechanism of integrating the AI and SB to engineer microbes of gut is shown in the schematic (Figure 4). The design of a genetic circuit involves enriched understanding about the gene regulation mechanism in different microbes inhabiting a gut, molecular basis of host-microbes and microbe-microbe interactions. Toward this, metagenomic data of human gut microbiome was utilized to illustrate the potential of Pipeline for Operon Exploration in Metagenomes or POEM convoluted neural network (CNN) in identification of operons by Hu and Friedberg (Hu and Friedberg 2019). They used the knowledge of evolutionary conservation of co-transcribed genes in Operons to develop the above method which is based on AI algorithm named convoluted neural network. Upon identification of operons functional in healthy gut microbiome, a genetic sequence circuit can be designed in an engineered microbes that senses exogenous repressor or inducer molecules present in the gut microenvironment (Saltepe et al. 2018). Thereafter, the arrangement of other genetic elements that act as a toggle switch or logic circuit after obtaining the signal from the sensing exogenous repressor or inducer molecules and affect the genetic circuit for the production of transcription factors. The genetic circuit design can be achieved in an accelerated and robust way through efforts of ML has been discussed in detail in the review by Michael J V et al (Volk et al. 2020). These transcription factors regulate the gene expression of the designed genetic circuit, triggering metabolic/regulatory pathway to express the suitable molecules inside or outside of a cell (Chen et al. 2012; Lugagne et al. 2017). Later, this engineered microbial community can be transplanted in dysbiosis patients. Several parameters of genetic circuit design need to be considered such as the relative abundance of a promoter, repressor, initiator, gene sequence, their order of arrangements, their way of interaction (Boolean), method of gene trigger and others. As these genetic circuits need to be selected and sorted from a large database based on the above design criteria, ML and AI can assist in a robust selection of genetic elements (Randall et al. 2011).

Further, interactions among the genetic elements to produce the desired effect need to be optimized through mathematical modeling, considering all the constrained parameters. The tools of optimization is to enable proper selection of bio-elements in a genetic circuit such that the yield of the final product is maximized (Cloney 2016; Dasika and Maranas 2008). This cause and effect can be deciphered through metagenome of gut microbiota. The designing of optimal bio-element sequences and their interactions require supervised and unsupervised learning techniques of AI. In addition, AI can effectively assists in fishing out these bio-elements from a large data pool of metagenomics (Hiscock 2019). The AI and ML have already been observed to be successful in implementing multi-parametric design like protein design, drug design, and genetic circuit design (AlQuraishi 2019; Hiscock 2019). The knowledge-based approaches to design and optimize genetic regulatory network topologies from the behavioral specifications through where the motif-based technique was demonstrated by (Beal, Lu, and Weiss 2011). The aging biomarkers discovery through transcriptome data set through the efforts of the ML algorithm showed success by 80% (Mamoshina et al. 2018). Some of the current AI-based solutions and challenges in SB that require the help of AI or ML are enumerated in the Table 2.

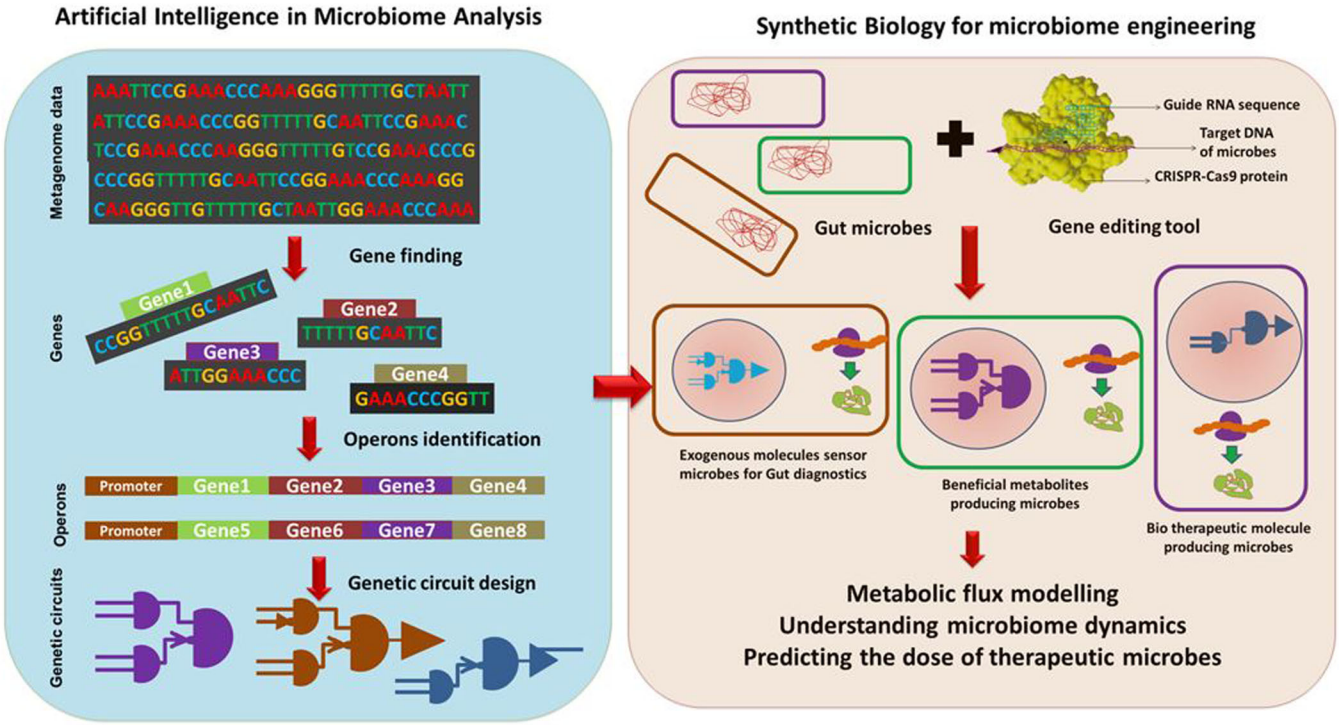

Figure 4. Schematic showing the mechanism of integration of AI for accelerated synthetic biology.

The engineered probiotics can turn human gut into a reservoir of beneficial microbes. Nevertheless, intake of probiotics cannot be promoted indiscriminately as the live microbes interaction with the host primarily depends upon the gut microenvironment of an individual. Hence, microbiome data are used in predictive algorithms based on AI techniques to examine and envisage the efficacy of existing and novel treatments against diseases by maintaining diverse microbiota of human gut through engineered probiotics. Further, the electronic health records (EHR), data related to clinical cohort studies, metagenomics data about the relative abundance of microbiota and metabolomics data can be utilized to build computational models with advance analytics that can help in potential identification of bio signatures, predict the efficacy of engineered probiotics as therapeutics and test the potential outcome of clinical studies before any foray in clinical trials. For instance, it has been demonstrated that modeling system when integrated with advance analytics and AI can assist in identifying new molecular signatures for gut diseases like *Clostridium difficile* infection (CDI) from the data obtained from EHR, clinical cohort studies and relative abundance of intestinal microbiota (Leber et al. 2017). On one hand, these molecular biomarkers facilitate the selection of therapeutics strategies and on the other, modeling assists in further predicting the clinical outcomes, evaluates dosage effects, and predicts antagonisms/synergisms of any combinational therapies. The modeling approach successfully demonstrated that pathogenic removal or direct treatment of inflammation in CDI can be avoided by simple approach of restoring immune tolerance through an LANCL2 ligand molecule or by populating the gut microbiome with healthy microbiota to outcompete the pathogenic *C. difficile* strains. This study proved the use of engineered probiotics in successful deployment of precision medicine. The typical one-size-fits-all approach of medicine can therefore be replaced by precision medicine that optimizes treatment design on a patient-by-patient basis. However, recent scientific findings on the use of computational models used in evaluating the probiotic efficacy and identifying the role of parameters like patients physiology, dosing formulations, microbe selection and therapeutic production have started emerging (Mays and Nair 2020).

Application of synthetic biology in the food and nutrition sector

The type of food intake has a significant effect on the production of biochemicals and nutraceuticals that affect human health (Sánchez et al. 2012; Santana-Gálvez, Cisneros-Zevallos, and Jacobo-Velázquez 2019). Hence, the role of dietary intake cannot be negated on defining the population of gut microbiota. Further, the role of food (texture, complexity, and composition) that acts as a prebiotic and its assimilation through gut microbiota has been reported in over-all health and well-being of an individual. It is highly recommended to have diet and food composition that facilitates the development of a healthy gut flora (Binns 2013). However, engineered microbes (probiotics) can confer variety of beneficial roles like desired biochemical production, sensing microenvironment, activating immune system against pathogenic strains, releasing drugs and others to take care of human gut.

Table 2. Current solutions and AI approaches to synthetic biology problems.

S. No	Synthetic Biology requirements	Current solutions	Current Challenges	Potential ML/ AI Approaches	Ref.
1	Development of reproducible protocols for genetic circuits design	SBOL for genetic circuit design GLAMM assists in visualization and search of novel transgenic pathways in databases Eugene allows textual specification of both parts and combinatorial assemblies of the parts BioCompiler to design gene motifs GenoCAD facilitate to specify part of DNA sequences to ensure functionality based on grammar of DNA assembly	Many biological processes are poorly understood Stochastic behavior in genetic expression and their variation with time Availability of redundant uncharacterized information in various databases Greater precision required for computation and managing complexity of data	Curate databases through ontologies, semantic networks, Statistical modeling, natural language processing methodologies	(Beal et al. 2012)
2	Optimization of genetic circuit under constraints	MatchMaker converts the Abstract Genetic Regulatory Network obtained from the BioCompiler to genetic regulatory Network having signal compatiability BioBrick assembly optimizes across multiple genetic circuit designs Combinatorial strategies in genetic circuit design	Incomplete or missing data in the databases Designing biological requirements under constraint conditions Identification of necessary and sufficient conditions during optimization Complex nature of the Objective functions used in optimization, Heuristics search algorithm require a lot of domain-specific biology expertise	Genetic algorithm, supervised, semi-supervised and Unsupervised leaning through Artificial neural network	(Naseri and Koffas 2020; Sleight et al. 2010)
3	DNA assembly planning and automated assembly	Liquid-Handling Robots operated though Puppeteer software Puppeteer Gene Assembly feature allows the genes picking from library of DNA parts (SynBioHub)	Some data collection methods destroy the cells, Different trials of the same experiment may have different results, Modeling enough to guarantee predictability, Communication rate and reaction time are slow, Need to model cell-to-cell communication for complex aggregate behavior Test and validation of differentiated cells	Mathematical and Empirical modeling, machine learning, qualitative reasoning	(Walsh et al. 2019)
4	Planning, Scheduling and execution	Puppeteer converts the genetic circuit assembly instruction to robot specific instruction Automated assembly of genetic circuit in vitro (Biomek 3000)	Reliance on existing paper lab notebooks, software, etc. Closed source lab management software, Complex scheduling including living cells that must be maintained and measured at certain times Sampling cells and pipetting small volumes, Effective human-machine collaborations, Assembly techniques that will require more automation	Genetic algorithm, quantitative reasoning	(Beal, Adler, and Yaman 2016)

Engineering probiotics as factory of biochemicals

Probiotics when taken as dietary supplement can restore the normal microflora of the human gut. With the advent of SB, the genetic information of a healthy gut can be used to create engineered bacteria, which can then be taken as dietary supplements (Tyagi et al. 2016). The engineered bacterial strains have been demonstrated to produce nutrients, enzymes, antioxidants, chemical molecules as potent drugs and they can be included in human diets as probiotics (Liu et al. 2017). The essential metabolites produced by microbes in maintaining human health are listed in Table 3. *L. plantarum* Lp91 strain that produces bile salt hydrolase can be used as a probiotic for the management and the evaluation of the anti-hypercholesterolaemic conditions. The antioxidant capabilities of *Lactobacilli* spp. of an Indian gut origin highlighted the highest antioxidative activity among *Lactobacillus* spp. S3 followed by *Lactobacillus* spp. Lp55 in inhibiting linolenic acid oxidation (Achuthan et al. 2012). The pro, anti-inflammatory cytokines produced by *L plantarum* Lp91 harbored in 2, 4, 6-trinitrobenzene sulfonic acid-induced colitis mouse model demonstrated strong immune-modulatory properties (Duary et al. 2012). Further, the broad spectrum preservatives were also demonstrated to be produced in a cost-effective manner in *Bacillus subtilis* by introduction of Nisin resistant genes nisFEG and nis I from *L. lactis* (Hansen et al. 2009). In addition, SB approaches can also modulate bacteria in dairy products like yogurt and buttermilk to render them naturally sweet by expression of Monellin, a sweetening protein. This can prevent the growth of harmful microbes that utilizes sugar, a sweetening agent as their dietary preference. The engineered bacterial protein was observed to be heat and pH stable, eventually leading their storage and transport under ambient conditions (Chen et al. 2011). Metabolic pathway restructuring using the tools of SB has already shown the possibility of the development of microbial strains capable of production of tetraterpenoids, Gamma-aminobutyric acid (GABA), Hyaluronic acid (HA), Vitamin B12, Folate, glucosamine (GlcN) and N-acetylglucosamine (GlcNAc) that has high nutritional and health benefits (Wang et al. 2016).

It has been witnessed in the past that apart from the benefits of engineered bacteria in dietary supplements, several plant-based biochemicals that serve as antioxidants or proteins can be produced through the metabolic reengineering of microbes (Yuan and Alper 2019). Few selected microbes like yeast, algae, spirulina have already been used for the production of biochemicals due to their ease of tailoring their metabolic pathway, ability to handle large genetic constructs, already established process engineering parameters, high productivity and rapid growth rate (Wang et al. 2016). Out of these, yeast has been demonstrated as a classical platform for the production of semi-synthetic artemisinin, a potential anti-malarial compound after much deliberation into cytotoxicity and hurdles of its biosynthetic pathway (Paddon et al. 2013). Similarly, a strain of yeast was developed for the production of fragrant raspberry ketone, which was originally obtained in 1-4 gm/kg of raspberries (Lee et al. 2016). Other essential food items like cannabinoids, opioids, and cocoa butter have also been demonstrated to be produced in yeast by tailoring their metabolic pathways to meet the growing demand (Goold, Wright, and Hailstones 2018).

Engineered probiotics as sensors for diagnostics, and therapeutic agents

Efforts have been driven to engineer probiotics to become an efficient system that can sense any increased level of toxin in a human gut, increased the load of pathogenic bacteria, altered micro-environment of the human gut and rectify gut microbiome through drug releasing microbes (Hwang et al. 2014). The genetic circuits of microbes inhabiting the gut, taken as probiotics, can be modulated to introduce promoter gene that gets activated after assimilation of the exogenous chemical/biomarkers molecules released due to an altered micro-environment of a human gut. This triggers initiation of transcription factors binding to the promoter region of the desired gene. Eventually, the microbe responds to the exogenous signal by expression of fluorescence proteins or any genomic signal. Thereafter, these proteins conjugate with substrates to give fluorescence signal for the diagnosis of gut health. Thus, they can act as a biosensor for gut health monitoring (Daeffler et al. 2017). It has been reported that gut transplanted with sensor microbes can suggest the well-being of gut through stool analysis through colorimetric, fluorescent, or luminescent readout (Wang et al. 2019). For instance, an EcN gut inflammation biosensor was developed by engineering *Salmonella typhimurium* and *Shewanella baltica* capable of detecting tetrathionate and thiosulfate, respectively (Daeffler et al. 2017). These microbes can be collected from human excreta and analyzed for fluorescence by flow cytometry analysis. Similarly, an engineered *E. coli* with programmable EcN platform with potential of reporting hepatic tumors in rodents has been developed (Danino et al. 2015). The EcN includes a luminescence expression genes attached to an inducible lacZ reporter. This engineered *E. coli* selectively migrates to the tumor site and gets converted into an active form by combining with the co administered luciferin-conjugate substrate. Eventually, this active form produces a compound detectable in the urine of a rodent. However, such diagnostic engineered microbes that colonize tumors have been reported only in mice, and human testing still in question. This could be a potential way of diagnosing the gut dysbiosis. However, the sensitivity of the diagnosis is questionable as when concentration of analyte in vivo is below the limits of detection for the sensor.

The therapeutic engineered microbes also respond to exogenous chemical and bioelements produced by pathogens by transcribing genes for antimicrobial peptides/compounds to destroy the pathogenic bacteria. The volume of antimicrobial peptides/compounds released in quantity such that the exogenous chemical and bioelements produced by pathogens is reduced. This acts in a feedback loop system to turn off/on these engineered microbes for accurate diagnosis and effectively responding to several disease conditions (Zhou et al. 2020). For instance, engineered probiotics have

Table 3. Metabolites produced by gut microbial species and their health implications.

S. No	Metabolites produced from their substrates	Microbial species	Role in human health	Reference
1	Short-chain fatty acids (SCFAs) from carbohydrates	*Bifidobacteria* and *Lactobacillus*	Energy homeostasis; synthesis of glucagon-like peptide 1; Increase leptin production; Lowering of pH; Enhance glucose tolerance and insulin sensitivity; Regulation of intestinal cell Proliferation; Intestinal gluconeogenesis, lipogenesis, suppression of fasting-induced adipose factor; Immunomodulatory effect, activate dendritic cells, gut immunity; increase in Mg and Calcium absorption	(Chambers et al. 2018; Dl 1996)
2	Indole derivatives from tryptophan amino acid	*E. coli* K12 variant, *Bacteroides* and *Lactobacillus* sp.	Act as a powerful antioxidant, inhibitor of amyloid-beta fibril formation, and exhibits neuro-protective and cyto-protective effects against a variety of oxidotoxins. IPA regulates intestinal barrier function, regulates intestinal permeability and mucosal integrity	(Sonowal et al. 2017)
3	Bile acid metabolites from Bile acid	*Bacteroides, Clostridium, Lactobacillus*, and *Bifidobacterium*	Activate host nuclear receptors and cell signaling pathways: regulation of bile acid, cholesterol, glucose, lipid, and energy metabolism. Exhibit antimicrobial effects	(Molinero et al. 2019)
4	Choline metabolites from animal proteins	*Anaerococcus hydrogenalis, Clostridium asparagiforme, Clostridium hathewayi, Clostridium sporogenes, Escherichia fergusonii, Proteus penneri, Providencia rettgeri, and Edwardsiella tarda*	Modulate lipid metabolism and glucose homeostasis. Contribute to nonalcoholic fatty liver disease and cardiovascular disease	(Romano et al. 2015)
5	Phenolic derivatives from aromatic amino acid like phenylalanine	*Bacteroides, Eubacterium hallii,* and *Clostridium barlettii*	Repress pathogenic microbes, influence gut microbiota composition, intestinal health maintenance Protection from oxidative stress. Estrogen-modulating effect. Platelet aggregation inhibition effect	(Rowland et al. 2018)
6	Vitamins	*Propionibacteria, Lactobacillus, Spirulina, Lactococcus, Bacillus subtilis, Candida famata*	Energy production, red blood cell formation, as an enzymatic cofactor for diverse biochemical reactions; DNA replication, repair, and methylation, regulating cell proliferation; Production of nucleotides, vitamins and amino acids; Enhance immune functioning; Sustain high proliferation rate of Intestinal epithelial cells	(Liu et al. 2017)
7	Polyamines from protein diet	*Bacteroides* spp. and *Fusobacterium* sp.	Dysregulated polyamine metabolism possibly enhances cancer development, Augment intestinal barrier integrity and function via stimulating synthesis of	(Tofalo, Cocchi, and Suzzi 2019)

(*continued*)

Table 3. Continued.

S. No	Metabolites produced from their substrates	Microbial species	Role in human health	Reference
			Intercellular junction proteins [occludin, zonula occludens-1 (ZO-1), E-cadherin]; boost maturation of intestinal and systemic adaptive immune system, Spermine inhibits pro-inflammatory M1 macrophage activation	

demonstrated that they in response to the inflammation in gut of a colitis animal model, release short hairpin RNA as a therapeutic molecule. This RNA gets successfully transfected to murine intestinal cells where they bring about RNA interference-mediated down regulation of an inflammatory response protein (Spisni et al. 2015). Further, it is possible to engineer a microbe through SB approaches that can communicate with the gut microenvironment and undertake potential measures by expression of drug able biomolecules. These biomolecules act as a potent drug against pathogenic microbes producing the toxins (Jones and Versalovic 2009). The burden of antibiotic resistance can also be lowered using such SB approaches. In this, an engineered antimicrobial called 'eligobiotics' can be programmed to eradicate pathogenic bacteria based on genetic sequence and spare the beneficial bacteria (Citorik, Mimee, and Lu 2014; Menzella et al. 2005; Yosef et al. 2015). Through SB, it can also be possible to populate the gut with microbes producing substrates essential for growth and development of therapeutic and sensory microbes.

Challenges in SB approaches to develop beneficial microbes

The clinical use of probiotics has been primarily used to treat gastrointestinal (GI) problems. There have been strong clinical evidences on the use of probiotics for the treatment of acute diarrhea and pouchitis. However, currently limited data is available that demonstrate the efficacy of probiotics in treating non-GI diseases. (Islam 2016) has discussed the composition and make of probiotics along with their recommended doses to treat GI related problems (Islam 2016). These probiotics are not regulated by Food and Drug Administration (FDA) as they are labeled as health food. With the advent of engineered microbes for use as biotherapeutics/sensing/diagnostics, limited regulations are there to guide its production and applications. Although majority of probiotics belongs to the naturally occurring microbes in human gut, emergence of SB can result in engineered microbes with specific functions. Therefore, intake of such microbes as probiotics need to pass through thorough regulatory scheme of FDA to decide upon doses, efficacy, therapeutic actions, side effects and safety before adopted as mainstream probiotics. However, *E. coli* and *Lactobacillus* sp. are the major class of engineered bacteria used a probiotics. They have provided enough hope in gut health management. Despite this, they are quite challenging to manipulate due to their anaerobic nature and compromised efficacy due to strain dependence (Barra, Danino, and Garrido 2020). The challenges of lateral gene transfer from engineered microbes to native gut microbes may pose risk of antibiotic resistance. Although autolysis through quorum sensing and incorporation of bacterial kill switches have been introduced to resolve the issue of gene transfer (Chowdhury et al. 2019). The understanding of gut microbiome through AI approaches can help to design consortium of engineered microbes having certain beneficial gene element that can synergistically co-exist and assist their survival. This will resolve the problems of single strain supplements being out competed by the native gut microbiome when taken as probiotic biotherapeutics or biosensors. Due to the lack of regulatory on the variations during manufacturing of these probiotics, doctors and patients confidence over an adoption of probiotics as treatment strategy in number of diseases remains low irrespective of the volume of literature available (de Simone 2019).

Conclusion and future direction

The human gut microbiome holds a prime position in maintaining the health and well-being of an individual. Owing to the advancements in AI techniques, handling of voluminous data created through inexpensive genome sequencing techniques has become a reality. However, the inherent challenges associated with sample preparation and genome sequencing result in incompleteness of the data set. Although AI is capable of handling these inconsistencies, the choice of AI methods to be adopted rests on the shoulders of researchers. Further, due to some or other limitations of each AI tool, the results of metagenome, metaproteome, metabolome varies across labs. Moreover, the majority of the studies on metagenomics have been time independent and systematic microbial population dynamics with time need to be studied in healthy and diseased individual for better understanding of the role of microbiome in healthy gut. Nevertheless, AI has enabled a better understanding of healthy gut microbiome in addition to the microbiota of dysbiosis patients through the curation of metadata. The AI is still evolving to accommodate the complexity and volume of metadata before it can be used as a diagnostic tool. Further, these understanding of healthy microbiota could be translated into potential therapies through the efforts of SB by the renewal of the gut of dysbiosis patients. The SB tools have the potential to create synthetic microbes, having engineered

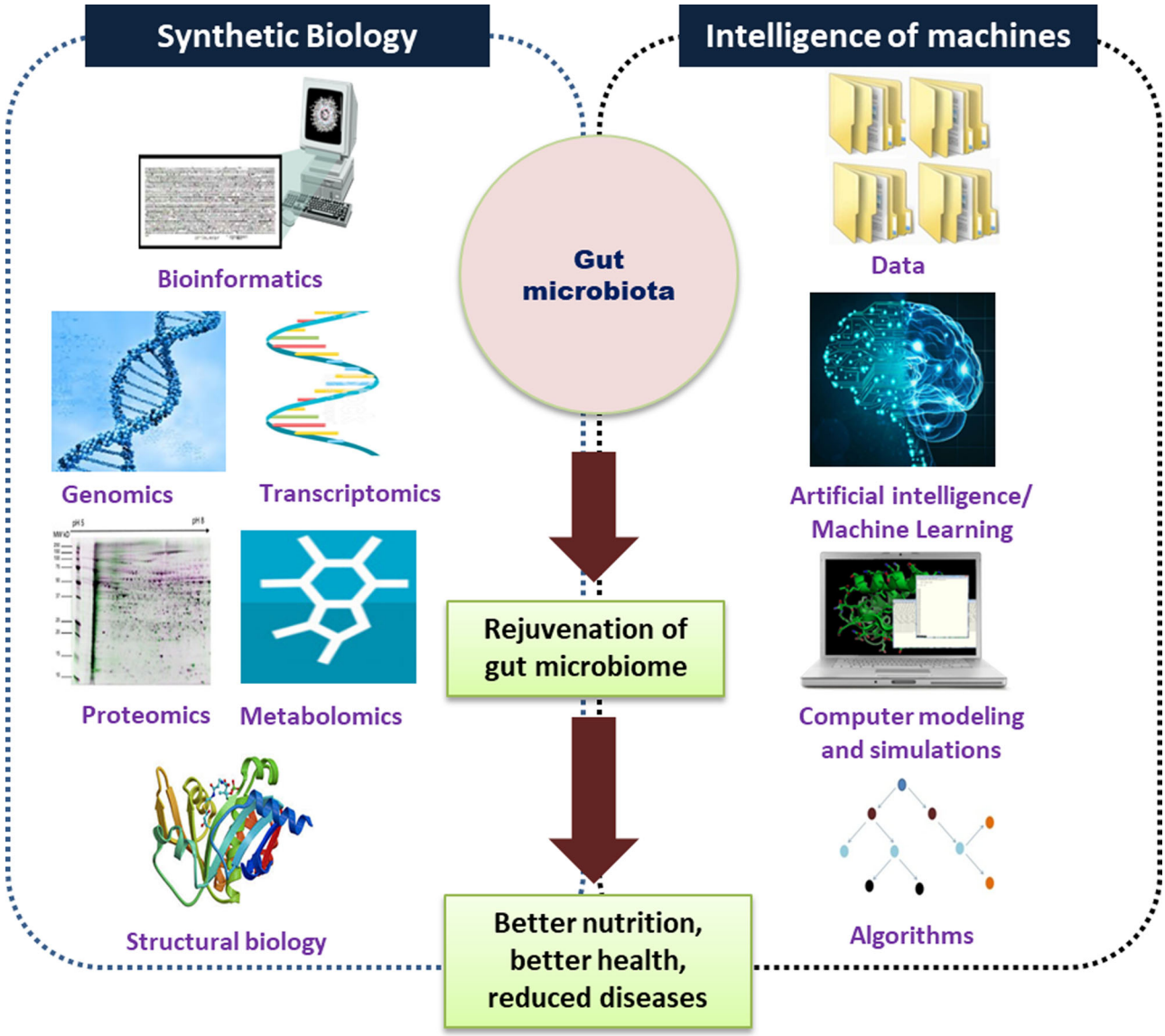

Figure 5. A snapshot of combinatory tools of Artificial Intelligence and synthetic biology for gut microbiome.

metabolic pathways to produce biochemical, neutraceuticals, medicine, and others. These engineered life forms can be taken in the form of probiotics that can exogenously provide useful prebiotics. The current SB tools are capable of engineering one type of microbe at a time. Hence, probiotics rich in particular bacterial species is today's reality. However, SB can potentially impact human health only when it can produce synthetic forms of large variety of beneficial microbes taking account of the dynamic interaction between these microbes and microbes and the host. Further, cost and scalability of SB approaches for producing engineered microbes need to be addressed. These dietary supplements, in conjunction with appropriate food type, can harbor beneficial microbes and facilitate their growth and development as an intelligent bioreactor. Although AI and SB have reached a significant position to impact the human health through gut renewal, these technologies are still evolving and should be adopted only after thorough scrutiny and approval from the concerned food regulatory authority (Suppan 2014). A snapshot of combinatory AI-SB techniques for gut microbiome is represented in the Figure 5. The safety of these tools in diagnosis and therapeutics need to be ascertained before they could be adopted as major tools in a healthcare industry. In addition, regulatory authorities across the globe are required to maintain a standard for these engineered probiotics through SB approaches to be used as biotherapeutics. Thus, AI and SB combined with food science in the future can be helpful in maintaining healthy gut flora and hence, facilitate human health and well-being.

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References

Achuthan, A. A., R. K. Duary, A. Madathil, H. Panwar, H. Kumar, V. K. Batish, and S. Grover. 2012. Antioxidative potential of

lactobacilli isolated from the gut of Indian people. *Molecular Biology Reports* 39 (8):7887–97. doi: 10.1007/s11033-012-1633-9.

AlQuraishi, M. 2019. End-to-end differentiable learning of protein structure. *Cell Systems* 8 (4):292–301.e293. doi: 10.1016/j.cels.2019.03.006.

Arango-Argoty, G., E. Garner, A. Pruden, L. S. Heath, P. Vikesland, and L. Zhang. 2018. DeepARG: A deep learning approach for predicting antibiotic resistance genes from metagenomic data. *Microbiome* 6 (1):23. doi: 10.1186/s40168-018-0401-z.

Arnold, J. W., J. Roach, and M. A. Azcarate-Peril. 2016. Emerging technologies for gut microbiome research. *Trends in Microbiology* 24 (11):887–901. doi: 10.1016/j.tim.2016.06.008.

Barra, M., T. Danino, and D. Garrido. 2020. Engineered probiotics for detection and treatment of inflammatory intestinal diseases. *Frontiers in Bioengineering and Biotechnology* 8:265–265. doi: 10.3389/fbioe.2020.00265.

Beal, J., A. Adler, and F. Yaman. 2016. Managing bioengineering complexity with AI techniques. *Bio Systems* 148:40–46. doi: 10.1016/j.biosystems.2015.08.006.

Beal, J., T. Lu, and R. Weiss. 2011. Automatic compilation from high-level biologically-oriented programming language to genetic regulatory networks. *Plos ONE* 6 (8):e22490. doi: 10.1371/journal.pone.0022490.

Beal, J., R. Weiss, D. Densmore, A. Adler, E. Appleton, J. Babb, S. Bhatia, N. Davidsohn, T. Haddock, J. Loyall, et al. 2012. An end-to-end workflow for engineering of biological networks from high-level specifications. *ACS Synthetic Biology* 1 (8):317–331. doi: 10.1021/sb300030d.

Beck, D., and J. A. Foster. 2014. Machine learning techniques accurately classify microbial communities by bacterial vaginosis characteristics. *Plos ONE* 9 (2):e87830. doi: 10.1371/journal.pone.0087830.

Bharti, R., and D. G. Grimm. 2019. Current challenges and best-practice protocols for microbiome analysis. *Briefings in Bioinformatics* 1–16. doi: 10.1093/bib/bbz155.

Binns, N. 2013. Probiotics, prebiotics and the gut microbiota. In *ILSI Europe concise monograph series*, eds. M. E. S. Glenn and R. Gibson, 40. Brussels, Belgium: ILSI Europe.

Bober, J. R., C. L. Beisel, and N. U. Nair. 2018. Synthetic biology approaches to engineer probiotics and members of the human microbiota for biomedical applications. *Annual Review of Biomedical Engineering* 20:277–300. doi: 10.1146/annurev-bioeng-062117-121019.

Caesar, R., V. Tremaroli, P. Kovatcheva-Datchary, P. D. Cani, and F. Bäckhed. 2015. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. *Cell Metabolism* 22 (4):658–668. doi: 10.1016/j.cmet.2015.07.026.

Camp, J. G., C. L. Frank, C. R. Lickwar, H. Guturu, T. Rube, A. M. Wenger, J. Chen, G. Bejerano, G. E. Crawford, and J. F. Rawls. 2014. Microbiota modulate transcription in the intestinal epithelium without remodeling the accessible chromatin landscape. *Genome Research* 24 (9):1504–1516. doi: 10.1101/gr.165845.113.

Cañez, C., K. Selle, Y. J. Goh, and R. Barrangou. 2019. Outcomes and characterization of chromosomal self-targeting by native CRISPR-Cas systems in Streptococcus thermophilus. *FEMS Microbiology Letters* 366 (9) fnz105. doi: 10.1093/femsle/fnz105.

Chambers, E. S., T. Preston, G. Frost, and D. J. Morrison. 2018. Role of gut microbiota-generated short-chain fatty acids in metabolic and cardiovascular health. *Current Nutrition Reports* 7 (4):198–206. doi: 10.1007/s13668-018-0248-8.

Chen, Z., Z. Li, N. Yu, and L. Yan. 2011. Expression and secretion of a single-chain sweet protein, monellin, in Saccharomyces cerevisiae by an α-factor signal peptide. *Biotechnology Letters* 33 (4):721–725. doi: 10.1007/s10529-010-0479-2.

Chen, S., H. Zhang, H. Shi, W. Ji, J. Feng, Y. Gong, Z. Yang, and Q. Ouyang. 2012. Automated design of genetic toggle switches with predetermined bistability. *ACS Synthetic Biology* 1 (7):284–290. doi: 10.1021/sb300027y.

Chowdhury, S., S. Castro, C. Coker, T. E. Hinchliffe, N. Arpaia, and T. Danino. 2019. Programmable bacteria induce durable tumor regression and systemic antitumor immunity. *Nature Medicine* 25 (7):1057–1063. doi: 10.1038/s41591-019-0498-z.

Chu, D. M., J. Ma, A. L. Prince, K. M. Antony, M. D. Seferovic, and K. M. Aagaard. 2017. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nature Medicine* 23 (3):314–326.

Citorik, R. J., M. Mimee, and T. K. Lu. 2014. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. *Nature Biotechnology* 32 (11):1141–1145. doi: 10.1038/nbt.3011.

Cloney, R. 2016. Synthetic biology: Automating genetic circuit design. *Nature Reviews. Genetics* 17 (6):314–315. doi: 10.1038/nrg.2016.50.

Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske, and J. M. Tiedje. 2014. Ribosomal database project: data and tools for high throughput rRNA analysis. *Nucleic Acids Research* 42 (Database issue): D633–D642. doi: 10.1093/nar/gkt1244.

Daeffler, K. N. M., J. D. Galley, R. U. Sheth, L. C. Ortiz-Velez, C. O. Bibb, N. F. Shroyer, R. A. Britton, and J. J. Tabor. 2017. Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation. *Molecular Systems Biology* 13 (4):923–923. doi: 10.15252/msb.20167416.

Dangi, A. K., R. Sinha, S. Dwivedi, S. K. Gupta, and P. Shukla. 2018. Cell line techniques and gene editing tools for antibody production: A review. *Frontiers in Pharmacology* 9:630. doi: 10.3389/fphar.2018.00630.

Danino, T., A. Prindle, G. A. Kwong, M. Skalak, H. Li, K. Allen, J. Hasty, and S. N. Bhatia. 2015. Programmable probiotics for detection of cancer in urine. Science *Translational Medicine.* 7:289ra284.

Dasika, M. S., and C. D. Maranas. 2008. OptCircuit: An optimization based method for computational design of genetic circuits. *BMC Systems Biology* 2:24. doi: 10.1186/1752-0509-2-24.

de Simone, C. 2019. The unregulated probiotic market. *Clinical Gastroenterology and Hepatology* 17 (5):809–817. doi: 10.1016/j.cgh.2018.01.018.

DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* 72 (7):5069–5072. doi: 10.1128/AEM.03006-05.

Dl, T. 1996. Short-chain fatty acids produced by intestinal bacteria. *Asia Facific Journal of Clinical Nutrition* 5:9–15.

Dou, J., and M. R. Bennett. 2018. Synthetic biology and the gut microbiome. *Biotechnology Journal* 13 (5):e1700159–e1700159. doi: 10.1002/biot.201700159.

Duan, F., and J. C. March. 2010. Engineered bacterial communication prevents Vibrio cholerae virulence in an infant mouse model. *Proceedings of the National Academy of Sciences* 107:11260.

Duary, R. K., M. A. Bhausaheb, V. K. Batish, and S. Grover. 2012. Anti-inflammatory and immunomodulatory efficacy of indigenous probiotic Lactobacillus plantarum Lp91 in colitis mouse model. *Molecular Biology Reports* 39 (4):4765–4775. doi: 10.1007/s11033-011-1269-1.

Elkrief, A., L. Derosa, L. Zitvogel, G. Kroemer, and B. Routy. 2019. The intimate relationship between gut microbiota and cancer immunotherapy. *Gut Microbes* 10 (3):424–428. doi: 10.1080/19490976.2018.1527167.

Ercolini, D., and V. Fogliano. 2018. Food design to feed the human gut microbiota. *Journal of Agricultural and Food Chemistry* 66 (15): 3754–3758. doi: 10.1021/acs.jafc.8b00456.

Espinoza, J. L. 2018. Machine learning for tackling microbiota data and infection complications in immunocompromised patients with cancer. *Journal of Internal Medicine* 284 (2):189–192.

Fricke, W. F., and D. A. Rasko. 2014. Bacterial genome sequencing in the clinic: Bioinformatic challenges and solutions. *Nature Reviews. Genetics* 15 (1):49–55. doi: 10.1038/nrg3624.

Goold, H. D., P. Wright, and D. Hailstones. 2018. Emerging opportunities for synthetic biology in agriculture. *Genes* 9 (7):341. doi: 10.3390/genes9070341.

Grenham, S., G. Clarke, J. F. Cryan, and T. G. Dinan. 2011. Brain-gut-microbe communication in health and disease. *Frontiers in Physiology* 2:94–94. doi: 10.3389/fphys.2011.00094.

Gupta, S. K., and P. Shukla. 2016. Advanced technologies for improved expression of recombinant proteins in bacteria: Perspectives and applications. *Critical Reviews in Biotechnology* 36 (6):1089–1098. doi: 10.3109/07388551.2015.1084264.

Han, X., A. Lee, S. Huang, J. Gao, J. R. Spence, and C. Owyang. 2019. Lactobacillus rhamnosus GG prevents epithelial barrier dysfunction induced by interferon-gamma and fecal supernatants from irritable bowel syndrome patients in human intestinal enteroids and colonoids. *Gut Microbes* 10 (1):59–76.

Hansen, M. E., R. Wangari, E. B. Hansen, I. Mijakovic, and P. R. Jensen. 2009. Engineering of Bacillus subtilis 168 for increased nisin resistance. *Applied and Environmental Microbiology* 75:6688.

Henriques, D., A. F. Villaverde, M. Rocha, J. Saez-Rodriguez, and J. R. Banga. 2017. Data-driven reverse engineering of signaling pathways using ensembles of dynamic models. *PLoS Computational Biology* 13 (2):e1005379. doi: 10.1371/journal.pcbi.1005379.

Hidalgo-Cantabrana, C., A. B. Crawley, B. Sanchez, and R. Barrangou. 2017. Characterization and Exploitation of CRISPR Loci in Bifidobacterium longum. *Frontiers in Microbiology* 8:1851–1851. doi: 10.3389/fmicb.2017.01851.

Hiscock, T. W. 2019. Adapting machine-learning algorithms to design gene circuits. *BMC Bioinformatics* 20 (1):214. doi: 10.1186/s12859-019-2788-3.

Hu, X., and I. Friedberg. 2019. Identifying core operons in metagenomic data. *bioRxiv*, 2019.2012.2020.885269.

Huttenhower, C., C. Gevers, D. Knight, R. Abubucker, S. Badger, J. H. Chinwalla, A. T. Creasy, H. H. Earl, A. M. FitzGerald, M. G. Fulton, et al. 2012. Structure, function and diversity of the healthy human microbiome. *Nature* 486:207.

Hwang, I. Y., E. Koh, A. Wong, J. C. March, W. E. Bentley, Y. S. Lee, and M. W. Chang. 2017. Engineered probiotic Escherichia coli can eliminate and prevent Pseudomonas aeruginosa gut infection in animal models. *Nature Communications* 8:15028–15028. doi: 10.1038/ncomms15028.

Hwang, I. Y., M. H. Tan, E. Koh, C. L. Ho, C. L. Poh, and M. W. Chang. 2014. Reprogramming microbes to be pathogen-seeking killers. *ACS Synthetic Biology* 3 (4):228–237. doi: 10.1021/sb400077j.

Islam, S. U. 2016. Clinical uses of probiotics. *Medicine* 95 (5): e2658–e2658. doi: 10.1097/MD.0000000000002658.

Jiménez-Avalos, J. A., G. Arrevillaga-Boni, L. González-López, Z. Y. García-Carvajal, and M. González-Avila. 2020. Classical methods and perspectives for manipulating the human gut microbial ecosystem. *Critical Reviews in Food Science and Nutrition* :1–25. doi:10.1080/10408398.2020.1724075. PMC: 32114770

Jones, S. E., and J. Versalovic. 2009. Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors. *BMC Microbiology* 9:35–35. doi: 10.1186/1471-2180-9-35.

Kanchiswamy, C. N., M. Maffei, M. Malnoy, R. Velasco, and J.-S. Kim. 2016. Fine-Tuning Next-Generation Genome Editing Tools. *Trends in Biotechnology* 34 (7):562–574. doi: 10.1016/j.tibtech.2016.03.007.

Kearney, J. 2010. Food consumption trends and drivers. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 365 (1554):2793–2807. doi: 10.1098/rstb.2010.0149.

Kim, S., S. J. Kerns, M. Ziesack, L. Bry, G. K. Gerber, J. C. Way, and P. A. Silver. 2018. Quorum sensing can be repurposed to promote information transfer between bacteria in the mammalian gut. *ACS Synthetic Biology* 7 (9):2270–2281. doi: 10.1021/acssynbio.8b00271.

Knight, R., A. Vrbanac, B. C. Taylor, A. Aksenov, C. Callewaert, J. Debelius, A. Gonzalez, T. Kosciolek, L.-I. McCall, D. McDonald, et al. 2018. Best practices for analysing microbiomes. *Nature Reviews. Microbiology* 16 (7):410–422. doi: 10.1038/s41579-018-0029-9.

Knights, D., E. K. Costello, and R. Knight. 2011. Supervised classification of human microbiota. *FEMS Microbiology Reviews* 35 (2): 343–359. doi: 10.1111/j.1574-6976.2010.00251.x.

Kong, W., V. S. Kapuganti, and T. Lu. 2016. A gene network engineering platform for lactic acid bacteria. *Nucleic Acids Research* 44 (4): e37–e37. doi: 10.1093/nar/gkv1093.

Krom, R. J., P. Bhargava, M. A. Lobritz, and J. J. Collins. 2015. Engineered Phagemids for Nonlytic, Targeted Antibacterial Therapies. *Nano Letters* 15 (7):4808–4813. doi: 10.1021/acs.nanolett.5b01943.

Leber, A., R. Hontecillas, V. Abedi, N. Tubau-Juni, V. Zoccoli-Rodriguez, C. Stewart, and J. Bassaganya-Riera. 2017. Modeling new immunoregulatory therapeutics as antimicrobial alternatives for treating Clostridium difficile infection. *Artificial Intelligence in Medicine* 78:1–13. doi: 10.1016/j.artmed.2017.05.003.

Lee, D., N. D. R. Lloyd, I. S. Pretorius, and A. R. Borneman. 2016. Heterologous production of raspberry ketone in the wine yeast Saccharomyces cerevisiae via pathway engineering and synthetic enzyme fusion. *Microbial Cell Factories* 15:49–49. doi: 10.1186/s12934-016-0446-2.

Lee, H. L., H. Shen, I. Y. Hwang, H. Ling, W. S. Yew, Y. S. Lee, and M. W. Chang. 2018. Targeted approaches for in situ gut microbiome manipulation. *Genes* 9 (7):351. doi: 10.3390/genes9070351.

Liu, L., N. Guan, J. Li, H-d Shin, G. Du, and J. Chen. 2017. Development of GRAS strains for nutraceutical production using systems and synthetic biology approaches: Advances and prospects. *Critical Reviews in Biotechnology* 37 (2):139–150. doi: 10.3109/07388551.2015.1121461.

Liu, J.-J., I. I. Kong, G.-C. Zhang, L. N. Jayakody, H. Kim, P.-F. Xia, S. Kwak, B. H. Sung, J.-H. Sohn, H. E. Walukiewicz, et al. 2016. Metabolic engineering of probiotic Saccharomyces boulardii. *Applied and Environmental Microbiology* 82 (8):2280–2287. doi: 10.1128/AEM.00057-16.

Lugagne, J.-B., S. Sosa Carrillo, M. Kirch, A. Köhler, G. Batt, and P. Hersen. 2017. Balancing a genetic toggle switch by real-time feedback control and periodic forcing. *Nature Communications* 8 (1): 1671 doi: 10.1038/s41467-017-01498-0.

Ma, N., P. Guo, J. Zhang, T. He, S. W. Kim, G. Zhang, and X. Ma. 2018. Nutrients mediate intestinal bacteria–mucosal immune crosstalk. *Frontiers in Immunology* 9(5). doi: 10.3389/fimmu.2018.00005.

Ma, C., M. Han, B. Heinrich, Q. Fu, Q. Zhang, M. Sandhu, D. Agdashian, M. Terabe, J. A. Berzofsky, V. Fako, et al. 2018. Gut microbiome–mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* 360 (6391):eaan5931. doi: 10.1126/science.aan5931.

Mali, P., L. Yang, K. M. Esvelt, J. Aach, M. Guell, J. E. DiCarlo, J. E. Norville, and G. M. Church. 2013. RNA-guided human genome engineering via Cas9. *Science (New York, N.Y.)* 339 (6121):823–826. doi: 10.1126/science.1232033.

Ma, N., and X. Ma. 2019. Dietary amino acids and the gut-microbiome-immune axis: Physiological metabolism and therapeutic prospects. *Comprehensive Reviews in Food Science and Food Safety* 18 (1):221–242. doi: 10.1111/1541-4337.12401.

Mamoshina, P., M. Volosnikova, I. V. Ozerov, E. Putin, E. Skibina, F. Cortese, and A. Zhavoronkov. 2018. Machine learning on human muscle transcriptomic data for biomarker discovery and tissue-specific drug target identification. *Frontiers in Genetics* 9: 242. doi: 10.3389/fgene.2018.00242.

Markle, J. G. M., D. N. Frank, K. Adeli, M. von Bergen, and J. S. Danska. 2014. Microbiome manipulation modifies sex-specific risk for autoimmunity. *Gut Microbes* 5 (4):485–493. doi: 10.4161/gmic.29795.

Mays, Z. J. S., and N. U. Nair. 2018. Synthetic biology in probiotic lactic acid bacteria: At the frontier of living therapeutics. *Current Opinion in Biotechnology* 53:224–231. doi: 10.1016/j.copbio.2018.01.028.

Mays, Z. J. S., and N. U. Nair. 2020. A quantitative model for oral administration of living therapeutics. *bioRxiv*, 2020.2004.2001.020677.

McDonald, D., E. Hyde, J. W. Debelius, J. T. Morton, A. Gonzalez, G. Ackermann, A. A. Aksenov, B. Behsaz, C. Brennan, Y. Chen, et al. 2018. American gut: An open platform for citizen science

microbiome research. *mSystems* 3 (3):e00031-00018. doi: 10.1128/mSystems.00031-18.
Menzella, H. G., R. Reid, J. R. Carney, S. S. Chandran, S. J. Reisinger, K. G. Patel, D. A. Hopwood, and D. V. Santi. 2005. Combinatorial polyketide biosynthesis by de novo design and rearrangement of modular polyketide synthase genes. *Nature Biotechnology* 23 (9): 1171–1176. doi: 10.1038/nbt1128.
Mimee, M., A. C. Tucker, C. A. Voigt, and T. K. Lu. 2015. Programming a human commensal bacterium, bacteroides thetaiotaomicron, to sense and respond to stimuli in the murine gut microbiota. *Cell Systems* 1 (1):62–71. doi: 10.1016/j.cels.2015.06.001.
Moeller, A. H., Y. Li, E. Mpoudi Ngole, S. Ahuka-Mundeke, E. V. Lonsdorf, A. E. Pusey, M. Peeters, B. H. Hahn, and H. Ochman. 2014. Rapid changes in the gut microbiome during human evolution. *Proceedings of the National Academy of Sciences* 111 (46): 16431–16435. doi: 10.1073/pnas.1419136111.
Molinero, N., L. Ruiz, B. Sánchez, A. Margolles, and S. Delgado. 2019. Intestinal bacteria interplay with bile and cholesterol metabolism: Implications on host physiology. *Frontiers in Physiology* 10: 185. doi: 10.3389/fphys.2019.00185.
Mysara, M., P. Vandamme, R. Props, F.-M. Kerckhof, N. Leys, N. Boon, J. Raes, and P. Monsieurs. 2017. Reconciliation between operational taxonomic units and species boundaries. *FEMS Microbiology Ecology* 93 (4): fix029 doi: 10.1093/femsec/fix029.
Nandi, S., A. Subramanian, and R. R. Sarkar. 2017. An integrative machine learning strategy for improved prediction of essential genes in Escherichia coli metabolism using flux-coupled features. *Molecular bioSystems* 13 (8):1584–1596. doi: 10.1039/c7mb00234c.
Naseri, G., and M. A. G. Koffas. 2020. Application of combinatorial optimization strategies in synthetic biology. *Nature Communications* 11 (1):2446. doi: 10.1038/s41467-020-16175-y.
Neish, A. S. 2009. Microbes in gastrointestinal health and disease. *Gastroenterology* 136 (1):65–80. doi: 10.1053/j.gastro.2008.10.080.
Nikolski, H. S. a M. 2016. Machine learning for metagenomics: Methods and tools. Metagenomics 1 (1): 1-19 *arXiv:1510.06621v2 [q-bio.GN]* doi: 10.1515/metgen-2016-0001
Noguchi, H., J. Park, and T. Takagi. 2006. MetaGene: Prokaryotic gene finding from environmental genome shotgun sequences. *Nucleic Acids Res* 34 (19):5623–5630. doi: 10.1093/nar/gkl723.
Oh, J.-H., and J.-P. van Pijkeren. 2014. CRISPR-Cas9-assisted recombineering in Lactobacillus reuteri . *Nucleic Acids Research* 42 (17): e131–e131. doi: 10.1093/nar/gku623.
Paddon, C. J., P. J. Westfall, D. J. Pitera, K. Benjamin, K. Fisher, D. McPhee, M. D. Leavell, A. Tai, A. Main, D. Eng, et al. 2013. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* 496 (7446):528–532. doi: 10.1038/nature12051.
Qu, K., F. Guo, X. Liu, Y. Lin, and Q. Zou. 2019. Application of machine learning in microbiology. *Frontiers in Microbiology* 10: 827. doi: 10.3389/fmicb.2019.00827.
Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* 41 (Database issue):D590–D596. doi: 10.1093/nar/gks1219.
Rahman, S. F., M. R. Olm, M. J. Morowitz, and J. F. Banfield. 2018. Machine learning leveraging genomes from metagenomes identifies influential antibiotic resistance genes in the infant gut microbiome. *mSystems* 3 (1): e00123-17. doi: 10.1128/mSystems.00123-17.
Ramachandran, G., and D. Bikard. 2019. Editing the microbiome the CRISPR way. *Philosophical Transactions of the Royal Society B: Biological Sciences* 374 (1772):20180103. doi: 10.1098/rstb.2018.0103.
Randall, A., P. Guye, S. Gupta, X. Duportet, and R. Weiss. 2011. Chapter seven - design and connection of robust genetic circuits. In *Methods in enzymology , Synthetic Biology, Part A*. Vol. 497, ed. C. Voigt, 159–86. Amsterdam: Academic Press.
Romano, K. A., E. I. Vivas, D. Amador-Noguez, and F. E. Rey. 2015. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. *mBio* 6:e02481–02414.
Ronda, C., S. P. Chen, V. Cabral, S. J. Yaung, and H. H. Wang. 2019. Metagenomic engineering of the mammalian gut microbiome in situ. *Nature Methods* 16 (2):167–170. doi: 10.1038/s41592-018-0301-y.
Rothschild, D., O. Weissbrod, E. Barkan, A. Kurilshikov, T. Korem, D. Zeevi, P. I. Costea, A. Godneva, I. N. Kalka, N. Bar, et al. 2018. Environment dominates over host genetics in shaping human gut microbiota. *Nature* 555 (7695):210–215. doi: 10.1038/nature25973.
Round, J. L., and S. K. Mazmanian. 2009. The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews. Immunology* 9 (5):313–323. doi: 10.1038/nri2515.
Rowland, I., G. Gibson, A. Heinken, K. Scott, J. Swann, I. Thiele, and K. Tuohy. 2018. Gut microbiota functions: Metabolism of nutrients and other food components. *European Journal of Nutrition* 57 (1): 1–24. doi: 10.1007/s00394-017-1445-8.
Ruotsalainen, P., R. Penttinen, S. Mattila, and M. Jalasvuori. 2019. Midbiotics: Conjugative plasmids for genetic engineering of natural gut flora. *Gut Microbes* 10 (6):643–11.
Saltepe, B., E. Ş. Kehribar, S. S. Su Yirmibeşoğlu, and U. Ö. Şafak Şeker. 2018. Cellular biosensors with engineered genetic circuits. *ACS Sensors* 3 (1):13–26. doi: 10.1021/acssensors.7b00728.
Sánchez, B., L. Ruiz, M. Gueimonde, P. Ruas-Madiedo, and A. Margolles. 2012. Toward improving technological and functional properties of probiotics in foods. *Trends in Food Science & Technology* 26:56–63.
Sander, J. D., and J. K. Joung. 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology* 32 (4): 347–355. doi: 10.1038/nbt.2842.
Santana-Gálvez, J., L. Cisneros-Zevallos, and D. A. Jacobo-Velázquez. 2019. A practical guide for designing effective nutraceutical combinations in the form of foods, beverages, and dietary supplements against chronic degenerative diseases. *Trends in Food Science & Technology* 88:179–193.
Sekirov, I., S. L. Russell, L. C. M. Antunes, and B. B. Finlay. 2010. Gut microbiota in health and disease. *Physiological Reviews* 90 (3): 859–904. doi: 10.1152/physrev.00045.2009.
Singh, R. K., H.-W. Chang, D. Yan, K. M. Lee, D. Ucmak, K. Wong, M. Abrouk, B. Farahnik, M. Nakamura, T. H. Zhu, et al. 2017. Influence of diet on the gut microbiome and implications for human health. *Journal of Translational Medicine* 15 (1):73–73. doi: 10.1186/s12967-017-1175-y.
Sleight, S. C., B. A. Bartley, J. A. Lieviant, and H. M. Sauro. 2010. In-Fusion BioBrick assembly and re-engineering. *Nucleic Acids Research* 38 (8):2624–2636. doi: 10.1093/nar/gkq179.
Song, X., H. Huang, Z. Xiong, L. Ai, and S. Yang. 2017. CRISPR-Cas9 nickase-assisted genome editing in Lactobacillus casei. *Applied and Environmental Microbiology* 83 (22):e01259–01217. doi: 10.1128/AEM.01259-17.
Sonowal, R., A. Swimm, A. Sahoo, L. Luo, Y. Matsunaga, Z. Wu, J. A. Bhingarde, E. A. Ejzak, A. Ranawade, H. Qadota, et al. 2017. Indoles from commensal bacteria extend healthspan. *Proceedings of the National Academy of Sciences* 114 (36):E7506–E7515. doi: 10.1073/pnas.1706464114.
Spisni, E., M. C. Valerii, L. De Fazio, E. Cavazza, F. Borsetti, A. Sgromo, M. Candela, M. Centanni, F. Rizello, and A. Strillacci. 2015. Cyclooxygenase-2 silencing for the treatment of colitis: A combined in vivo strategy based on RNA interference and engineered Escherichia coli. *Molecular Therapy : Therapy* 23 (2): 278–289. doi: 10.1038/mt.2014.222.
Spor, A., O. Koren, and R. Ley. 2011. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews. Microbiology* 9 (4):279–290. doi: 10.1038/nrmicro2540.
Statnikov, A., M. Henaff, V. Narendra, K. Konganti, Z. Li, L. Yang, Z. Pei, M. J. Blaser, C. F. Aliferis, and A. V. Alekseyenko. 2013. A comprehensive evaluation of multicategory classification methods for microbiomic data. *Microbiome* 1 (1):11. doi: 10.1186/2049-2618-1-11.
Stephens, K., and W. E. Bentley. 2020. Synthetic biology for manipulating quorum sensing in microbial consortia. *Trends in Microbiology* 28 (8):633–643. doi: 10.1016/j.tim.2020.03.009.

Suppan, S. 2014. *From GMO to SMO: How synthetic biology evades regulation*. Minneapolis, MN: Institute of Agriculture and Trade Policy.

Thomas, T., J. Gilbert, and F. Meyer. 2012. Metagenomics - a guide from sampling to data analysis. *Microbial Informatics and Experimentation* 2 (1):3–3. doi: 10.1186/2042-5783-2-3.

Thompson, J. A., R. A. Oliveira, A. Djukovic, C. Ubeda, and K. B. Xavier. 2015. Manipulation of the quorum sensing signal AI-2 affects the antibiotic-treated gut microbiota. *Cell Reports* 10 (11): 1861–1871. doi: 10.1016/j.celrep.2015.02.049.

Thursby, E., and N. Juge. 2017. Introduction to the human gut microbiota. *The Biochemical Journal* 474 (11):1823–1836. doi: 10.1042/BCJ20160510.

Tofalo, R., S. Cocchi, and G. Suzzi. 2019. Polyamines and gut microbiota. *Frontiers in Nutrition* 6: 16. doi: 10.3389/fnut.2019.00016.

Treangen, T. J., and S. L. Salzberg. 2011. Repetitive DNA and next-generation sequencing: Computational challenges and solutions. *Nature Reviews. Genetics* 13 (1):36–46. doi: 10.1038/nrg3117.

Trimble, W. L., K. P. Keegan, M. D'Souza, A. Wilke, J. Wilkening, J. Gilbert, and F. Meyer. 2012. Short-read reading-frame predictors are not created equal: Sequence error causes loss of signal. *BMC Bioinformatics* 13:183–183. doi: 10.1186/1471-2105-13-183.

Tyagi, A., A. Kumar, S. V. Aparna, R. H. Mallappa, S. Grover, and V. K. Batish. 2016. Synthetic biology: Applications in the food sector. *Critical Reviews in Food Science and Nutrition* 56 (11): 1777–1789. doi: 10.1080/10408398.2013.782534.

van der Helm, E., H. J. Genee, and M. O. A. Sommer. 2018. The evolving interface between synthetic biology and functional metagenomics. *Nature Chemical Biology* 14 (8):752–759. doi: 10.1038/s41589-018-0100-x.

Vashistha, R., D. Chhabra, and P. Shukla. 2018. Integrated artificial intelligence approaches for disease diagnostics. *Indian Journal of Microbiology* 58 (2):252–255. doi: 10.1007/s12088-018-0708-2.

Volk, M. J., I. Lourentzou, S. Mishra, L. T. Vo, C. Zhai, and H. Zhao. 2020. Biosystems design by machine learning. *ACS Synthetic Biology* 9 (7):1514–1533. doi: 10.1021/acssynbio.0c00129.

Voorhees, P. J., C. Cruz-Teran, J. Edelstein, and S. K. Lai. 2020. Challenges & opportunities for phage-based in situ microbiome engineering in the gut. *Journal of Controlled Release : Official Journal of the Controlled Release Society* 326:106–119. doi: 10.1016/j.jconrel.2020.06.016.

Walsh, D. I., 3rd, M. Pavan, L. Ortiz, S. Wick, J. Bobrow, N. J. Guido, S. Leinicke, D. Fu, S. Pandit, L. Qin, et al. 2019. Standardizing automated DNA assembly: Best practices, metrics, and protocols using robots. *SLAS Technology* 24 (3):282–290. doi: 10.1177/2472630318825335.

Wang, J., S. Guleria, M. A. G. Koffas, and Y. Yan. 2016. Microbial production of value-added nutraceuticals. *Current Opinion in Biotechnology* 37:97–104. doi: 10.1016/j.copbio.2015.11.003.

Wang, W., L. Lin, Y. Du, Y. Song, X. Peng, X. Chen, and C. J. Yang. 2019. Assessing the viability of transplanted gut microbiota by sequential tagging with D-amino acid-based metabolic probes. *Nature Communications* 10 (1):1317 doi: 10.1038/s41467-019-09267-x.

Wang, W.-L., S.-Y. Xu, Z.-G. Ren, L. Tao, J.-W. Jiang, and S.-S. Zheng. 2015. Application of metagenomics in the human gut microbiome. *World J Gastroenterol* 21 (3):803–814. doi: 10.3748/wjg.v21.i3.803.

Wu, H., L. Cai, D. Li, X. Wang, S. Zhao, F. Zou, and K. Zhou. 2018. Metagenomics biomarkers selected for prediction of three different diseases in Chinese population. *BioMed Research International* 2018: 2936257–2936257. doi: 10.1155/2018/2936257.

Wu, Y., N. Ma, P. Song, T. He, C. Levesque, Y. Bai, A. Zhang, and X. Ma. 2019. Grape seed proanthocyanidin affects lipid metabolism via changing gut microflora and enhancing propionate production in weaned pigs. *The Journal of Nutrition* 149 (9):1523–1532. doi: 10.1093/jn/nxz102.

Yadav, M., and P. Shukla. 2020. Efficient engineered probiotics using synthetic biology approaches: A review. *Biotechnology and Applied Biochemistry* 67 (1):22–29. doi: 10.1002/bab.1822.

Yadav, R., and P. Shukla. 2017. Probiotics for human health: current progress and applications. In *Recent advances in applied microbiology*, ed. P. Shukla, 133–47. Singapore: Springer Singapore.

Yaman, F., A. Adler, and J. Beal. 2018. AI challenges in synthetic biology engineering. In *AAAI Conference on Artificial Intelligence*, North America.

Yazdani, M., B. C. Taylor, J. W. Debelius, W. Li, R. Knight, and L. Smarr. 2016. Using machine learning to identify major shifts in human gut microbiome protein family abundance in disease. In *2016 IEEE International Conference on Big Data (Big Data)*, 1272–1280. doi: 10.1109/BigData.2016.7840731.[]

Yoon, B.-J. 2009. Hidden Markov models and their applications in biological sequence analysis. *Current Genomics* 10 (6):402–415. doi: 10.2174/138920209789177575.

Yosef, I., M. Manor, R. Kiro, and U. Qimron. 2015. Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria. *Proceedings of the National Academy of Sciences* 112 (23):7267–7272. doi: 10.1073/pnas.1500107112.

Yuan, S.-F., and H. S. Alper. 2019. Metabolic engineering of microbial cell factories for production of nutraceuticals. *Microbial Cell Factories* 18 (1):46. doi: 10.1186/s12934-019-1096-y.

Zeevi, D., T. Korem, N. Zmora, D. Israeli, D. Rothschild, A. Weinberger, O. Ben-Yacov, D. Lador, T. Avnit-Sagi, M. Lotan-Pompan, et al. 2015. Personalized nutrition by prediction of glycemic responses. *Cell* 163 (5):1079–1094. doi: 10.1016/j.cell.2015.11.001.

Zhou, Z., X. Chen, H. Sheng, X. Shen, X. Sun, Y. Yan, J. Wang, and Q. Yuan. 2020. Engineering probiotics as living diagnostics and therapeutics for improving human health. *Microbial Cell Factories* 19 (1): 56 doi: 10.1186/s12934-020-01318-z.

Zhou, Y.-H., and P. Gallins. 2019. A review and tutorial of machine learning methods for microbiome host trait prediction. *Frontiers in Genetics* 10:579–579. doi: 10.3389/fgene.2019.00579.