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Next Generation Probiotics: An Extended Arena of Probiotic Science

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Abstract

Gut microbiome has an important role for the maintenance of good individual health. Any compromised situation in the ecology of gut microflora manifest disease condition. Manipulation of these microflora with probiotic has been emerged as an treatment to prevent dysbiosis. With the advancement of the new technologies a new range of commensal microorganisms from human microbiome have been reported and treated as Next Generation Probiotics. Upon supplementation these microorganisms can restore a healthy homeostasis condition within the human gastrointestinal tract in a natural way. These nest generation probiotics were subjected to preclinical studies and shown positive response for some specific diseases.

Keywords: next generation probiotic; microbiome; dysbiosis; gastro intestinal tract; immunomodulation

Introduction

Second brain is the new terminology used to relate the gut microbiota because of its role to control an individual's health. So, it turns out that looking after the second brain may be the key to better health and improved mood by manipulating the gut microbiota composition. Several therapeutic approaches like oral administration of probiotic, prebiotic, symbiotic, fecal microbiota transplantation(FMT) have been emerged to prevent dysbiosis. But these are highly generic and non-specific. Depending on this situation, there is an urgent need to design disease specific personal medicine according to individual specific microbiome. Commensal gut bacteria could be the new cusp in this probiotic arena.

The research and development activity in the field of probiotics research expanded in the last few years due to increased knowledge of the human microbiome and their functions. This has expanded the scope of new discovery with new potential probiotic taxa (Cunningham, 2021). Rapid developments of better microbial culturing techniques, affordable genomic and metagenomic techniques for sequencing and editing bacterial genomes have allowed isolation and characterization of a new range of beneficial microorganisms from our human microbiome with potential health benefits and the opportunity to be developed as Next Generation Probiotics (O'Toole el al, 2017). Application of next generation probiotics can restore a healthy homeostasis stimulation within the gastrointestinal tract in a natural way. These candidates represent a significant proportion of the currently cultivable human gut microflora and offer physiological functions that are not always directly compared by bifidobacteria and lactobacilli such as production of butyrate, propionate and other bioactives (Blaak et al, 2020) (Table:1). Converting these species into industrially viable probiotics offers challenges as their requirements for suitable growth media and anaerobic conditions add cost and complexity as well as investment in determining optimal fermentation and manufacturing processes over time (Cunningham et al, 2021).

Traditional Probiotics	Next Generation Probiotics
• Isolated from gut, breast milk, fermented foods	• Mainly derived from commensal
• Belong to limited genera; mainly *Lactobacillus sp.* (Fermicutes), *Bifidobacteria sp* (Actinabacteria), *Sspp, Bacillus spp*.(Fermicutes), *Escherichia coli* (Protobacteria) and *Sachharomyces cerevisiae*(yeast)	• Belong to diverse genera
• Target general health of the population	• Target specific disease
• Generally Recognized As safe (GRAS) status from US FDA and Qualified Presumption of Safety (QPS) status from European Food safety Authority (EFSA)	• Strict safety and regulation yet to be evaluated
• Long history of safe use	• Identified from comparative result between healthy and disease animals/humans

Table 1: Comparison of Traditional Probiotics and Next Generation Probiotics (Lin et.al.,2019)

The United Nations Food and Agricultural Organization (FAO) define probiotic in a broad sense which permit the flexibility in terms of phylogenetic origin of probiotic organisms (Hage et al, 2017). Information generated from the studies facilitates the selection of next generation probiotics. *Clostridium* species, *Faecalibacterium prausnitzii*, *Akkermansia muciniphlia*, *Bacteroides fragillis*, *Bacteroides uniformis* (Neef and Sanz, 2013, Patel and Dupont, 2015) and *Eubacterium halli* (Udayappan et al, 2016) are getting priority due to their probiotic attributes. These next generation probiotics were evaluated preclinical studies and shown positive outcome for inflammatory and metabolic disorders (Neef and Sanz, 2013; Patel and Dupont, 2015). (Table:2)

Organism	Key features	Health benefits	Reference
Faecalibacterium prausnitzii	Gram positive, obligate anaerobe, endospore former, butyric acid producer	Controls obesity and diabetes mellitus	Duo et al.(2016)
Akkermancia muciniphila	Gram negative, obligate anaerobe, non-motile, non-spore former, oval shaped, mucin producing bacteria	Ameliorates HFD-induced obesity and type2 diabetes	Plovier et al.(2017)
Eubacterium hallii	Gram positive, obligate anaerobe, non-spore former, bacilli	Ameliorates effect of obesity and diabetes	Udayappan et al.(2016)
Clostridia(clusters IV and XIVa)	Gram positive, obligate anaerobe, endospore former, butyric acid producing bacteria	• Improves efficiency and reduce the chemotherapy induced issue in patient with lung cancer • Reduces cancer	Guo et al.(2020)
Bacteroids • *B. fragilis* • *B. uniformis*	Gram negative, obligate anaerobe, rod-shaped	Immunoregulatory function Prevent viral encephalitis in mice Antiobesity, antidiabatic immunoregulatory effect in high fat diet fed mice	Ramkrishna et al.(2019) Gauffin et al.(2012)

Table:2 Selected examples of Next Generation Probiotics

Candidates of next generation probiotics

Some potential NGP candidates are discussed here

Faecalibacterium prausnitzii

Faecalibacterium prausnitzii is a gram positive, extreme oxygen sensitive bacterium belonging to the family Ruminococcaceae (the Clostridia class and Firmicute phylum). It is the only known species of the *Faecalibacterium* genus. This organism accounts for 3-5% of the total fecal bacteria (Breyner et al, 2017). *F. prausnitzii* can ferment glucose and produce short chain fatty acids(SCFAs) such as butyrate, formic acid and D-lactate (Duncan et al, 2002). Due to the production of butyrate the intestinal homeostatis and integrity and health are maintained (Wrzosek et al, 2013). Some researchers have claimed this species as a biomarker of choice to assist in ulcerative colitis (UC) and Crohn's disease(CD)(Lopez-Siles et al. 2017).

F. prausnitzii showed invivo and invitro anti-inflammatory activities. It was confirmed that anti-inflammatory properties of mucosa-associated microbiome (NAM) and their ability to reduce TH1 and Th17 proinflammatory cytokines and Mesenteric Lymphatic Node (MLN) and colon tissues in both DNBS and DSS colitis model (Breyner et al, 2017). MAM was also able to improve TGFβ cytokine which effects NF-κB activity in DNBS model thus protecting the host and decreasing intestinal inflammation (Breyner et al, 2017).

Gopalakrishnan et al (2018) reported the role of *F. prausnitzii* in the gut and $CD8^+$ T cell infiltration within the tumor environment, in addition to the frequency of effector $CD4^+$ and T cells the periphery. An enrichment with this bacterium positively correlated with expression of inducible T cell co-stimulator (ICOS) on the surface of circulating effector $CD4^+$ T cells and negatively correlated with the numbers of regulatory T cells and levels of pro-inflammatory proteins such as IL-6, IL-8 and soluble IL-2 receptor (IL-2Rα) in the blood at baseline.

All these studies and reports suggest and highlight the importance of *F. prausnitzii* which can be considered as promising candidate as probiotic for use in therapeutic purposes.

Akkermansia muciniphila

Akkermansia is a gram negative, strictly anaerobic, non-motile, non-sporeforming, symbiotic bacterium of the mucus layer (phylam Verrucomicrobia). This organism occupies upto 5% of total intestinal microbiome and utilizes mucin as its sole carbon, nitrogen and energy source for proliferation (Cani and deVos, 2017). Researchers have discovered that *Akkermansia muciniphila* could be used to control obesity and diabetes mellitus (Dao et al, 2016; Li ert al, 2016) and hence associated with healthier metabolic status.

The mechanism of action to combat obesity and type 2 diabetes by *A. muciniphila* was identified and it was reported that an immunomodulatory protein "Amuc_1100" located in the bacterium outer membrane (Plovier et al, 2017}. The bacterium can do modulate endocannabinoid (eCB) system. This is an important regulatory system in respect of controlling obesity, type 2 diabetes and inflammation (Cani et al, 2014). Schnecberger et al, (2015) reported the effect of high fat diet on metabolic parameter and nature of gut microbiota composition over time and it was found the presence of *Akkermansia muciniphila* was associated with controlled lipid metabolism, inflammatory markers in adipose tissue, insulin resistance and plasma triglyceride.

A. muciniphila may also be used anticancer immunotherapy (Wang et al, 2018). Li et al (2016) observed that administration of *Akkermansia muciniphila* could cure the atherosclerotic lesions, impose metabolic endotoxemia-induced inflammation and ultimately restore gut barrier. In addition to the important move from "bench to beside", pasteurized *A. muciniphila* is the first next generation beneficial bacterium to receive green light from European Food Safety Authority to be used safely as a novel food in Sep 2021.

Eubacterium hallii

Eubacterium hallii belonging to the Firmicutes phylum is a Gram positive, strict anaerobic bacterium that can be found in mucin and human feces (Louis et al.2010). It is now considered as an important microorganism in respect to maintenance of intestinal metabolic balance. The organism has the ability to utilize glucose and the fermented intermediate acetate and lactate to form butyrate and hydrogen in a low pH environment (Duncan, Louis & Flint,2004)). It is also observed that *Eubacterium halii* is capable of metabolizing glycerol to 3-hydroxy propionaldehyde (3-HPA reuterin) with reported antimicrobial activity. This bacterium *has* also ability to produce cobalamin (Engels et al, 2016). All these versatile characters may enhance the host-gut-microbiota homeostasis (Engels et al, 2016).

Clostridium species

The bacteria representing genus *Clostridium* are rod-shaped, gram positive and spore forming, strict anaerobes. In the intestine of human *Clostridium* species are one of the richest bacterial cluster are mainly composed of cluster IV and XIVa. These two groups account for 10-40% of total bacteria (Nagano et al, 2012). *Clostridium* cluster IV also called *Clostridium leptum* group with 4 members, i.e. *Clostridium leptum*, *Clostridium sporosphaeroides*, *Clostridium cellulosi* and *Faecalibacterium prausnitzi*. The other one *Clostridium* cluster XIVa also known as *Clostridium coccoides* group consists of 21 species. *Clostridium* are one of the members of the early-colonizing bacteria and they can be detected in feces within the first week of birth (Guo et al, 2020).

Clostridium species are predominant cluster of our gut and exert a lot of beneficial effects on our intestinal homeostasis as commensal bacteria. As a result they are the potent candidate to alleviate dysfunction and disorders in intestine. They are reported to attenuate inflammation and allergic diseases effectively. The cellular components of *Clostridium* species and metabolites like butyrate, secondary bile acids and indolepropionic acid can play probiotic role primarily through energizing intestinal epithelial cells, strengthing intestinal barrier and interacting with immune system (Guo et al, 2020). It is reported that *C. butyricum* reduces chemotherapy induced diarrhea in patients with lung cancer, reduces the systemic inflammatory response.

Bacteroides

Bacteroides species (belonging to the phylum Bacteroidetes) are anaerobic, gram negative, bile resistant, non-spore forming, rod shaped bacteria. *Bacteroides* can be passed from mother to the child during vaginal delivery and thus become the part of human flora in the earliest stage of life. These bacteria maintain a complex and generally beneficial relationship with the host when retained in the gut (Wexler, 2007). However in some cases *Bacteroides* species escape from the gut due to rupturing of gastrointestinal tract or during intestinal surgery. These condition can cause significant pathogenesis including absceaa formation in multiple body parts (e.g. the abdomen, brain, liver, pelvis and lungs) as well as bacteremia (Wexler, 2007).

Bacteroides fragilis is a good candidate for use as probiotic. The establishment of bacterial colonization in gut modulates the immune system either by direct host-bacteria interation or molecule produced by our commensal bacteria (Hage et al, 2017).

A zwitterionic polysaccharide (ZPS) produced by *Bacteroides fragilis* can activate $CD4^+$ cells (i.e. T helper cells expressing the CD4 glycoprotein). Polysaccharide A and B (PS-A and PS-B) of B. fragilis capsular polysaccharide complex are both ZPS. It was reported that PS-A of *B. frgilis* is necessary and sufficient to mediate the generation of a normal mature immune system (Mazmanian and Kasper, 2006). It was also observed that *Bacteroides fragilis* activates Toll-like receptor (TLR) pathways and thus regulatory T-cells can boost immunogenic tolerance (Round et al, 2011). So from the scientific evidences it is evident that PS-A can be treated as a model symbiosis factor, because it preserves the balance between T cell types and maintains the immune system homeostasis (Round et al, 2011).

Bacteroides uniformis is found in feces of healthy breast fed infants and considered as emerging probiotic. This organism when administered orally to high fat fed mice, it can able to maintain improved lipid profile, reduced glucose, insulin levels, increased TNF-α production by dendritic cells (DCs) in response to LPS stimulation and increased phagocytosis (Gauffin cano et al, 2012).

Safety and Regulatory Aspects

Significant emphasis have to be placed for the investigation on the safety of the newly discovered probiotic strains for obtaining Generally Recognized As safe (GRAS) status from US FDA and Qualified Presumption of Safety (QPS) status from European Food safety Authority (EFSA). This will enable the new probiotic organism to qualify for commercialization for pharmaceutical applications (Cunningham et al, 2021). A complete characterization of strains from these new species will likely be required, comprising retrospective analysis of possible human diseases linked with the taxa considered. The full genome retrospective analysis and possible human disease linked with the taxa are to be considered for safety evaluation. The antibiotic resistance genes, toxin genes, transferable genetic elements, virulence factors, proven safety in animal models, pharmacokinetics, pharmacodynamics and phase I-III trials are also the parameters for assessment for safety regulation. These products are required to comply Good Manufacturing Practice guidelines for commercial application. It is also important that the public health officers and medical professionals continue a post market surveillance of the product as recommended by FAO/WHO.

Japan is a global market leader in probiotic business in both food and drug segments. According to Japanese regulations, probiotic products are in different category than foods and Foods for Specific Health Uses (FOSHU). Efficacy claims for probiotic products are prohibited on the labeling until the product gets the permission from the Ministry of Health and Welfare (MHLW) to be considered as FOSHU, for which efficacy and safety validation is mandatory. The Japanese government usually divide FOSCHU health claim into subcategories in which their effect could be in gastrointestinal tract, metabolism, cholesterol moderation or bone health (Baldi and Arora, 2015).

Conclusion

The probiotic industry whether it is food or pharmaceutical industry growing very fast and entirely depends on the new products being taken to the market. New probiotic organisms with their enhanced therapeutic activity posses challenges towards the scientific and regulatory definition. With the availability of new technologies, the improved understanding of gut microbiome interaction will enable the scientific community to the discovery of next generation probiotic strains. The clinical applications of these strains will widen the horizon of probiotic therapy like respiratory system, urogenital tract, skin, immune system, nervous system, oral cavity, weight management as well as cardio metabolic system. More research work is needed to demonstrate whether these new probiotic strains can be applicable to human, as safety study have only been conducted in animal.

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