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Next-Generation Probiotics and Secreted Extracellular Vesicles in Disease Amelioration

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Review Article

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ABSTRACT

Growing evidence has emphasized the close association of non-homeostasis in gut microbiota (dysbiosis) with the pathogenic development of various intestinal ailments such as Inflammatory Bowel Disease (IBD) and coeliac disease. Additionally, extra-intestinal diseases, such as the metabolic syndrome, cardiovascular disease, and neurodegenerative disease, are also concomitant with gut dysbiosis. Beneficial microbes have been often isolated from intestinal commensal bacteria that contribute to human health. In the preceding decades, most people have preferred the lactic acid bacteria belonging to the genera *Lactobacillus* and *Lactococcus* in addition to strains from *Bifidobacterium* genus as probiotic supplements, to improve the uncomfortable condition and maintain a healthy condition. Although generally considered safe, traditional probiotics have been shown to contribute subtly in sub-healthy conditions *via* undefined mechanisms. Recently, emerging Next-Generation Probiotics (NGPs), such as *Akkermansia muciniphila*, *Bacteroides fragilis*, *Faecalibacterium prausnitzii*,

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Eubacterium hallii, *Odoribacter laneus*, *Parabacteroides* species as well as *Clostridium* species have been acknowledged as prospective novel microbial therapeutics for various diseases. Particularly, certain NGPs have been also categorized as live biotherapeutic products that are expected to function as therapeutic drugs for certain disease conditions. Thus, there is a more serious demand for NGPs than for traditional probiotics. Accordingly, unlike traditional probiotics, it is essential to understand the underlying mechanism of NGPs in maintaining health. Furthermore, Extracellular Vesicles (EV) released beneficial probiotics have also been established to ameliorate diseases and related immune disorders. Hence, in this review, we aim to deliberate the characteristics of various NGP candidates and bacteria EVs that exhibit promising roles in the maintenance of health and explore their functions as defined treatment strategies.

INTRODUCTION

Traditional probiotics and Next Generation Probiotics (NGPs)

The human gut microbiota is a critical ecosystem with various microbes, including bacteria, viruses, and fungi. It is estimated that there are more than one trillion bacteria in the human intestine, which is comparable to the number of host cells [1,2]. Further research has revealed that fluctuations in the diversity, community, and stability of the gut microbiota; the so-called gut dysbiosis are associated to gastrointestinal and systemic diseases [3]. Thus, maintaining the ecosystem homeostasis of the intestinal microbiota is of critical importance, where the usage of probiotics serves as one of the frequent ways to maintain favourable balance in gut microbiota. According to the international scientific association of probiotics and prebiotics, probiotics are defined as living microorganisms that promote health upon administration in adequate amounts [3]. Lactic Acid Bacteria (LAB) are considered as traditional probiotics and are prevalent in the environment, naturally fermented products, and the gastrointestinal tract of humans and animals [4]. Most microbial strains used as traditional probiotics primarily belong to the genera *Lactobacillus* and *Bifidobacterium*. In addition, a few traditional probiotic supplements also employ other strains belonging to *Lactococcus*, *Streptococcus*, *Enterococcus*, and *Bacillus spp*., genera as well as the genus Saccharomyces [5]. The use of tradition probiotics is considered harmless for humans due to their generally regarded as safe status in the United States or qualified presumption of safety status by the european food safety authority [5]. LAB strains evidently exhibit systemic health maintenance through various biological activities, including Short Chain Fatty Acid (SCFA) production, modulation of bile acid production and secretion, inflammatory regulation, production of bacteriocin-like molecules against pathogenic infections, modulation of intestinal microbiota, and enhancement of intestinal integrity [6,7]. Traditional probiotics comprise of microbial strains from a limited number of microbial genera and species that were initially designated safe for human consumption based on their prolonged history. Moreover, these traditional probiotics are generally marketed by companies for general class of human population. Although LAB strains exhibit various biological functions, their ameliorative effects on disease are uncertain and not disease-specific.

Specific gut microbial communities have been identified by cutting-edge high-throughput and multiple-omics technologies and further established as the innovative NGPs on the basis of previous definitions of probiotics, the

abundance of NGPs is accompanied by specific diseases and clinical outcomes. Unlike the limited genera of the microbial strains comprising traditional probiotics, NGPs comprise of strains from diverse genera and species. The main goal of NGP usage is to attain effective biological resources to alleviate specific microbiota disorders and related illnesses. NGPs fall into the category of Live Biotherapeutic Products (LBPs); the definition of LBPs according to the US Food and Drug Administration (FDA) states that they are biological formulations that (1) contain live organisms like bacteria; (2) have the capability to prevent, treat, and cure specific diseases or conditions in humans; and (3) does not function as a vaccine. At present, NGPs have not attained any safety authorizations from international agencies. Hence, it is essential to confirm their safety for the establishment of their potential therapeutic applications. To characterise the safety of NGPs, not only the virulence and anti-microbial resistance genes, but also the genes associated with the transfer of antibiotic resistance *in vitro*, as well as their toxicity assessment in healthy and immune-deficient murine models in vivo is critical [8,9]. In addition to the characterization of safety, a comprehensive understanding of the target disease is also very imperative to support the development of specific NGP. Moreover, it is necessary to elucidate the underlying molecular mechanisms of the NGP-host interaction. Additionally, it is essential to understand the NGP tolerance in gastric and bile acids, which will facilitate subsequent gut colonisation by the NGPs [10]. Table 1 shows a widespread comparison between the traditional probiotics and NGPs.

Table 1. The comparison between traditional probiotics and next generation probiotics.

Classification	Traditional probiotics	Next generation probiotics
Taxonomy	Lactic acid bacteria (*Lactobacillus* and *Bifidobacterium*, limited genera)	Diverse genera and species
Safety	Generally Regarded as Safe (GRAS) status in the United States or qualified presumption of safety status by the European Food Safety Authority (EFSA)	Strict safety test *in vitro* and *in vivo*
Resource	Natural fermented products, environment and gastrointestinal of human and animal	Derive from commensal bacteria dominantly
Exploit	Long history of use	Advanced high-throughput and multiple-omics technologies such as NGS sequence
Application	As food or food supplements	Fit well within the US Food and Drug Administration (FDA) definition of a LBP
Mechanism	NA	Clarify the underlying molecular mechanism
Disease-targeting	NA	Yes

Note: LBP=Live biotherapeutic products; NA=Not Applicable.

LITERATURE REVIEW

NGP candidates

The advancement in the field of metagenomics along with the application of emerging "multi-omics" technologies in combination with innovative microbial cultivation techniques have identified several presumably beneficial bacterial species in the human gut microbiome. Extensive studies have revealed that diverse species of bacteria can

function as NGPs to ameliorate various diseases depending on the dosage used for specific-treatments. The following sections deliberate on some of the important NGP candidates, which are also listed in Table 2.

Table 2. Biological activity of NGP.

Organism	Disease	Functions and mechanisms	References
Akkermansia muciniphila	Obesity and metabolic syndrome	Outer-membrane protein, Amuc_1100 are responsible for improvement of gut permeability, decrease of endotoximea and glucagon-like peptide production	[20,23,24]
Akkermansia muciniphila	Inflammatory bowel disease	Prevention of cytokines and chemokines production and alteration of gut microbiota	[25-27]
Bacteroides fragilis	Inflammatory bowel disease and colorectal cancer	A capsular polysaccharide PSA induced Foxp3 (+) regulatory T cells and IL-10 production by recognition of TLR-2	[33-35]
Faecalibacterium prausnitzii	Inflammatory bowel disease	Ameliorative effect of butyrate	[42,43]
Faecalibacterium prausnitzii	Colorectal cancer	Infiltration of CD8 T cell	[46]
Eubacterium hallii	Obesity and metabolic syndrome	Improvement of gut permeability and insulin resistance, increase of energy expenditure	[49]
Odoribacter laneus	Obesity and metabolic syndrome	Improvement on insulin resistance and inflammation	[53]
		Decrease of serum succinate	
Parabacteroides goldsteinii	Obesity and metabolic syndrome	Improvement of gut permeability, decrease of endotoximea and increase of regulatory T cells and IL-10	[56,57]
Parabacteroides goldsteinii	Chronic obstructive pulmonary disease	Anti-inflammatory lipopolysacchride	[58]
Parabacteroides distasonis	Inflammatory bowel disease	Induction of regulatory T cells	[62]
Parabacteroides distasonis	Obesity and metabolic syndrome	1. Secondary bile acid, lithocholic acid and ursodeoxycholic acid	[60]
		2. Succinate	
Clostridium butyricum CBM588	Inflammatory bowel disease	IL-10 production	[67]

Akkermansia muciniphila: *Akkermansia muciniphila*, belonging to the phylum *Verrucomicrobia*, is a commensal gram-negative anaerobic bacterium enriched in the mucus layer of the human intestine (comprising of 0.5%–5.0% abundance of the total bacteria) that has been characterised for its mucin-degradation property [11-13]. Formerly, Everard, et al. had reported an instigation of over 100-folds abundance of *Akkermansia muciniphila* upon digestion of prebiotics [14]. Furthermore, the abundance of *Akkermansia muciniphila* was found to be lesser in healthy non-obese controls as compared to both genetics and High-Fat Diet (HFD)-induced obese mice as well as obese individuals [15,16]. In addition to obesity, several studies have indicated a reverse association between the

abundance of *Akkermansia muciniphila* and various diseases such as type 2 diabetes, inflammatory bowel diseases, hypertension, and liver diseases [17-19]. Everard, et al. provided evidences to support that *Akkermansia muciniphila* is a probiotic with an anti-obesogenic effect as evidenced by the improvement in body weight, glucose tolerance, inflammation, and endtoxaemia in HFD-induced obese mice upon daily supplementation of 2×10^8 CFU of live *Akkermansia muciniphila*. Furthermore, the study revealed that the decrease in body weight was independent of food intake and faecal fat absorption [20]. Metformin and bariatric surgery are used for the treatment of type I diabetes and obesity, respectively. Studies have shown that both these interventions are associated with an increased abundance of *Akkermansia muciniphila* [21,22]. Glucagon-Like Peptide-1 and Glucagon-Like Peptide-2 (GLP-1 and GLP-2) are bioactive hormones produced from intestinal L cells that further potentiate insulin secretion and the gut barrier, respectively. It has been reported that administration of live *Akkermansia muciniphila* results in the activation of endocannabinoid system that is involved in the production of GLP-1 and GLP-2 [23]. In contrast, administration of *Akkermansia muciniphila* has been shown to upregulate the expression of tight-junction proteins, including occludin, claudins, and Zonula Occludens (ZO) in mice with fatty liver and HFD-induced obese mice as compared to the untreated mice. Furthermore, not only live but pasteurised *Akkermansia muciniphila* also exhibited beneficial effects on the improvement of glucose tolerance and body weight in mice. The outer-membrane protein, Amuc_1100, which is highly expressed in pasteurised *Akkermansia muciniphila*, contributes to a potential mechanism of its anti-obesogenic effect [24]. The development of Inflammatory Bowel Diseases (IBDs) upsets gut microbiota, which is also known to be affected by various elements such as genes, innate immunity, and environmental factors. Many studies have shown a reverse association between IBD and fluctuation of *Akkermansia muciniphila* abundance. For instance, *Akkermansia muciniphila* abundance was reported to be significantly decreased in IBD patients as compared to that in healthy controls [17]. Furthermore, the administration of *Akkermansia muciniphila* was found to result in the suppression of cytokines and chemokines, in addition to inducing gut microbiota alterations in Dextran Sulphate Sodium (DSS)-induced colitis mouse model [25]. In contrast, conflicting studies have reported an increase in *Akkermansia muciniphila* abundance in DSS-treated C57BL/6 and STAT knockout mouse models as compared to that in their respective control mice [26,27]. Furthermore, the abundance of *Akkermansia muciniphila* has been reported to be higher in patients with constipation-type irritable bowel syndrome than in healthy controls [28]. Collectively, most studies have reinforced that *Akkermansia muciniphila* can be regarded as a promising NGP candidate with the capability of acting on intra-intestinal and extra-intestinal organs in metabolic syndrome and inflammatory conditions.

Bacteroides fragilis: *Bacteroides fragilis*, an obligate anaerobic gram-negative bacterium belonging to the genus *Bacteroides* in the phylum *Bacteroidetes*, is a predominant commensal that constitutes 1%-2% of the human gut microbiota [29]. Enterotoxigenic *Bacteroides fragilis* (ETBF) is a pathogen that has been attributed to severe inflammation and known to contribute towards the development of various diseases, such as intraperitoneal abscesses [30], IBD [31], and colorectal cancer (CRC) [32]. Interestingly, non-enterotoxigenic *Bacteroides fragilis* (NTBF), considered as a potential NGP candidate, demonstrates beneficial health properties. Studies have shown that *Bacteroides fragilis* modulates inflammation by directing the conversion of $CD4^+$ T cells into $Foxp3^+$ regulatory T cells (tregs), thereby increasing the production of IL-10. In particular, *Bacteroides fragilis* induces functional $Foxp3^+$ treg cells and IL-10 expression *via* interaction between the immunomodulatory molecule (polysaccharide A {PSA}), a capsular polysaccharide from *Bacteroides fragilis*, and toll-like receptor 2 (TLR2) on dendritic cells during intestinal inflammation [33,34]. Another study has also demonstrated that *Bacteroides fragilis* induces immune regulatory functions by interacting with TLR2 and directly acting on TLR2 expressing $Foxp3^+$ regulatory T cells [35]. In addition to its anti-inflammatory benefits, *Bacteroides fragilis* has been demonstrated to restore intestinal integrity

by enhancing tight junction protein expression in *Clostridium* difficile-infected mice [36] and mice with DSS-induced colitis [37]. Interestingly, *Bacteroides fragilis* has been documented to exhibit anti-cancer properties. For instance, the protective effect of *Bacteroides fragilis* against colon cancer has been supported by the activation of the PSA-induced TLR2 signalling pathway, which further suppresses the expression of C-C chemokine receptor 5 (CCR5) in the gut of mice subjected to azoxymethane (AOM)/DSS-induced colitis-associated colon cancer [38]. Further, *Bacteroides fragilis* evidently contributes to the anti-tumour effects of CTLA-4 blockade. The defective anti-tumour response in antibiotic-treated or germ-free mice subjected to CTLA-4 blockade has been demonstrated to be effectively reversed upon *Bacteroides fragilis* and PSA administration [39]. Briefly, it should be noted that the PSA of *Bacteroides fragilis* has a potential therapeutic effect on inflammatory diseases.

Faecalibacterium prausnitzii: *Faecalibacterium prausnitzii* is a gram-positive bacterium belonging to the family *Ruminococcaceae* in the phylum *Firmicutes*. The abundance of *Faecalibacterium prausnitzii* in the human intestinal faecal microbiota is 5% [40]. Remarkably, the decreased abundance of *Faecalibacterium prausnitzii* is highly associated with patients suffering from intestinal diseases, such as IBD and CRC [41,42]. Furthermore, studies have demonstrates that *Faecalibacterium prausnitzii* can utilize polysaccharides to produce butyrate that supports the energy metabolism of colon cells and protects them against inflammation by inhibiting the NF-κB pathway as well as the production of cytokines, such as interleukin 12 (IL-12) and interferon-gamma (IFN-gamma) [42,43]. Moreover, due to its close association with IBD, *Faecalibacterium prausnitzii* serves as a useful diagnostic and prognostic biomarker for IBD [44]. Meta-analysis studies have reported that *Faecalibacterium prausnitzii* was excreted in lower amounts by patients with type 2 diabetes [45]. Additionally, *Faecalibacterium* has been documented to present beneficial activity against CRC formation by increasing infiltration of $CD8^+$ T cells into the tumour microenvironment of colorectal cancer patients with good clinical response to ipilimumab [46]. Overall, these findings highlight the potential role of *Faecalibacterium prausnitzii* as a probiotic for intestinal disease therapy.

Eubacterium hallii: *Eubacterium hallii* is a gram-positive anaerobic bacterium that colonizes the intestinal tract. *Eubacterium hallii* belong to butyrate-producing *Lachnospiraceae* family, is able to produce butyrate from lactate and monosaccharides in low PH condition [47,48]. Udayappan, et al. administered alive form of *Eubacterium hallii* presented modification of bile acid metabolism and amelioration on insulin resistance, as well as increase of energy expenditure compared to heat-inactive *Eubacterium hallii* in db/db mice [49]. In addition, administration of alive form of *Eubacterium hallii* improved intestinal permeability and regulate the community of gut microbiota in db/db mice. Previous studies have been demonstrated the closed correlation between hyperglycemia, leaky gut and systemic inflammation [50,51]. Therefore, insulin sensitivity, bile acid metabolism, gut permeability were improved by *Eubacterium hallii* that account for beneficial effect from potential NGP.

Odoribacter laneus: *Odoribacter laneus* is gram-negative anaerobic bacterium that colonizes the intestinal tract and isolated from human stool of Japanese adult [52]. *Odoribacter laneus* may be considered as potential NGP due to its improvement on insulin resistance and inflammation in diet-induced HFD mice and db/db mice [53]. High level of serum succinate has an association with T2DM and obesity that present systemic low-grade inflammation [54]. Excess succinate also contributes to disruption of gut barrier, especially in cases with gut dysbiosis. Succinate receptor 1 (SUCNR1) also play important role for progression of T2DM and obesity. For example, macrophage infiltration characterized by SUCNR1 activation leads to inflammation of adipose tissue [55]. Overall, *Odoribacter laneus* may be a NGP dependent on succinate-consuming properties.

***Parabacteroides* species**: *Parabacteroides* species are a group of gram-negative anaerobic bacteria that commonly colonise the gastrointestinal tract of numerous species. The genus *Parabacteroides* is considered to be a probiotic. For instance, *Parabacteroides goldsteinii* was identified in mice that were administered 300 kDa polysaccharides

from medical fungi. Moreover, they have been shown to exert anti-obesogenic and anti-inflammatory effects [56,57]. Recently, LPS from *Parabacteroides goldsteinii* has been documented to presented anti-inflammatory properties against Chronic Obstructive Pulmonary Disease (COPD) induced by cigarettes in mice [58]. However, a case report has revealed that *Parabacteroides goldsteinii* causes abdominal infection in patients with lymphoma [59]. Furthermore, *Parabacteroides distasonis* may be considered as NPG candidate because of their biological activities. *Parabacteroides distasonis* have been demonstrated the ability of reduce indicators of metabolic dyfuntion such as cholesterol, free fatty acids and triglyceride in obese and HFD mice [60]. Furthermore, *Parabacteroides distasonis* also enhanced production of succinate and secondary bile acids in the gut that reduce weight gain, hyperglycemia and hepatic steatosis [60]. *Parabacteroides distasonis* has also reported anti-inflammatory properties and prevented gut barrier permeability [61]. In addition to improvement of metabolic syndrome, *Parabacteroides distasonis* has also been demonstrated to ameliorate intestinal disease. *Parabacteroides distasonis* has been reported to alleviate 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice *via* induction of regulatory T cells from naïve T cells [62]. However, a study has also highlighted depressive-like behaviour in mice with colitis upon *Parabacteroides distasonis* administration [63]. Briefly, most studies have reported that *Parabacteroides* species exhibit beneficial characteristics.

***Clostridium* species**: *Clostridium* cluster XIVa and IV are commensal bacteria that belong to the phylum *Firmicutes* and account for 10%-40% of the total bacteria in the gut of healthy individuals [64]. *Clostridium* species has been documented to digest a broad spectrum of nutrients, such as carbohydrates, which cannot be consumed in the intestine of the host and produce Short-Chain Fatty Acids (SCFAs) with immunomodulatory properties [65]. The reduction of *Clostridium* clusters III, IV, and XIVa species is often detected in intestinal disorders. The oral administration of *Clostridium* has been reported to exert ameliorative effect on colitis and allergic diarrhoea by inducing the proliferation of interleukin (IL)-10-producing regulatory T cells [66]. *Clostridium butyricum* CBM588 is one of the popular strains of *Clostridium* species, which is an obligate spore-type bacterium. It has been reported to significantly inhibit inflammation in a colitis model of germ-free mice. The mono-colonization of *Clostridium butyricum* CBM588 has been reported to be effective in protecting intestinal inflammation by instigating the production of IL-10 from macrophages in mice subjected to DSS-induced colitis [67]. Furthermore, *Clostridium butyricum* CBM588 has been shown to inhibit *Enterococcus faecalis*-induced proinflammatory cytokines, such as IL-12/IL-23 p40, IL-23, tumour necrosis factor α (TNF- α), and IL-6, in mice with crohns disease [68]. Taken together, these studies indicate that *Clostridium butyricum* CBM588 may be recognised as an NGP beneficial for the treatment of inflammatory diseases.

DISCUSSION

Extracellular Vesicles (EVs)

EVs are lipid bilayer membraned structures that are secreted by parent cells into the extracellular space for facilitating cell-cell communication with neighbouring/distant cells [69]. Exosomes, microvesicles, and apoptotic bodies are the three main subtypes of EVs, depending on their size and release mechanism. EVs isolated from body fluids obtained *via* liquid biopsies reflect the status of parent cells and serve as biomarkers through identification of cargo components, such as lipids, nucleic acids, proteins, and metabolites [70-73]. EVs are responsible for intracellular communications *via* real-time component transfer from parent cells as the encapsulated structure of EVs protects its components from enzymatic degradation in the extracellular environment. EVs have been reported to regulate innate and adaptive immunity by facilitating the recognition of various components and their corresponding receptors [74]. In addition to EVs released by the eukaryotes, EVs, called Membrane Vesicles (MVs), have also been identified from bacteria. MVs derived from gram-negative bacteria are referred to as Outer

Membrane Vesicles (OMVs). MVs are similar to EVs from eukaryotic host cells and are preferred mode of communication between bacterial cells, depending on their cargoes [75]. The contents of MVs have been shown to be taken up by the host cells, mainly through endocytosis in case of non-phagocytic cells. In the case of phagocytes, such as macrophages and Dendritic Cells (DCs), the MVs are engulfed *via* phagocytosis. MVs carrying various cargos belonging to Microbe-Associated Molecular Patterns (MAMPs) are recognized by pattern recognition receptors such as Toll-Like Receptors (TLRs) and Nucleotide-binding Oligomerization Domain protein (NOD) expressed on the surface and cytoplasm of host cells [76]. Previous studies have shown that MVs exert a dichotomous effect on immunity, which is dependent on the bacterial characteristics [77].

OMVs from *Akkermansia muciniphila*: *Akkermansia muciniphila* has been reported to exert beneficial effects for the amelioration of various diseases as described already in the preceding sections. Interestingly, the OMVs secreted from *Akkermansia muciniphila* have also been documented to exert protective effects against various diseases. For instance, the OMVs have been observed to reduce the infiltration of immune cells in the colon tissue and the production of pro-inflammatory cytokines in mice subjected to DSS-induced colitis [78]. The OMVs from *Akkermansia muciniphila* also show ameliorative effects on gut permeability in HFD-induced mice with metabolic symptoms by increasing colonic tight junction proteins [79]. Furthermore, they have also been reported to restore intestinal permeability by enhancement of tight junction protein expression such as ZO-1 and claudin-5 in colon cells both *in vivo* and LPS-induced Caco-2 cell monolayers *in vitro* [79]. Another study also supports the anti-obesogenic effect of OMVs from *Akkermansia muciniphila* [80]. The study reports that OMVs from *Akkermansia muciniphila* also enhance the expression of other tight junction proteins such as ZO-2 and claudin-4 in HFD-fed mice [80]. Another study indicated pasteurized *Akkermansia muciniphila* and its extracellular vesicles were more noticeable than its active form. The increased genes of inflammation and lipid metabolism were inhibited in mice that were administered pasteurized *Akkermansia muciniphila* and its EVs. Furthermore, some genus were restored and trend to control group by pasteurized *Akkermansia muciniphila* and its EVs [81]. In mouse model of HFD/CCL4 induced liver injury, *Akkermansia muciniphila* and its EVs prevent liver inflammation *via* enhancement of intestinal integrity [82]. *Akkermansia muciniphila* OMVs presented immuno-modulatory property *via* regulation of microRNA such as microRNA-155, microRNA-34a, and microRNA-146a involved in inflammatory/anti-inflammatory pathways in human dendritic cells [83]. Overall, these findings indicate that *Akkermansia muciniphila* OMVs alleviate inflammatory diseases by reducing inflammation and enhancing gut integrity.

OMVs from *Bacteroides fragilis*: There are two main types of *Bacteroides fragilis*: nontoxigenic and toxigenic. This section specifically deliberates on the functions of OMVs derived from non-toxigenic *Bacteroides fragilis* as it is considered to be a potential NGP candidate. PSA is a major component responsible for the *Bacteroides fragilis*-induced anti-inflammatory effects *via* interaction with TLR2 on the cell surface. It has been demonstrated that PSA is packaged in OMVs from *Bacteroides fragilis* in colitis and internalised into DC-induced Foxp3$^+$ regulatory T cells, thereby suppressing cytokine production and T cell proliferation [35]. Furthermore, the ameliorative effects of OMVs released from wild-type *Bacteroides fragilis* have been demonstrated to be better than that of OMVs from PSA-deficient mutants. The underlying mechanism was confirmed to be the activation of treg cells that result in the induction of OMV-treated DCs. Moreover, the adoptive transfer of OMV-treated DCs has been shown to result in suppression of inflammatory symptoms in experimental colitis mice. Similarly, the immunomodulatory effects of OMVs-containing PSA have been reported to be mediated by the TLR2 pathway in DCs [84]. In addition to OMVs from *Bacteroides fragilis* interacting with TLR2 receptors on DCs, they have also been demonstrated to affect TLR2 and TLR4 expression in human intestinal epithelial cells [85]. Overall, these studies reveal a unique mechanism of host-bacterium communication facilitating mutualism.

OMVs from *Faecalibacterium prausnitzii*: *Faecalibacterium prausnitzii* is a critical butyrate producer in the intestine. Moreover, it is reasonable to subscribe to the notion that *Faecalibacterium prausnitzii* prevents IBD through the anti-inflammatory properties of butyrate and contributes to the diagnostic approach for IBD dependent on their lower counts. OMVs released from *Faecalibacterium prausnitzii* also present certain beneficial effects. For instance, *Faecalibacterium prausnitzii*-derived OMVs have been reported to increase serotonin production in human epithelial cells in a dose-dependent manner and are involved in the maintenance of the serotonin pathway [86]. Furthermore, OMVs from *Faecalibacterium prausnitzii* have been documented to maintain permeability homeostasis by upregulating tight junction proteins in intestinal epithelial cells and regulating peroxisome proliferator-activated receptor gene expression that potentially cures dysbiosis [87]. Interestingly, the OMVs derived from *Faecalibacterium prausnitzii* have been shown to exhibit stronger efficacy in regulating the balance of cytokines than the *Faecalibacterium prausnitzii* itself [88]. Overall, these studies reflect the beneficial effects of *Faecalibacterium prausnitzii* and its OMVs in the treatment of inflammatory diseases.

OMVs from other probiotics: The commensal bacteria *Escherichia coli* Nissle 1917 (EcN) have been studied for their involvement in the homeostasis of microbiota to prevent intestinal illness due to their immunomodulatory and anti-inflammatory properties [89]. Recently, internalisation of EcN-derived OMVs has been reported to be associated with clathrin-mediated endocytosis in epithelial cells and regulate the activation of immunological signaling through the intestinal epithelial barrier *in vitro* [90,91]. A previous study has demonstrated that OMVs from EcN suppress pro-inflammatory cytokine expression and prevent gut permeability in experimental colitis mice. Moreover, the EcN-derived OMVs have been reported to induce more anti-inflammatory cytokines than pro-inflammatory cytokines as well as antibacterial activity in RAW 264.7 macrophages [92]. However, other studies have demonstrated that EcN-derived OMVs interact with the NOD1 receptor and subsequently turn on NF-κB-mediated cytokine production in intestinal epithelial cells [93]. Overall, these findings implicate that OMVs from EcN are a safe strategy for preserving gastrointestinal health by modulating intestinal inflammatory processes.

CONCLUSION

A large number of studies and growing evidence from international research groups reinforce a stronger association between gut microbiota and various diseases. Thus, a large repertoire of NGPs has been identified from commensal bacteria facilitated by recent technological advances in the omics field. However, safety of NGP candidates is the most important issue that requires comprehensive due diligence. Recently, MVs isolated from NGPs have received considerable attention as therapeutic strategies to prevent intestinal inflammatory diseases and immune-related disorders in pre-clinical studies. The size of MVs in the scale of nanometres facilitates their easy penetration into the circulation systems of patients and healthy individuals, thereby forming the basis of a novel diagnostic approach for the prediction of intestinal microbiota. In addition, various reports have supported the beneficial effects of preserving healthy status by facilitating understanding about the complex communication between microbiota and the host. Regardless of the various health benefits of NGP candidates or their MVs, there is still a long way for them to be actively employed as drugs in clinical applications to improve chronic inflammation-related diseases.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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