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Targeting patients' microbiota with probiotics and natural fibers in adults and children with constipation

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Abstract. – OBJECTIVE: **Functional constipation (FC) is a common condition in which the gut microbiota composition plays a fundamental role. The increasing knowledge on the role of gut microbes in the regulation of gut motility and stool consistency has allowed reconsidering, with a new scientific-based approach, the possibility to target the composition of intestinal bacterial populations for FC treatment. In this review, we evaluate recent attempts that used prebiotics, natural fibers or probiotics to treat FC, with a deep microbiome-based focus.**

MATERIALS AND METHODS: This is a literature review of articles published in Medline, Web of Science, and the Cochrane Library. Studies on FC in adults and children were identified using the following terms: constipation AND probiotics OR prebiotics OR synbiotics PR fibers OR microbiome OR microbiota. Selected animal studies were also considered if showing mechanistic observations.

RESULTS: FC is associated with alteration in microbiome composition. Motility and fecal consistency are affected with different efficacy by the type of fiber, prebiotic or probiotic strain used in patients.

CONCLUSIONS: Selected bacterial strains, mainly belonging to the *Bifidobacterium* genus, and some poorly or non-fermented natural fibers, such as *Psyllium*, may significantly improve FC and may represent the basis for an effective supplementation.

Key Words:
Microbiota, Probiotics, Natural fibers, Constipation.

Introduction

Constipation can be defined as infrequent (<3 bowel movements [BMs] per week) elimination of small/hard stools that are difficult to pass. Constipation is a common worldwide condition, with a prevalence ranging from 2.5% to 79% in adults and in elderly patients, and from 0.7% to 29.6% in children1. Defecation can be still considered in the normal range if at least three BMs per week and up to 3/day occur, that allow elimination of bulky/soft/easy-to-pass stools. According to Rome IV criteria for adults and the more recent criteria for children, chronic constipation can be classified into three broad categories: normal-transit constipation, slow-transit constipation (STC), and defecatory or rectal evacuation disorders[2,3]. Among these, STC is the most frequent condition, characterized by increased colonic transit time[2].

Recent data[4,5] collected through newly available culture-independent technologies (such as next generation sequencing) have shown that chronic constipation is accompanied with intestinal dysbiosis in both adults and children. Bacteria account for 50% of the stool volume and prolonged stasis in the colon of constipated patients may affect microbial ecology per se. However, novel data suggest an important interaction between microbes and gut physiology. Despite some discrepancies, most papers show that patients with chronic constipation have a lower abundance of *Bifidobacterium* and *Lactobacillus genera*, but increased the abundance of *Bacteroidetes* and *Enterobacteriaceae*[6,7]. Some groups have also shown that patients with irritable bowel syndrome, when constipation is the predominant manifestation (IBS-C), have a significant increase of *Bacteroides* and *Enterobacteriaceae*[5]. Interestingly the bacterial endotoxin lipopolysaccharide, present in Gram-negative bacteria (such as *Enterobacteria*), may influence intestinal motility by delaying gastric emptying and inducing sphincter dysfunction[8]. Other groups have found that the concen-

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tration of *Bifidobacteria, Clostridium leptum* and *Faecalibacterium prausnitzii* decreases in IBS-C patients[9]. Recently generated microbiome analyses (when analyses were conducted on the microbiota genome) on samples collected from patients with constipation, have shown that some patients present also a significantly decreased abundance in Prevotella strains and an increased representation of Firmicutes as compared with controls[6,7,10]. The abundance of methanogens bacteria, such as Methanobrevibacter strains, was increased in harder stools and in adult subjects with constipation[11,12]. This matches with the higher than normal methane production occurring in patients with chronic constipation[13,14].

Very few microbiome composition analyses are available from studies conducted on children suffering from constipation. In one study1[4], several bacterial species varied in quantity as compared to control subjects even if differences were not statistically significant: *Bacteroides fragilis*, *Bacteroides ovatus*, *Bifidobacterium longum*, *Parabacteroides* species were increased, while *Alistipes finegoldii* was decreased. When compared with previous microbiome data, the differences observed in this study may reside in the still immature and more dynamic composition of children's microbiome or on the different technique used to characterize the microbiome composition (not through ultra-deep next generation sequencing), which still needs complete independent validation to estimate sensitivity as well as the specificity of the results obtained, especially on the complexity of patients' samples.

A causal relationship between the altered composition of commensal microbes (the microbiota) and constipation was elegantly demonstrated by recent papers that showed that gnotobiotic animals (germ-free animals re-colonized with human fecal material) receiving fecal microbiota from patients with constipation, had a reduced intestinal peristalsis and abnormal defecation parameters[15]. Commensal bacteria play a key role in the development and maintenance of intestinal immune, vascular, sensory and motor functions as demonstrated in germ-free mice that show a significantly impaired gut development as well as delayed gastric emptying and gastrointestinal transit time as compared with conventionally raised animals[16].

Recent experiments have addressed the possible mechanisms explaining how specific bacterial species or strains affect gut physiology and motility. Re-colonizing of intestinal tract of germ-free rats showed that Lactobacilli and *Bifidobacteria* can reduce the migrating myoelectric complex period and accelerate small intestinal transit, in part due to the increased release of serotonin (5-HT) that has pro-motility effects[17]. Opposite effects were obtained with *Micrococcus luteus* and *Escherichia coli* strains that showed an inhibitory effect[17]. Notably, lipopolysaccharide (LPS), present on the surface of -negative bacteria (such as *E. coli*), influences intestinal motility by inducing sphincter dysfunction and delaying gastric emptying[8]. Other microbes, such as *Bacteroides thetaiotaomicron*, stimulate gut motility by increasing the expression of γ-aminobutyric acid, vesicle-associated protein-3 and enteric γ-actin. Moreover, host microbiota and its metabolic activity, including some metabolites or enzymes produced by some gut bacteria, are known to affect colonic motility. The short chain fatty acids (SCFAs), such as butyrate, acetate, and propionate, produced in anaerobe conditions by some gut microbes (including *Bifidobacteria, Clostridia*, and *Faecalibacterium prausnitzii* during fermentation), trigger GLP-1 or polypeptide YY release by gut mucosal cells and stimulate the enteric cholinergic reflex[18,19]. SCFAs promote the vagal sensory fibers to induce the release of 5-HT from enterochromaffin cells (ECs), thus accelerating colonic transit[20]. Several other taxa (e.g., some *Clostridia*) were correlated with faster colonic transit, butyrate production and were found reduced in constipated patients. *Bacteroidetes* in feces are negatively associated with dietary fiber intake, while *Faecalibacterium* is directly correlated with the amount of fiber intake but some papers have described an increased amount of *Faecalibacterium prausnitzii* in constipated patients[21]. This observation and other results generated in animal models suggest that the beneficial effects of butyrate might be observed only at its high concentrations. In fact, at physiological concentrations, butyrate decreases colonic mucin secretion and increases colonic water and electrolyte absorption, predisposing to constipation[22,23]. Another paradoxical observation that was described in gnotobiotic animals receiving feces from constipated patients was the higher amount of *Akkermansia muciniphila* as compared to animal receiving feces from healthy donors[14]. *Akkermansia muciniphila* is the only member belonging to *Verrucomicrobia* phylum that colonizes humans and reduction of this microbe was shown to be correlated with the development of metabolic diseases and obesity in humans[24]. Hence, it is now under evaluation as next generation probiotic in human clinical trials after the promising results obtained in an animal model of obesity and metabolic disorders[24]. Unfortunately, *Akkermansia* resides in the gut mucus layers, producing many enzymes that help this microbe to degrade mucins in order to collect carbon and nitrogen as an energy source. It is thus possible that degradation of the mucus layer (especially in association with weight management regimens and weight loss) may contribute to dryer stool or to a harder stool transit observed in the animal fecal-transfer experiment. Next generation probiotics, such as *F. prausnitzii* or *A. muciniphila*, are still lacking clinical trials to support their beneficial usage in many disease conditions, but future trials will help to understand their effects also on gut motility or constipation when used as supplements[25].

Apart from SCFAs, other metabolic functions of gut microbes may directly affect gut motility. Bile acids produced in the liver from cholesterol, are released in the duodenum where they play an essential role in lipid emulsion and absorption. A fraction of bile acids is hydrolyzed, by enzymes produced by some bacterial species, into secondary bile acids (BAs) that are no more re-absorbed in the small bowel. BAs stimulate the release of 5-HT and calcitonin gene-related peptide (CGRP) from ECs and intrinsic primary afferent neurons via activating the G protein-coupled bile acid receptor (known as TGR5), that trigger the bowel peristaltic reflex[26,27]. 5-HT binds the 5-HT3 receptors triggering the inositol 1,4,5-trisphosphate pathway that induces the release of Ca^{2+} from the sarcoplasmic reticulum and trigger the contraction of colonic myocytes. In a recent experiment where mice were transplanted with the microbiota from humans with diverse diet traditions and diet composition, the amount of secondary BAs was correlated with faster gut transit[26,27]. The increasing knowledge on the role of the gut microbiome in the regulation of gut motility and stool consistency has allowed to reconsider, with a new scientific-based approach, the possibility to target the composition of intestinal bacterial populations (in a microbiome-driven healthy direction) for constipation treatment. In fact laxatives, prosecretory agents and prokinetic drugs are frequently prescribed since the beginning of this condition even if high percentages of patients do not achieve remission or discontinue treatment at various times due to loss of response or side-effects, with psychological frustration[28,29]. That is why new therapeutic options and new mechanistic targets to alter this condition worth to be continuously pursued. Many different dietary interventions can be attempted to increase the abundance of species that are lost or reduced in constipated patients that may have positive effects on gastric motility. Moreover, probiotics, prebiotics, fibers, herbal medicinal products and synbiotics, have over the years been used alone or complementary to traditional pharmacological treatments, including lactulose, for the management of constipation[29,30]. Their role has been addressed by several randomized controlled trials (RCTs), many systematic reviews and meta-analyses. Despite many drawbacks, such as either the paucity of included studies, or the poor quality of some studies and their heterogeneity, most results support the role of selected pre-and probiotic compounds to treat constipation. Moreover, most trials were designed in the pre-microbiome era and some discrepant results may be due to the different strains, dosages and duration of probiotic or synbiotic treatment used in each trial. Some older studies may have also been biased by factors that nowadays we know independently affect microbiota composition such as gender, age, BMI or immunometabolic comorbidities.

Materials and Methods

We performed a literature search in Medline, Embase, the Web of Science and the Cochrane Library that included articles published up to October 2017. Studies on functional constipation in adults and children were identified using the following terms: constipation AND probiotics OR prebiotics OR synbiotics OR fibers OR microbiome. Constipation AND microbiota OR microbiome. Meta-analysis and review articles were considered and the studies listed in their bibliography and analysed in this paper were individually checked for the number of subjects and to identify probiotic and prebiotic used in each study. Pubmed, Embase and Cochrane libraries were the main source of papers. Only papers in English were considered. Animal studies were analysed to discuss the potential mechanism of action of both fibers and probiotics, but only RTC trials were considered for efficacy analysis and to generate Table I.

Probiotics. In 2001, the Food and Agriculture Organization of the United Nations and the World Health Organization (WHO)[31] defined probiotics as live microorganisms, which, if administered in an adequate amount, confer a health benefit to

Table I. Clinical trials with probiotics in adults (A) and children (B).

Probiotic Strains	Daily dose	Subjects	Duration	Ref.	Results
Bifidobacterium lactis BI-07	10^8 CFU/d	30	30 d	63	Beneficial effects
B. lactis DN 173 010	$1.25x10^{10}$ CFU	126	2 w	64	Beneficial effects on stool frequency, defecation condition, and stool consistency
B. lactis DN 173 010	$1.25\ x10^{10}$ CFU	159	2 w	65	Beneficial effects
B. lactis GCL2505	10^{10} CFU	17	2 w	36	Beneficial effects
B. lactis GCL2505	>10^7 CFU	62	2 w	36	GCL2505-fermented milk contributes to an increase in intestinal bifidobacteria, defecation frequency and stool quantity increased significantly
B. lactis DN-173 010	$4.25x10^9$ CFU	159	5w	66	Increased stool frequency, but not statistically significant compared with control group
B. lactis HN019	$17.2x10^9$ CFU	88	2w	67	Decreased whole gut transit time in a dose-dependent manner
Lactobacillus casei Shirota	$6.5x10^9$ CFU	54	4 w	68	Beneficial effects on self-reported severity of constipation and stool consistency
L. casei Shirota	$3x10^{10}$ CFU	87	4 w	69	Improvement in constipation severity
Lactobacillus reuteri DSM 17938	$2x10^8$ CFU	60	4 w	70	L. reuteri is effective in improving bowel MOVEMENTS in adult patients with functional constipation but have no effect in stool consistency
Lactobacillus paracasei IMPC 2.1 (LMGP22043)	$2x10^{10}$ CFU	85	15 d	71	Beneficial effects
Lactobacillus GG (LGG)	1 mL/kg/day of lactulose + 10^9 CFU of LGG	84	12 w	72	LGG was not an effective adjunct to lactulose in treating constipation in children.
Lactobacillus casei rhamnosus, Lcr35	$8x10^8$ CFU	45	4 w	73	Lcr35 was effective in reducing abdominal pain in children with chronic constipation
Lactobacillus casei rhamnosus Lcr35 (Lcr35)	$8x10^8$ CFU	94	4w	74	Probiotic was not more effective than placebo on constipation
Lactobacillus reuteri (DSM 17938)	10^8 CFU	44	8 w	75	Positive effect on bowel frequency, even when there was no improvement in stool consistency
Bifidobacterium longum	10^9 CFU	59	5 w	76	Improvement in defecation frequency and abdominal pain
Lactobacillus casei PXN 37, *Lactobacillus rhamnosus* PXN 54, *Streptococcus thermophiles* PXN 66, *Bifidobacterium breve* PXN 25, *Lactobacillus acidophilus* PXN 35, *Bifidobacterium infantis* (child-specific) PXN 27, and *Lactobacillus bulgaricus* PXN 39	10^9 CFU	56	4w	77	Probiotics had a positive role in increasing the frequency and improving stool consistency
Bifidobacterium lactis strain DN-173 010	$4.25x10^9$ CFU	159	3w	78	Probiotics did increase stool frequency

the host. Even if *Bifidobacterium* and *Lactobacillus* strains are still the most widely used probiotic genera included in many functional foods and dietary supplements, the yeast *Saccharomyces boulardii* and some *E. Coli* and *Bacillus* species are also used (and more recently a *Clostridium* *butyricum* strain that was recently approved in European Union)[32].

A probiotic strain is identified by the genus, species, subspecies and an alphanumeric designation that identify a specific strain. Correct identification of the strain present in the probiotic preparations should be clearly indicated. The beneficial functional characteristics can be species or even strain specific, as demonstrated *in vitro* or in preclinical models. This should be especially addressed when the supplement contains complex blends of probiotics that may even exert opposite effects on gut motility. Moreover, strain vitality and the number of bacteria at the time of administration may affect the clinical efficacy of the treatment and the reproducibility of results. Lack of adequate probiotic supplement characterization and, sometimes, poorly standardized outcome measure in many heterogeneous trials has delayed official probiotics recommendation for chronic constipation. The term probiotics include a plethora of compounds that needs accurate analyses to select only those supplements with real demonstrated beneficial activity on constipation. However, a recent meta-analysis of RCTs analyzed the efficacy of the most commonly used probiotics on functional constipation: *Bifidobacterium lactis, Escherichia coli Nissle, Lactobacillus casei Shirota, Lactobacillus reuteri* and *Lactobacillus paracasei* (Table I) agreed on the conclusion that probiotics significantly improved whole intestinal transit time, stool frequency and consistency[33]. However, in the subgroup analysis, only Bifidobacterium lactis (*Bifidobacterium animalis*, subspecies lactis strain BB12, Chr. Hansen, Hoersholm, Denmark, and probably its phylogenetic nearest probiotic strains such as Bifidobacterium lactis BLC1 and a few others such as B. lactis DN173010 or GCL2505) was confirmed to be significantly effective in treating constipation, through reduction of whole gut transit time (by more than 12 hours) and increasing stool frequency (about 1.5 bowel more movements/week)[33-36].

Probiotic supplementation may affect the intestinal ecosystem of constipated patients by several mechanisms. Probiotics may replenish depleted beneficial resident microbes, such as Bifidobacteria, that have beneficial demonstrated biological activity against constipation or insufficient gut motility[37]. Moreover, probiotics can affect the gut environment impacting on mucosal immune mechanisms and down-regulating inflammation. Most probiotic strains generate metabolic end products, such as SCFAs, that in sufficient amount interact with host mucosal, immune and neural cells, promoting cholinergic reflex and gut motility[19,38]. Some probiotics produce lactate that reduces luminal pH but that can be converted into short-chain fatty acids by commensal anaerobes such as *Bifidobacteria*[39].

Natural Fibers

Several natural and synthetic fibers have shown efficacy in the management of both adults and children with constipation. Despite the recently documented efficacy of semi-synthetic or synthetic fibers and polymers, such as methylcellulose (a derivative of cellulose in which some of the hydroxyl groups are substituted with methoxide groups), calcium polycarbophil (polyacrylic acid crosslinked with divinyl glycol) or polyethylene glycol (PEG, a polymer of ethylene oxide that can be synthesized to reach high molecular weight), several natural compounds are now available. Synthetic fibers are available as OTC (over the counter drugs that do not require a prescription in most countries) but due to their chemical nature do not belong to the category of nutraceuticals and cannot be considered supplements. The main mechanism of action responsible for their efficacy is their osmotic activity that allows these compounds to hold water into the intestinal lumen resisting dehydration. They are usually prescribed for short periods (2/3 weeks) and excessive usage or dosage can be accompanied by several side-effects including diarrhoea, discomfort or rarely neurological symptoms. Nevertheless, these compounds have been used successfully as medication for decades, most frequently under medical supervision. Today, the availability of natural fibers that share the same or comparable osmotic/laxative mechanisms of action deserves attention and represent novel natural approaches to treat, even daily (or for longer periods), children or adults with constipation[40]. Moreover, some fibers may help to affect the dysbiotic composition of constipated patients by changing the gut environment and the composition of the gut microbiota.

Natural fibers are polymers of carbohydrates with three or more monomers that are not hydrolysed by human enzymes in the small bowel, as defined by the Commission of European Communities in the "Commission Directive 2008/100/EC amending Council Directive 90/496/ EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions" in the Official Journal of the European Union. Within this category, several prebiotic com-

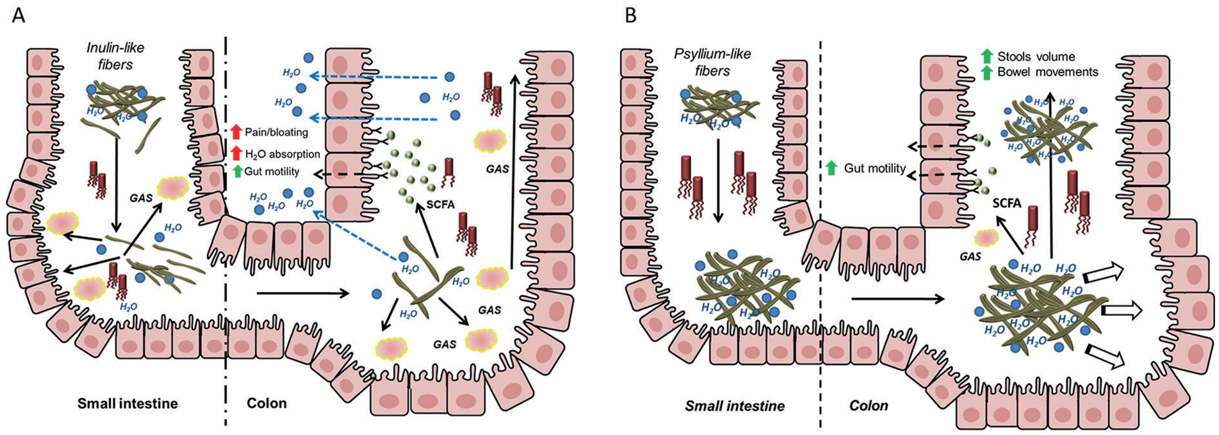

Figure 1. Fermentable and poorly/non-fermentable natural fibers in the small and large bowel. ***A,*** Inulin-like fibers are not rapidly degraded in the small bowel and often reach the terminal ileum or the colon intact. However, if assumed in greater amounts (especially if used as synbiotics with fiber-fermenting probiotic strains), sufficient fermentation may occur in the small bowel causing SCFAs production as well as gas production that may cause abdominal distention and pain. When colon anaerobe bacteria complete inulin-like fermentation, fibers are degraded and lose their water holding capacity. ***B,*** Psyllium-like fibers hold waters during their whole transit through the small and large intestine promoting a hydrated colon environment, increasing faeces volume and softness. Limited fermentation may still promote some SCFA production in the colon.

pounds can also be included41. Dietary fiber includes long-chain carbohydrates, such as cellulose, hemicelluloses, β-glucans, fructans, among which inulin, pectins, dextrins, gums and resistant starch, and short-chain carbohydrates, such as FOS and galacto-oligosaccharides (GOS)[42]. All these compounds are frequently included in commercially available nutritional supplements for their supposed beneficial direct or indirect activity through promoting beneficial commensals.

Fibers are further classified based on solubility, viscosity and fermentability by gut microbiota. Highly branched polymers with multiple branches at irregular intervals that do not pack in a regular array, are referred to as nonviscous (e.g., inulin, fructooligosaccharides, and wheat dextrin). In contrast, linear polymers pack into a regular array, and become more viscous the longer the polymeric chain is. If between linear polymer chains cross-links form, fibers form a gel (e.g., b-glucan, psyllium, and raw guar gum) in the intestinal tract. This latter characteristic may be responsible for the beneficial effects of this kind of fibers in softening faeces as well as in favoring expulsion despite their often-modest fermentability in the gut (Figure 1A).

Fibers that are fermented by some host commensal anaerobes lead to the production of gases and SCFAs30. SCFA, if produced in adequate amount, can induce the previously mentioned beneficial immunomodulatory and pro-motility effects[43,44]. However bloating, flatulence and fastidious gut discomfort may limit their usage (Figure 1A), especially if fermentation occurs already in the small bowel[45]. These side effects often occur even at the currently EFSA recommended dosage (at least 12 g/day)[40]. In fact highly fermentable fibers, including FOS, GOS and inulin, are used as prebiotics in several conditions since they positively affect the composition and function of host microbiota[30,46,47]. Unfortunately, according to recent clinical evidence, soluble fermentable fibers (e.g., inulin, fructooligosaccharides, and wheat dextrin) do not provide enough laxative effect, and some fibers can even result to promote constipation in clinical trials[48]. Moreover, most of the evidence regarding the beneficial effect of inulin-type prebiotics in constipation comes from animal studies. Chicory inulin in 2006 received an EU health claim for the improvement of bowel function and the maintenance of normal defecation by increasing stool frequency. More recently EFSA confirmed the claim related to "native chicory inulin" (EFSA https://www.efsa.europa.eu/en/efsajournal/pub/3951, 2015). The amount requested to assume to use the claim is at least 12 g/day. Interestingly several small trials and a more recent larger trial conducted on sixty constipated women, concluded that there was no significant difference in relief of constipation between inulin-type fermentable fibers and placebo group[49]. Similarly, some recent meta-analyses concluded that some FOS, except from inulin, may have some efficacy on constipation[40].

Table I. Synbiotics for constipation.

Probiotic Strains	Daily dose	Subjects	Duration	Ref.	Results
Lactobacillus casei, L. rhamnosus, L. plantarum, and B. lactis, polydextrose, FOS, GOS	4x10^9 CFU and 2 g prebiotics	146 functional constipation	4 w	58	Significant increase in weekly number children of defecation in the group receiving synbiotic
L. rhamnosus, L. plantarum, and B. lactis, inulin	5x10^9 and 900 mg inulin	71 IBS children	4 w	79	Administration of synbiotics and probiotics resulted in significant improvements in initial complaints when compared to prebiotics
Lactobacillus rhamnosus PXN 54, *Bifidobacterium bifidum* PXN 23, *Lactobacillus acidophilus* PXN 35, *Lactobacillus plantarum* PXN 47, *Lactobacillus bulgaricus* PXN 39, *polydextrose,* FOS, GOS*, psyllium*	nd	69 adults with functional constipation	4w	80	Improvement of constipation symptoms
Lactobacillus paracasei, Bifidobacterium lactis, Lactobacillus acidophilus Psyllium	2.4x10^{10} and 2.5 g psyllium	29 adults with severe functional constipation	2 w	59	Improvement of the main clinical parameters of functional constipation
Bacillus coagulans lilac-01 okara [soy pulp] powder	1x10^8 CFU	297 healthy adult with tendency for constipation	2 w	81	Lilac LAB was effective in improving bowel movements and fecal properties in functionally constipated persons
Lactobacillus paracasei (Lpc-37), *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (NCFM) *Bifidobacterium lactis* (HN019) FOS	6 g of FOS and 10^8–10^9 cfu	100 constipated adult women	30 d	57	Improved evacuation parameters and constipation intensity of chronically constipated women
Lactobacillus casei NCIMB1 30185 *Lactobacillus rhamnosus* NCIMB 30188 *Streptococcus thermophilus* NCIMB 30189 *Bifidobacterium breve* NCIMB 30180 *Lactobacillus acidophilus* NCIMB 30184 *Bifidobacterium longum* NCIMB 30182 *Lactobacillus bulgaricus* NCIMB 30186 FOS	10^8 colony forming units	Adult men with functional constipation (66)	4 w	56	Effective in increasing stool frequency and improving consistency
Bifidobacterium longum W11 FOS	nd	Chronic constipation adults undergoing diet	60 d	82	Improving constipation during hypocaloric diet
Bifidobacterium longum W11 FOS	3 g prebiotics	636 patients diagnosed with constipation-type IBS	36d	83	Symbiotic increase stool frequency in patients with constipation
Bifidobacterium animalis DN-173010) FOS	10^8 CFU and 0.5 g FOS	Constipated woman (266)	4 w	84	Significant improvement in the parameters related with bowel evacuation
Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis, Bifidobacterium bifidum, Bifidobacterium longum and Bifidobacterium infantis and FOS	6x10^{10} CFU	Constipated adults (120)	1 w	85	Synbiotic is effective in increasing stool frequency and improving stool consistency

Interestingly and unexpectedly from what was previously observed in other conditions, fermentable fiber supplementation was not sufficient to increase *Bifidobacteria* but caused a reduction in the amount of *Clostridia* when compared to placebo in most cases. Despite some discordant results, this may suggest that the stasis and the harder stool matrix present in constipated patients may limit their efficacy for this indication or that they may require dosage less tolerable for patients. Indeed, nor inulin or GOS seem to be beneficial over placebo in increasing stool frequency[40].

Long chain natural fibers used as supplements, with no or only modest fermentability, contain psyllium and wheat bran. The term 'psyllium' refers to the seed husk of the plant genus Plantago, more frequently from the *Plantago ovata* species. The husk is the mucilaginous fraction of the seed coat and contains soluble, viscous and moderately fermentable fibers. It can retain water for several times its weight, swell and form a gelatinous mass, which softens and increases the volume of stools effectively, thus helping to stimulate intestinal peristaltic movements (Figure 1b)[42]. Several RCTs[33,50,51] described psyllium as the natural fiber with the greatest effect on stool consistency and good patients' compliance. Despite its poor fermentability, mostly limited to few bacterial families (*Bacillus strains,* for example), psyllium fibers were shown to promote beneficial microbiota changes, to increase faecal biomass and increase the production of SCFAs in the colon. Moreover, the stool softening effect, as a direct consequence of its ability to form a gel, suggests that psyllium promotes an overall beneficial activity on the gut environment[52]. Psyllium has robust clinical evidence as an effective treatment in constipated patients with clinical trials showing its superiority to fermentable fibers[50]. In fact GOS and inulin, despite being soluble and with high water-holding capacity, when reach the colon, are almost completely fermented and lose their water-holding capacity and their beneficial effect on stool consistency (Figure 1A). If psyllium is used at high fiber dose (>15 g) it increases stool frequency with very large effect size. However such high dosage needs to be taken with caution, especially for prolonged daily supplementation where 2-5 g/die should more safely be used in adults and always ingested with water. Special caution should be used with children where prudential dosage for daily supplementation should probably limit the quantity between 0.5 and 1.5 g/die[50].

Wheat bran is another natural fiber collected from the hard outer layers of wheat grain and is composed of approximately 45-50% of fibers (cellulose and hemicelluloses)[42]. Differently from psyllium, wheat bran is almost insoluble and not fermentable. Its laxative effects are probably the consequence of the increased faecal mass with large/coarse insoluble fiber particles that mechanically irritate the gut mucosa stimulating the intestinal mucosal peristalsis[42]. However more robust clinical trials are needed to understand if wheat bran can beneficially alter microbiota composition, thus promoting a healthier gut environment, and to establish the amount to be used in adults or children.

Synbiotics

The combination of probiotic strains and prebiotic fibers may provide synergistic beneficial effects to the host when combined in synbiotic preparations. Some groups have tested synbiotic preparations also in constipated patients[53,54]. Prebiotics may improve survival of probiotic strains during the transit through the upper digestive tract or they may favour probiotic intestinal colonization and growth, together with the growth of resident beneficial microbes[55].

It was shown that synbiotics composed by a mixture of *Lactobacillus casei* NCIMB1 30185, Lactobacillus rhamnosus NCIMB 30188, Streptococcus thermophilus NCIMB 30189, *Bifidobacterium breve* NCIMB 30180, *Lactobacillus acidophilus* NCIMB 30184, *Bifidobacterium longum* NCIMB 30182, *Lactobacillus bulgaricus* NCIMB 30186 and FOS had a beneficial effect on improving stool frequency and consistency in male subjects affected by functional constipation[56]. The same effect was found in another study by Waitzberg et al[57] performed on a population of chronically constipated women where the administration of a synbiotic composed by *Lactobacillus paracasei* (Lpc-37), Lactobacillus rhamnosus (HN001), *Lactobacillus acidophilus* (NCFM) and *Bifidobacterium lactis* (HN019) and FOS improved frequency of evacuation as well as stool consistency.

Moreover, the beneficial effects of synbiotics were observed also in the pediatric population: the administration of *Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus plantarum,* and *Bifidobacterium lactis* and *prebiotics* (fiber, polydextrose, fructo-oligosaccharides, and galacto-oligosaccharides) resulted in a significant improvement in the weekly number of defecations, abdominal pain and painful defecation[58]. A recent meta-analysis performed on 7 RCT showed a significant increase in stool frequency and softer stools in patients treated with synbiotics composed of FOS and probiotic strains compared to placebo[40].

As psyllium seems to have the highest independent activity on constipation, it has been recently tested in a synergic mixture with five probiotic strains, belonging to different *Lactobacillus* and *Bifidobacterium species*, confirming an improvement of main clinical parameters of functional constipation in patients with chronic constipation[59].

Conclusions

Some fibers and some probiotic strains showed a beneficial effect on constipation in several clinical studies both in children and adults. Moreover, several new mechanisms in pre-clinical studies may now explain their activity in patients[17,18,23,27,42,46]. When making recommendations for a supplement or for designing the most effective combination, it is essential to select components based on current scientific evidence. Nowadays, the science of pre-and probiotics must face the growing knowledge of the role that the microbiota plays in human physiology and disease. The role of gut microbiome was demonstrated to be crucial in the maintenance of a healthy gut environment, including a proper and effective motility[60,61]. The composition of microbes that constitute a relevant fraction in faeces mass may also affect faeces consistency and host mucus secretion. In constipated patients a dysbiotic environment is a common finding and *Bifidobacteria* are the most affected group of microbes that is frequently reduced when compared with healthy controls[6,7]. Bifidobacteria were demonstrated to promote gut motility also in animal models and strain-specific data are available for some *Bifidobacteria*-containing probiotics[62]. Bifidobacteria-containing probiotics may replenish, at least in part, the lost populations or their metabolic activity observed in patients. This is also the reason to avoid recommending probiotic or synbiotic products where the strains are not clearly indicated. In fact current evidence suggests that only some bacterial species or strains may give beneficial effects in constipation management[62]. However, many probiotic preparations contain blends of strains, where *Bifidobacteria* may be a minor fraction of the living bacterial population. In fact other species, like Streptococcus salivarius or thermophilus, are more resistant to industrial processing and are often included in the blends to support the claimed quantity of living cells in the final product. Despite good manufacturing guidelines recommending showing the number of living cells for each strain, most products contain only the total number of bacteria. Moreover, bacteria are lyophilized and dehydrated in the commercial preparations. Any excipient or prebiotic compound present in the delivery vehicle (capsule, sachet, etc.) must be fully dried and perfectly sealed, to preserve bacteria vitality. Thus, the quality of the synbiotic preparation may make a big difference in term of expected efficacy. Psyllium, or other fibers that are only poorly degraded in the gut, have shown beneficial effects on constipated patients[50]. At the same time, as suggested by recent pilot studies, it may boost the efficacy of probiotic strains through its water-holding capacity, that allow these compounds to form a gel within the intestine and to hydrating the faecal mass[42]. In fact, when given alone, psyllium or other poorly fermentable fibers, affect the gut microbiota composition and may thus ameliorate the efficacy of probiotic activity allowing a better microbial metabolism and diffusion of nutrients or metabolites in hydrated faeces. This osmotic mechanism requires that the fiber resist sufficiently to bacterial fermentation, especially if used in synbiotic formulations. Fibers must remain relatively intact throughout the small and especially the large bowel, to promote bulky/soft/easy-to-pass stools. For this reason, if the prebiotic fibers are fermented by probiotics present in the same synbiotic preparation when they are still in the small bowel, bloating and abdominal pain can occur affecting patients' compliance. Moreover, fermentation in the colon leads to the loss of inulin-like fibers water holding capacity that explain their modest or null activity when given alone for constipation management (Figure 1)[48]. Special caution should also be paid when treating children not to limit their access to effective natural treatments for a fastidious condition. Self-administration of unstandardized amounts of fibers (such as psyllium or other insoluble fibers) should be avoided especially in children. In fact available data suggest that poorly fermented fibers should be used within safety ranges in children (0.5-1.5 g/day) to minimize risks of gel formation early in the upper gastrointestinal tract. Moreover, the availability of this natural compound suggests that also in adults for a daily and prolonged usage, as frequently required, a low dose of poorly fermentable fibers should be similarly effective and safe (between 2 and 5 g/day). This may cause a delay (2-3 days) in effective bowel movements to occur when compared to pharmacological treatments but will minimize the risk of side effects. For this reason, synbiotic preparations containing selected *Bifidobacteria* strains and *Psyllium-like* fibers may represent promising effective natural combinations for treatment of constipated patients as suggested by current clinical and microbiome-based evidence.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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