Interplay between probiotics and prebiotics for human nutrition and health

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Abstract

In the last years, probiotics, prebiotics and synbiotics have been exploited as microbiota-management tools to enhance human health. In this context, a vast array of probiotic, prebiotic and synbiotic products have been developed and provided in pharmaceutical forms or food/nutraceutical supplements. In this chapter, we will present an overall perspective of probiotics and prebiotics considering their benefits for the human health, underlying mechanisms of action and the criteria for their selection. In addition, the interplay between probiotics and prebiotics and their importance in human nutrition and health will be discussed, namely regarding the emergence of synbiotics as a combination of both.

Keywords

Food; Gut microbiota; Health; Prebiotic; Probiotic; Synbiotic

1. Introduction

The increasing knowledge in the field of human nutrition and health has demonstrated the pivotal role of probiotics, prebiotics and synbiotics in achieving health beneficial effects and improving people's welfare and quality of life. The word "probiotic", which comes from Greek and has the connotation "for life", is currently used as a broad concept to designate microorganisms with beneficial effects in humans and animals (Hill et al., 2014; Markowiak & Ślizewska, 2017). The perception of the benefits of lactic acid fermentation on human health goes back to ancient times, with reports of the consumption of different fermented milk types by ancient Roman, Greek and Egyptian communities (Markowiak & Ślizewska, 2017). However, scientific knowledge regarding this matter was only obtained by the observations of the Russian Nobel Laureate Élie Metchnikoff,

with his work at the Pasteur Institute, at the beginning of the 20th century. Metchnikoff suggested that the regular intake of fermented dairy products with lactic acid bacteria could be related with improved health and longevity in Bulgarian and Russian Steppes nomads. In his work, Metchnikoff proposed that it is possible to adopt measures to change the intestinal flora of human body, substituting the harmful microorganisms by beneficial ones (Metchnikoff, 1907). Since then, probiotic concept has been related to beneficial microorganisms for the host health (Martín & Langella, 2019). Regarding to prebiotics, they may be used as substitutes of probiotics or to provide them an additional support in improving human health, due to their high potential to modulate the intestinal microbiota (Markowiak & Ślizewska, 2017). The combination of probiotics and prebiotics in a single product is commonly referred as synbiotic (Martín & Langella, 2019). This chapter describes the current knowledge regarding probiotics and prebiotics, exploring their beneficial effects into improving human health, underlying mechanisms of action, the possible sources for their purification/production and the criteria involved in their selection. Also, the interaction between probiotics and prebiotics is highlighted, as well as their emergent combination as synbiotics.

2.1. Probiotics

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). In the last years, the scientific community proposed the probiotics as therapeutic and prevention options of a wide panoply of human diseases (Zommiti, Feuilloley, & Connil, 2020). In this scope, several clinical trials emphasizing beneficial effects of probiotics on human health are available in literature (see Table 1).

59 **Table 1.** Examples of clinical trials involving probiotics and their human health benefits, published in 2021.

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Target condition	Probiotic	Key findings	Reference
Anxiety and depression	*Lactobacillus plantarum* PS128	Daily administration of this probiotic may reduce depressive symptoms, fatigue level, cortical excitation, and an enhancement in sleep quality during the deep sleep stage among insomniacs.	(Ho, Tsai, Kuo, & Yang, 2021)
Antibiotic-induced faecal short-chain fatty acid (SCFA) and microbiota composition disruptions	*Bifidobacterium animalis* subsp. *lactis* BB-12	Simultaneous administration of amoxicillin/clavulanate and BB-12 yogurt to healthy participants was related with a significantly smaller diminution in the faecal SCFA levels and a more stable taxonomic profile of the microbiota over time than the control group.	(Merenstein et al., 2021)
Type 2 diabetes	*Lactobacillus plantarum* HAC01	An eight-week course of *L. plantarum* HAC01 supplementation significantly improved certain metabolic parameters (HbA1c and 2h-postprandial glucose levels) relative to placebo in prediabetic individuals and there were no reports of the serious adverse effects.	(Oh et al., 2021)
Constipation	*Lacticaseibacillus paracasei* strain Shirota	Daily consumption of this probiotic for 9 weeks seemed to alleviate constipation and improve the potentially depressive symptoms in patients with depression and significantly reduce IL-6 levels. Furthermore, this probiotic supplementation appeared to regulate the gut microbiota associated to mental disease.	(Zhang et al., 2021)
Rheumatoid arthritis	*Lactobacillus acidophilus* La-14, *Lactobacillus casei* Lc-11, *Lactococcus lactis* Ll-23, *Bifidobacterium lactis* Bl-04 and *Bifidobacterium bifidum* Bb-06	The mixture of these probiotic strains reduced inflammatory biomarkers and enhanced oxidative/nitrosative profile in patients with rheumatoid arthritis.	(Cannarella et al., 2021)
Stress	*Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, *Levilactobacillus brevis*	The intake of beverage fermented with these probiotic bacteria may reduce the stress and modulate the intestinal microbiota (correcting dysbiosis) in university students, without interfering with their lifestyle or diet.	(Márquez-Morales et al., 2021)

Target condition	Probiotic	Key findings	Reference
Allergic rhinitis	*Bifidobacterium animalis* subsp. *lactis* BB12 and *Enterococcus faecium* L3	When administered as a prophylactic treatment, a mixture of these probiotic strains reduced signs and symptoms of allergic rhinitis and diminished the need of conventional therapy.	(Anania et al., 2021)
Atopic dermatitis and cow's milk protein allergy	*Lactobacillus rhamnosus* ŁOCK 0900, *Lactobacillus rhamnosus* ŁOCK 0908, and *Lactobacillus casei* ŁOCK 0918	Supplementation of the children's diet with the probiotic preparation for three months led to a significant improvement in atopic dermatitis symptom severity. Furthermore, this probiotic preparation is safe, and triggers benefits especially in allergen sensitised patients.	(Cukrowska et al., 2021)
Fibromyalgia syndrome	*Lactobacillus rhamnosus* GG, *Lactobacillus paracasei*, *Lactobacillus acidophilus* and *Bifidobacterium bifidus*	Probiotic treatment produced an improvement in attention by reducing errors on an attention task, but it had no effect on memory, in patients with fibromyalgia syndrome.	(Cardona, Roman, Cañadas, & Sánchez-Labraca, 2021)
Irritable bowel syndrome	*Bacillus coagulans* LBSC	Probiotic intervention was safe for human consumption and efficacious in relieving overall pathophysiological symptoms of irritable bowel syndrome and thereby improving inclusive quality of life.	(Gupta & Maity, 2021)
Halitosis	*Weissella cibaria* CMU	Oral ingestion of *W. cibaria* CMU may aid in reduction subjective halitosis and improve oral-health-related quality of life, being reported the possibility of oral-probiotic use by college students without any difficulties or adverse effects.	(D. S. Lee, Kim, Nam, Kang, & Lee, 2021)
Pancreatitis	*Bacillus subtilis* and *Enterococcus faecium*	Probiotic supplementation seemed to be safe and effective in reducing the duration of hospitalization in patients with mild acute pancreatitis.	(Wan, Zhu, Bian, & Sun, 2021)
Community-acquired common colds	*Lactiplantibacillus plantarum* HEAL9 and *Lacticaseibacillus paracasei* 8700:2	Intake of this probiotic product may be protective against multiple colds in subjects prone to getting colds.	(Ahrén, Hillman, Nordström, Larsson, &

Target condition	Probiotic	Key findings	Reference
			Niskanen, 2021)
Constipation, diarrhoea, acute respiratory infections and nutritional status	*Lactobacillus casei* strain Shirota	Daily intake of fermented milk containing this probiotic bacterium strongly prevented the incidence of constipation and acute respiratory infections in Vietnamese children. Furthermore, this probiotic drink may contribute for diarrhoea prevention and nutritional status improvement.	(Mai et al., 2021)

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The exact mechanisms by which probiotics accomplish their benefits are diverse, heterogeneous and strain specific, remaining not fully clarified (Plaza-Diaz, Ruiz-Ojeda, Gil-Campos, & Gil, 2019). Nevertheless, four main mechanisms of action have been pointed, as depicted in Fig. 1: i) interference with pathogens; ii) strengthening of the barrier function of the epithelial lining; iii) modulation of the immune system; and iv) gut-brain axis interaction (Sánchez et al., 2017). For instance, probiotics play a key role in the prevention of pathogenic colonization. This can be mediated by binding of probiotics to the host adhesion sites, competition for available nutrients, coaggregation ability, production of antimicrobial compounds (such as bacteriocins, biosurfactants, hydrogen peroxide and organic acids), and/or the inhibition of flagella motility of pathogens (van Zyl, Deane, & Dicks, 2020). Also, probiotics contribute for the maintenance of the epithelial barrier integrity, through the stimulation of mucin glycoproteins secretion by the goblet cells that assemble into a thick mucus layer. Probiotics increase the secretion of antimicrobial proteins (defensins) by the intestinal epithelial cells, which help the clearance of pathogens that penetrate the mucus layer, and improve the stability of intercellular junctional complexes, which reduces the intestinal epithelial cells intercellular permeability to pathogens (van Zyl et al., 2020). Moreover, it is often described that probiotics may exert regulatory effects on the host's innate and adaptive immune responses, since these beneficial microorganisms may modulate the functions of dendritic cells, monocytes/macrophages, and T and B lymphocytes (Plaza-Diaz et al., 2019; van Zyl et al., 2020). In addition, probiotics may interact with the central nervous system (gut-brain axis), producing microbial metabolites, such as short-chain fatty acids, which are able to alter neuronal excitability, and neuroactive compounds (namely dopamine, gamma-aminobutyric acid, histamine, acetylcholine and tryptophan) (Plaza-Diaz et al., 2019).

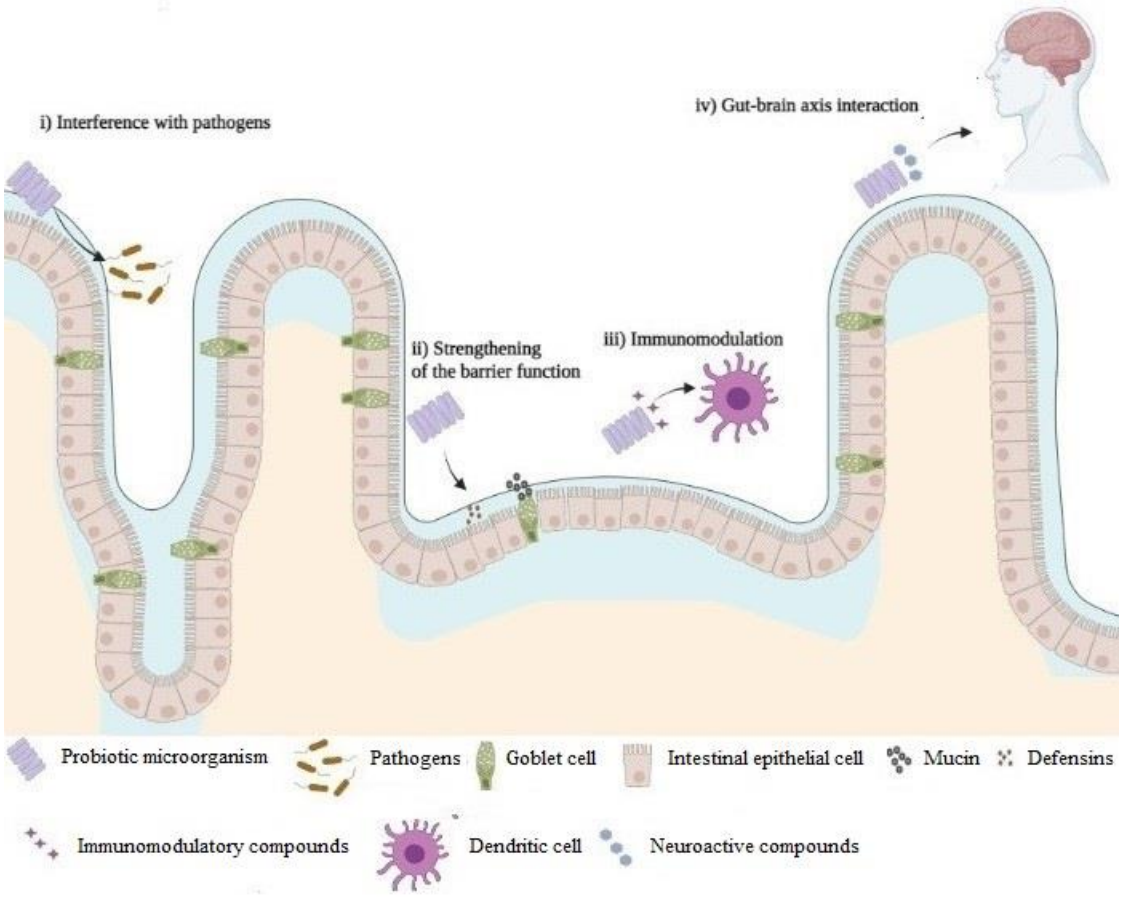

Figure 1. Probiotic mechanisms of action. This graphical representation illustrates the four main mechanisms underlying health benefits exerted by probiotics namely: i) interference with pathogens; ii) strengthening of the barrier function; iii) immunomodulation and iv) gut-brain axis interaction. Figure created in BioRender.com.

Probiotic microorganisms can be isolated from many sources including gastrointestinal tract, breast milk and fermented food products (Shokryazdan, Faseleh-Jahromi, Liang, & Ho, 2017; Zommiti et al., 2020). In addition, these microbes must meet certain criteria in terms of safety, functionality and technological utility in order to be qualified as a probiotic (Binda et al., 2020; Zommiti et al., 2020). Considering Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) guidelines, recently reiterated by International Scientific Association for Probiotics and Prebiotics (ISAPP), a probiotic microorganism should be sufficiently characterised at a genus, species and strain level. Furthermore, probiotics should be named according to scientifically valid nomenclature (including a strain designation) and deposited in an internationally recognised culture collection. In addition, a probiotic strain should be safe for the intended use; its health benefit must be proven from at least one study involving

humans; and the survival of adequate levels of the probiotic strains throughout the products' shelf life to deliver a health benefit must be guaranteed (Food and Agriculture Organization of the United Nations; World Health Organization, 2002; International Scientific Association for Probiotics and Prebiotics, 2018).

The major commercialised probiotic microorganisms are found within the *Lactobacillus*, recently reclassified into 25 genera (Zheng et al., 2020), and *Bifidobacterium* genera. Nonetheless, some members of *Bacillus* (*B. coagulans* and *B. subtilis*), *Streptococcus thermophilus* and yeast *Saccharomyces cerevisiae* variant *boulardii* have also been marketed (Gomes, Andrade, & Freitas, 2017). These probiotics, commonly designated as traditional probiotics, have been considered as Generally Regarded as Safe (GRAS) by the Food and Drug Administration (FDA) or as Qualified Presumption of Safety (QPS) by the European Food Safety Authority (EFSA), due to their long history of safe use (Martín & Langella, 2019). Nevertheless, the probiotic market demands the implementation and diversification of the available products. For this purpose, several studies targeting the selection of probiotic microorganisms with diverse functional properties have been performed (de Melo Pereira, de Oliveira Coelho, Magalhães Júnior, Thomaz-Soccol, & Soccol, 2018). In this alignment, various intestinal commensals, including *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Anaerobutyricum hallii*, *Prevotella copri* and *Christensenella minuta*, have emerged as potential novel probiotics, also termed as next generation probiotic candidates (Andrade et al., 2020). However, the novelty linked to such bacterial strains entails the need to clarify several key parameters regarding their effectiveness, safety, physiological, genomic, and metabolomic features, prior to their practical application. In addition, several technological aspects require substantial improvement, namely in terms of production, storage stability and delivery (Almeida et al., 2020; Andrade et al., 2020).

2.2. Prebiotic

In its first definition, a prebiotic was described by Gibson & Roberfroid (1995) as a 'non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'. More recently, this definition has been modified to 'a substrate that is selectively utilized by host microorganisms conferring a health benefit' Gibson et al., 2017), which broadens the definition to all ecosystems where a selective targeting of particular microorganisms is required for host well-being. Prebiotics are present in

natural products and may be extracted and applied to more common food preparations in order to improve their nutritional and health values. Acting as fermentable substrates for specific bacteria, prebiotics will lead to the stimulation of their growth and metabolic activity, which are linked to different health outcomes (Markowiak & Ślizewska, 2017; Voss, Campos, & Pintado, 2021).

Until recently, the main recognized prebiotics are carbohydrate-based, however, the new definition enables other substances such as polyphenols, peptides and polyunsaturated fatty acids to be included whenever these reveals proven prebiotic effects (Gibson et al., 2017).

Among the carbohydrate-based prebiotic substances, short and long-chain *β*-fructans (fructo-oligosaccharides and inulin, respectively), lactulose, galacto-oligosaccharides and *β*-glucans, are the most well-established (Markowiak & Ślizewska, 2017), and their supplementation in the diet has shown numerous health benefits. Thus, considering the importance of prebiotics on the human gut microbiota balance and their potential role on human health, the search for new prebiotics is continuous, and fruit, vegetables, cereals, and other edible plants are sources of carbohydrates constituting potential prebiotics (Markowiak & Ślizewska, 2017; Roberfroid et al., 2010; Voss et al., 2021).

2.2.1. Prebiotic substances and their selection criteria

Several food oligosaccharides and polysaccharides (including dietary fiber) have been claimed to have prebiotic activity, but not all dietary carbohydrates are prebiotics (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004); furthermore, not all prebiotics are carbohydrates, and two criteria can be used to differentiate fiber from carbohydrate-derived prebiotics: (i) fibers are carbohydrates with a degree of polymerization equal to or higher than 3 and (ii) endogenous enzymes in the small intestine cannot hydrolyse them (Davani-Davari et al., 2019). In addition, since other substances might fit the updated definition, such as the previously mentioned polyphenols and polyunsaturated fatty acids converted to respective conjugated fatty acids (Gibson et al., 2017), there is a need to establish clear criteria for classifying a food ingredient as a prebiotic. According to Gibson et al. (2004) and Wang (2009) there are four criteria for the classification of food ingredients such as prebiotics, as shown in Figure 2.

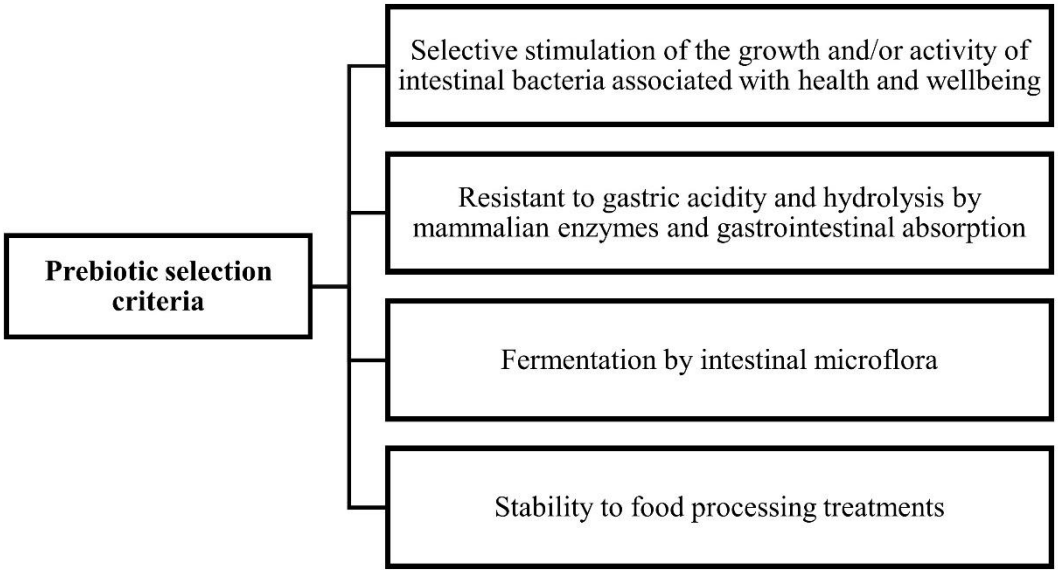

Figure 2. Criteria required for classification of a food ingredient as prebiotic (Gibson et al., 2004; Wang, 2009).

Prebiotic substances may serve as a medium for probiotics, stimulating their growth. Previous studies have demonstrated that prebiotics increase beneficial bacteria in the human gut, and inhibit pathogenic bacteria, mainly promoting the production of short-chain fatty acids (SCFAs) and lactic acid during their metabolic degradation, which confers a health benefit to the host (Roberfroid et al., 2010; Voss et al., 2021).

2.2.2. Novel prebiotic substances and sources

There are many natural whole sources of compounds with prebiotic activity. The most common include chicory, leeks, Jerusalem artichokes, garlic, onions, barley and oats which are sources of the well-established prebiotic fructans and β-glucans. As functional ingredients to be used in the fortification of specific foods these prebiotic compounds may be simply extracted from their natural sources or commercially produced via hydrolysis of polysaccharides or catabolic enzymatic reactions from lower molecular weight sugars (Scott et al, 2020). More recently, other natural products, including different fruits, seeds, vegetables, flowers, leaves and roots have been described as candidate prebiotics due to the presence in their constitution of some recognized prebiotics or even synergetic behaviours between them.

Several fruits have been largely studied for their prebiotic potential because of their richness in non-digestible carbohydrate compounds, such as the case of pineapple, bananas, apple, pear and even citrus fruits (Gómez-García et al., 2022). Generally, fruits

have a high content of pectin, medium to high molecular weight sugars, or inulin, all of which may be considered prebiotic substances. For instance, bananas are a good source of molecule mixtures with prebiotic potential, given their constitution rich in vitamins, minerals, and dietetic fibers, containing small quantities of inulin. Moreover, when consumed in an unripen form, the fruit contains high content of resistant starch, which is also known to promote a prebiotic effect. On the other hand, fruits containing high content of pectin and a high content of polyphenols, in free or complexed form, may also enable potential prebiotic effects. For instance, all varieties of apples and pears, as well as oranges and all of the citrus family are recognize for the improvement of the digestive tract health through enhancement of the human gut microbiota due to the synergies promoted by the pectins and polyphenols (Akter & Rabeta, 2021). Recent studies have demonstrated the potential prebiotic activity of pineapple by-products juice, associated with the presence of oligosaccharides and galactomannans, which were able to promote the growth of both *Bifidobacterium* and *Lactobacillus* genera, contributing towards the improvement of the fermentation conditions (Gómez-García et al., 2022). Further details will be presented in section 2.2.4 of this chapter.

In what concerns tubers, Jerusalem artichoke (*Helianthus tuberosus* L.), coming from a sunflower species has been described for its composition high in water and high in inulin, unlike other tuber-like foods that contain high amounts of starch and sucrose; such composition is associated with an important prebiotic potential of artichoke (Brkljača et al., 2014). The Konjac root (*Amorphophallus konjac*) is another tuber recognized for its health promoting effects directly associated with a prebiotic potential. Unlike artichoke, konjac root and derived flours contain between 70-90% of glucomannan fiber. The glucomannan fiber contains polysaccharides of high molecular weight and water-soluble, consisting mainly in units of D-glucose and D-mannose under a ratio of 1.6:1.0 connected by β(1-4)-glycosidic bonds. This structure enables the fiber to have a highly viscous nature which improves the carbohydrates metabolism and the growth promotion of probiotic bacteria (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al., 2017).

Besides roots and tubers some types of seeds have been recognized for their potential as prebiotic food, the most well-known being cocoa beans (the basis for chocolate production). Fresh cocoa beans contain a high variety of metabolites mainly polyphenols, but flavanols and procyanidins are the most representative. Several studies have shown the positive interaction between these molecules with the gut microbiota,

since flavanols influence positively the *Bifidobacterium* and *Lactobacillus* growth, while the growth of non-probiotic bacteria is not positively affected (Tzounis et al., 2008).

Flax seeds (*Linum usitatissimum*) are also recognized to be a health promoting food. These seeds are small oil seeds originally from Middle East with high content of α-linolenic acid (ALA), omega-3 fatty acids, lignans phytoestrogens, soluble fiber, digestible proteins and phenolic compounds and in combination these ingredients improve digestion and bowel mobility (Prasad, 2009).

While the cocoa beans' prebiotic potential is associated with specific polyphenols, in the case of flax seeds it is the overall impact of the food that potentiates the positive environment within the gut microbiota. So, the prebiotic foods show high potential to improve health and well-being and even for preventing illness.

2.2.3. Mechanism of action and health benefits associated with prebiotics

The presence of prebiotics in the diet may lead to various health benefits. Over the last few years, clinical evidence has demonstrated positive effects on the gastrointestinal tract (Guarino et al., 2020; Liong, 2008; Moreno, Corzo, Montilla, Villamiel, & Olano, 2017), on the cardiovascular system (Aliasgharzadeh et al., 2015; Wu & Chiou, 2021), on mental health (Chen et al., 2017; Johnstone et al., 2021; Pourjafar, Ansari, & Vaccine, 2020; Schmidt et al., 2015) and on bone (Mtasher, Abdulhussein, & Mutlag, 2018; Pandey, Naik, & Vakil, 2015; Whisner et al., 2013). Figure 3 presents the principal postulated mechanisms of prebiotic action and some of the possible health benefits on the host's health.

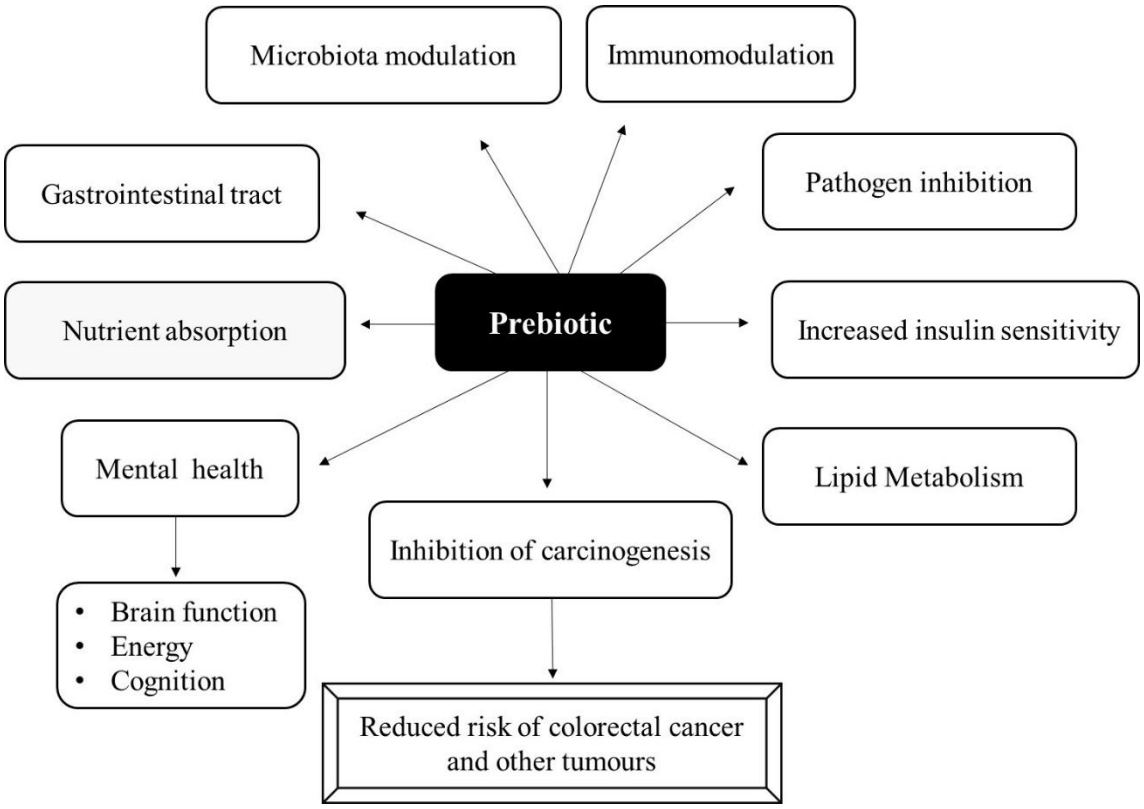

Figure 3. The possible health benefits and postulated mechanisms of action of prebiotics.

2.2.3.1. Modulation of the Gut Microbiota

The mechanism of a beneficial effect of prebiotics on immunological functions in the gut has not been established and possible hypotheses have been proposed. Currently, some studies have been indicated that the gut microbiota is deeply involved in the metabolic functions extending beyond the gut and prebiotics may play an important role in this system (Moreno et al., 2017). In addition, human clinical evidence has reported that prebiotics affect the composition of the gut microbiota. The non-digestible carbohydrate-based prebiotics can resist small-intestinal digestion and reach the colon intact, where they are efficiently metabolised leading to an increase in health-promoting organisms such as *Lactobacillus* and *Bifidobacterium* species (Guarino et al., 2020; Mtasher et al., 2018). In addition, some species are also able to ferment prebiotic substances and produce SCFAs (including acetate, butyrate, and propionate), which are important sources of energy for the host that can act to improve barrier function besides other benefits (Guarino et al., 2020). The chain length of the prebiotic can be considered an important factor for distinguishing between species, since although a few species can ferment substances with a high polymerization degree, such as inulin, several microorganisms are only able to

degrade prebiotics with polymerization degree below 10, such as FOS (Davani-Davari et al., 2019).

In relation to the metabolites produced during fermentation, butyrate is associated with different biological properties, however this metabolite is not an usual end-product from the fermentation of *Lactobacillus* and *Bifidobacterium* species. However, although *Bifidobacterium* species do not produce butyrate, they have been shown to stimulate butyrate-producing bacterial species such as eubacteria in the gut (Liong, 2008; Mtasher et al., 2018). Thus, the butyrate produced in the colon is related with the type of prebiotic compounds used (Liong, 2008). Gut microbial opportunists and pathogens are involved in the pathogenesis of several gastrointestinal disorders and different therapeutic strategies can help modify the negative effects caused by the gut microbiota imbalance. In this respect, the production of organic acids and the proliferation of beneficial bacteria through prebiotic modulation will result in a reduction in luminal pH, inhibiting growth of pathogens. Furthermore, nutrient availability as well adhesion receptors for pathogens are significantly reduced contributing to a reduced colonisation and infective capacity. Finally, the increase of beneficial bacteria will promote immunomodulation with the increase of secretory IgA that will reduce pathogens in the colon. Currently, there is an increasing interest in dietary strategies to modulate the composition and metabolic activity of gut microbiota, being dietary interventions using prebiotics one of the most applied (Moreno et al., 2017). Nevertheless, the prebiotics action on microbial diversity in the colon is still under debate (Guarino et al., 2020).

2.2.3.2. Inhibition of Colorectal Cancer

Evidence suggested that prebiotics can be protective against the development of cancer. Investigations have demonstrated that colorectal carcinoma occurs less commonly in individuals who often eat vegetables and fruit, and these effects can be commonly associated with prebiotics (Markowiak & Ślizewska, 2017). As already mentioned before, prebiotic compounds modify the microbiota by increasing the numbers of beneficial bacteria, such as *Lactobacillus* and/or *Bifidobacterium* species in the colon and often generate SCFAs, as acetate, propionate and butyrate, that have been shown to inhibit the development of the colon tumour cells (Mtasher et al., 2018). Furthermore, although the butyrate is produced in less amount (approximately 5% of total SCFAs), this metabolite has a particular interest, owing to its association with many biological properties in the colon and could have protective effects against the risk of colorectal cancer, as well as its

progression (Davani-Davari et al., 2019; Liong, 2008). In general, the products resulting from the prebiotics fermentation are mainly organic acids, leading to a decrease of the gut pH value. This alteration can modify the population of acid-sensitive species, such as *Bacteroides*, promoting the butyrogenic effect, through butyrate formation by *Firmicutes* (Davani-Davari et al., 2019). Moreover, when butyrate is present, apoptosis may be increased in transformed cells but inhibited in normal cells. Thus, an important mechanism of protection against carcinogen-enhanced colon cancer may be an enzyme induction by butyrate, or by the microbiota and increased activity by prebiotics (Wollowski, Rechkemmer, & Pool-Zobel, 2001).

2.2.3.3. Cardiovascular diseases

Some investigations have suggested that people consuming high amounts of prebiotics exhibited beneficial effects on cardiovascular diseases. Some possible explanations for these potential mechanisms of prebiotics have been suggested, such as modulation of gut microbiota and inflammatory responses (Wu & Chiou, 2021). In addition, the metabolites of intestinal microbiota, such as SCFAs have been demonstrated to be inversely associated with some risk factors for cardiovascular diseases. The production of SCFAs can improve cardiovascular homeostasis, indicating a potential target for reducing cardiovascular risk (Wu, Xu, Tu, & Gao, 2021). Previous studies using animal models showed that acetate and propionate are able to regulate blood pressure (Natarajan et al., 2016), while butyrate compounds attenuated blood pressure in angiotensin II-induced hypertensive rats, mainly via reducing the expression of renal protein receptors and renin (Wang et al., 2017). Therefore, prebiotic compounds and their metabolites (SCFAs) in the gut, exhibit beneficial effects on cardiovascular diseases through several mechanisms involved in alteration of the gut environment, histone deacetylation, improvement of gut epithelial permeability contributing to reduced total and LDL cholesterol and hs-CRP, and lowering the incidence of risk factors in cardiovascular diseases such as hypertension, obesity, and diabetes (Wu & Chiou, 2021).

Additionally, in a previous clinical trial performed by Aliasgharzadeh et al. (2015), the effects of oligofructose-enriched inulin were evaluated on some cardiovascular disease risk factors in women with type 2 diabetes. Based on these results, oligofructose-enriched inulin supplementation may improve glycaemic indices, lipid profiles, antioxidant status and decrease the malondialdehyde (a marker of lipid peroxidation and oxidative stress) concentrations in type 2 diabetic patients. Many studies have established the association

between inflammation and cardiovascular risks. Thus, prebiotics have a positive effect in preventing the development of these diseases as a result of their anti-inflammatory action (Wu & Chiou, 2021).

2.2.3.4. Mental health

In recent years, studies have been shown that prebiotics can also have an important role in neuro-immune processes (Pourjafar et al., 2020). The gut microbiota plays a role to understand the factors that influence brain function and behaviour, and the effects of prebiotics on brain health are related to the interactions between gastrointestinal microbiota and immune and nervous systems (Johnstone et al., 2021; Pourjafar et al., 2020). The gut and the brain are deeply connected via the gut-brain axis, which involves bidirectional communication via neural, endocrine and immune pathways and the study of their role in neuropsychiatric diseases is rapidly advancing (Pourjafar et al., 2020). Thus, fostering a beneficial gut microbiome with prebiotics, such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and inulin, is a viable approach, but under-investigated (Chen et al., 2017). In addition, nutrition has been increasingly recognized as an important factor that intermediates the composition and metabolic function of the gastrointestinal microbiota, and the ingestion of prebiotics is a path that modulates the microorganisms in the gastrointestinal tract (Pourjafar et al., 2020).

Previous findings have demonstrated that prebiotic treatment can change the behaviour across domains related to depression, anxiety, stress reaction, cognition, and social behaviour, thereby opening new prospects in the field of nutritional neuropsychopharmacology (Chen et al., 2017; Pourjafar et al., 2020). The effects of two prebiotics (FOS and Bimuno-galacto-oligosaccharides (B-GOS)) on the secretion of the stress hormone, cortisol and emotional processing in healthy adults was investigated over 3 weeks by Schmidt et al. (2015). The results showed a lower secretion of the stress hormone in the presence of the prebiotic compound in comparison to the placebo. The volunteers also exhibited decreased attentional vigilance to negative versus positive information in a dot-probe task after intake of B-GOS compared to placebo. However, no effects were found in volunteers after intake of FOS. In another study using a rodents model, Burokas et al. (2017) also studied FOS and GOS, and tested whether chronic prebiotic treatment modifies behaviour across domains relevant to anxiety, depression, cognition, stress response, and social behaviour. The results exhibited antidepressant and anxiolytic effects. In addition, the administration of GOS and the combination of FOS

and GOS reduced stress-induced corticosterone release. Thus, these data suggest a beneficial role of prebiotic treatment for stress-related behaviours. But, although prebiotics and probiotics can be offered as new potent antidepressants, more investigations are needed for confirmation.

2.2.3.5. Mineral absorption

Minerals are the micronutrients required for the smooth functioning of the body (Pandey et al., 2015). Previous works have demonstrated that the most significant health effects of prebiotics on mammalian physiology is the capacity to improve calcium, magnesium, iron and zinc absorption, and the attendant enhancement of bone mineralization (Mtasher et al., 2018). There are clinical trials on the impact of different prebiotics on calcium absorption, but the results are conflicting. In a previous study, Abrams et al. (2005) showed that prebiotic short- and long-chain inulin-type fructans increase calcium absorption and enhance bone mineralization during pubertal growth. However, in another study using milk enriched with FOS by López-Huertas et al. (2006) no significantly increased calcium absorption was observed. However, in another study, Whisner et al. (2013) investigated the dose-response relationship of GOS supplementation on calcium absorption in adolescent girls, and the results indicated that daily consumption of 5 g of GOS improved calcium absorption, which may be mediated by the gut microbiota. Many investigations have suggested that a fraction of calcium is absorbed from the colon, as a consequence of the fermentation of prebiotics by the intestinal microbiota. The fermentation leads to the production of SCFAs which lower the intraluminal pH generating an acidic environment where the insoluble and unabsorbed calcium is converted to the ionic soluble form. So, low intraluminal pH and SCFAs, lead to an expansion of the surface area of the intestine enabling improved calcium absorption (Cashman, 2003; Pandey et al., 2015).

2.2.4. Effects of prebiotics on the growth/survival and metabolism of probiotics

As previously described in section 2.2.1 and Figure 2, a compound can be considered a prebiotic if it follows the established requirements of being fermentable by the intestinal microbiota and stimulating bacteria in a selective way (only probiotics). In other words, a prebiotic must be capable of being fermented by saccharolytic bacteria (e.g. *Bifidobacterium* genus).

Saccharolytic bacteria are in general present in the bowel and can metabolize complex carbohydrates leading to bacterial multiplication, associated energy production and to the production of specific metabolites such as the previously mentioned SCFAs (formic, acetic, propionic, butyric, isobutyric, valeric, isovaleric and 2-methylbutyric acids) besides other organic acids (lactic, pyruvic and succinic acids) and ethanol (Loo, Clune, Bennett, & Collins, 2005; Miller & Wolin, 1979). These metabolites are of high importance in the regulation of gut health and functions including mucus barrier function of the gut epithelia, regulation of glucose and lipid metabolism, satiety and homeostasis (Cunningham et al., 2021). All of the aforementioned metabolites are essential for the interconnection of glycolysis pathways, more specifically the tricarboxylic acid/Krebs cycle, oxidative phosphorylation (OXPHOS), as well as, amino acids and fatty acids metabolisms, leading to a direct impact on the immune cells, mainly macrophages, which respond to different physiological and pathological stimulations, acquiring at the end, specific functions. The two most important types of macrophages are controlled by the different metabolism consumption of glucose (Belizário, Faintuch, & Garay-Malpartida, 2018). Thus, it is of high importance to understand the glucose metabolisms promoted by the fermentation of both *Bifidobacterium* and *Lactobacillus* genus.

Novel sources of prebiotic compounds also play a supportive role in what concerns metabolic activity of probiotic strains. As previously described pineapple peel juice played an important role on the fermentation capacity of three types of *Bifidobacterium* species and three types of *Lactobacillus* species (Gómez-García et al., 2022). The pineapple peel juice was characterized in terms of polyphenols where chlorogenic, ferrulic and caffeic acids were the main compounds found and in the case of carbohydrates, trisaccharides and oligosaccharides were the most common. *Bifidobacterium* species were able to ferment the pineapple peel juice and promote the production of significant levels of lactic and acetic acids in the medium. Furthermore, the samples also promoted the growth of *Lactobacillus* strains tested, which was also proven by the production of lactic acid and reduction of glucose in the medium. Although the fermentations were performed in an isolated single-strain form and the impact for the combined fermentation of strain consortia was not assessed, other studies have demonstrated the positive impact of prebiotic foods on the combined fermentation of *Lactobacillus* and *Bifidobacterium* genera (Campos, Ribeiro, Teixeira, Pastrana, & Pintado, 2020).

Schley & Field (2002) described that the direct immunological impact was not so clear as published by other authors, but described several possible models that empower the beneficial effect of prebiotics: i) regulation action upon hepatic lipogenic enzymes promoting the production of SCFAs; ii) butyric acid (from SCFAs) as a modulator of histone acetylation; iii) modulation of mucin production; iv) promotion of an increased amount of lymphocytes and/or leukocytes in the gut-associated lymphoid tissues (GALTs)[1] and peripheral blood; v) increase secretion of IgA by GALTs can stimulate the function of phagocytosis of the intra-inflammatory macrophages (Markowiak & Ślizewska, 2017).

But further work is required to enable a full comprehension of the prebiotic metabolism promotion and consequential impact in the associated cascade of signals and activation within the gut epithelia and immunological system. Nevertheless, it has been shown that the different glucose metabolisms are important and that prebiotics are able to promote the proliferation and metabolic modulation of positive bacteria in the human gut, as well as the impairment of non-probiotic bacteria.

3. Synbiotic emergent association between probiotics and prebiotics

Considering the recognised beneficial effects of both probiotics and prebiotics, their combination in a single product comes as a natural step, in an attempt to potentiate their impact in human health. Since the introduction of the term "synbiotic" by Gibson and Roberfroid, in 1995, the definition has evolved to designate these combined products, which are now defined as "a mixture comprising live microorganisms and substrate(s) selectively utilized by the host microorganisms that confers a health benefit on the host" [Figure 4; (G. R. Gibson & Roberfroid, 1995; Swanson et al., 2020)]. In this context, "host microorganisms" include both the resident/colonising microorganisms (autochthonous) and the externally applied (allochthonous), such as probiotics, that may be transient in the host microbiota (Swanson et al., 2020). Several types of probiotic and prebiotic combinations fall within this definition of synbiotics, which can be divided into two main groups: complementary synbiotics and synergistic synbiotics [Figure 4; (Cunningham et al., 2021; Swanson et al., 2020)]. Complementary synbiotics result from

[1] GALTs (Gut Associated lymphoid tissues) it is a very important structure present at the gut, being the biggest in size at the human body, responsible for the management of the immune response to the massive antigen exposure experienced, while maintaining a potent adaptive immune response to protect the host from mucosal pathogens.

the combination of accepted prebiotics and probiotics, meaning that each component must meet the criteria established for its own category; their mechanisms of action can be independent from each other, and each part must have its specific demonstrated health benefits. Despite that, evidence that the combination of both provides a health benefit in the target host is also required for the categorisation as a synbiotic (Cunningham et al., 2021; Swanson et al., 2020). Concerning the synergistic synbiotics, the substrate is designed to selectively support the growth or activity of the co-administered live microorganism(s), which, in turn, is selected based on its ability to provide a health benefit. Although their combination must have a demonstrated health benefit, the substrate and the live microorganism might not be able to elicit a health benefit independently from each other, which means that their previous establishment as probiotics or prebiotics is not required (Cunningham et al., 2021; Swanson et al., 2020).

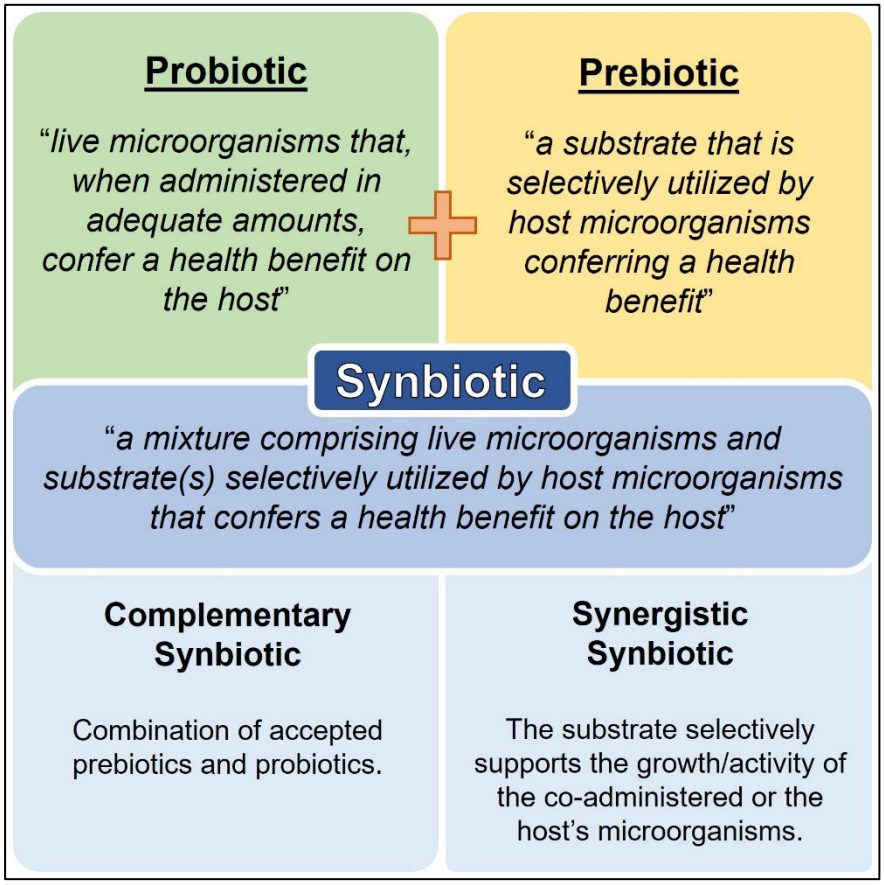

Figure 4. Definition of synbiotics. The production of synbiotic products appears as a natural evolution upon the establishment of probiotics and prebiotics (on the top). Two types of synbiotics were defined: complementary and synergistic synbiotics (on the bottom). Complementary synbiotics result from the direct combination of pro- and prebiotics, while synergistic synbiotics' components might not correspond to previously defined probiotics and prebiotics.

Regarding the characterisation of a synbiotic, it is required to ensure its safety and stability, starting with the characterisation of both the live microorganism and the substrate components. Even if not categorised as so, the live microorganism counterpart must meet most of the criteria required for a probiotic strain to be used for human consumption: it should be clearly identified, to the strain level; it should be deposited into at least a recognized international culture collection and its genome sequence and respective annotation should be available in public databases. In parallel, it should be assessed in terms of safety, both phenotypic and genotypically, concerning the risk of toxin production and resistance genes transferability. Also, the purity of the strain and its potency and specific attributed benefits must be assessed (Sanders et al., 2014; Swanson et al., 2020). In what concerns the substrates, their characterisation must include the appropriate chemical analysis to determine their structure and purity, including for the presence of contaminants of microbial origin, among others. Regarding substrates that might be digested and absorbed during the oro-gastro-intestinal tract, the actual amount of product that is delivered to the target should also be assessed and considered when calculating the dosage required to yield a given effect. Finally, the components of the synbiotic must be stable to ensure that the right dosage of live microorganism and substrate (if that is the case) reach the target in the gastrointestinal tract and exert their expected health benefit. This must consider the specific requirements of the components and also the production constraints, distribution chain and intended shelf-life of the final product, and ultimately, its passage through the gastrointestinal tract itself. This is particularly relevant for the live microorganisms, whose viability is dependent on several factors, including the matrix nature and water content, storage temperature, pH and oxygen levels. The safety of the synbiotic should also be assessed according to the legislation required for the intended use and country of commercialisation (Swanson et al., 2020).

As mentioned, even if the beneficial effects of the substrate and/or live components were already demonstrated, the effect of their combination must also be assessed, through specifically designed trials to demonstrate their health benefit in the host (Klurfeld et al., 2018; Moher et al., 2012). In an ideal synbiotic, the health benefit should be increased when comparing to the sum of its individual components. However, this might not happen with some synergistic synbiotics, since the observed effect might be delivered by the live microorganism, whose survival and growth were, in turn, supported by the substrate. For

synergistic probiotics, besides the health benefit on the host, appropriate studies must be conducted to demonstrate the utilisation of the substrate, either by the host microbiota or by the probiotic counterpart of the synbiotic itself (Swanson et al., 2020). The assessment of the synbiotic's activity is also essential to exclude potential – although improbable – antagonistic effects of the combination, which may decrease the expected health outcomes (Swanson et al., 2020). For instance, the metabolization of some carbohydrate substrates can increase the production of antimicrobial compounds by the probiotic strains, which might have a negative impact on the commensal microbiota upon delivery to the gut (Tzortzis, Baillon, Gibson, & Rastall, 2004).

Several randomized controlled trials were conducted in humans in the recent years to assess the health benefits of emerging synbiotics. These trials focus on a vast range of pathologies, which include overweight and obesity (Amir Hadi, Alizadeh, Hajianfar, Mohammadi, & Miraghajani, 2018), type 2 diabetes (Mahboobi, Rahimi, & Jafarnejad, 2018; Nikbakht et al., 2018), non-alcoholic fatty liver disease (A. Hadi, Mohammadi, Miraghajani, & Ghaedi, 2019; Sharpton, Maraj, Harding-Theobald, Vittinghoff, & Terrault, 2019), irritable bowel syndrome (Chlebicz-Wójcik & Śliżewska, 2021; Ford, Harris, Lacy, Quigley, & Moayyedi, 2018), surgical infections (Kasatpibal et al., 2017; Skonieczna-Żydecka et al., 2018), chronic kidney disease (McFarlane, Ramos, Johnson, & Campbell, 2019; Pisano, D'Arrigo, Coppolino, & Bolignano, 2018), and atopic dermatitis (Chang et al., 2016). The most used bacterial strains belong to the classical genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus*, and are usually applied as consortia instead of single-strain applications (Gyawali et al., 2019; Swanson et al., 2020). However, some next-generation probiotics are also being employed, namely *Akkermansia muciniphila* and *Anaerobutyricum hallii* (Perraudeau et al., 2020). Regarding the substrates, the most used are usually galacto-oligosaccharides, inulin or fructo-oligosaccharides, administered in variable doses [

547 Table **1.** Examples of clinical trials involving probiotics and their human health benefits,
548 published in 2021.
549

Target condition	Probiotic	Key findings
Anxiety and depression	*Lactobacillus plantarum* PS128	Daily administration of this probiotic m symptoms, fatigue level, cortical excita in sleep quality during the deep sleep s
Antibiotic-induced faecal short-chain fatty acid (SCFA) and microbiota composition disruptions	*Bifidobacterium animalis* subsp. *lactis* BB-12	Simultaneous administration of amoxic 12 yogurt to healthy participants was re smaller diminution in the faecal SCFA taxonomic profile of the microbiota ov group.
Type 2 diabetes	*Lactobacillus plantarum* HAC01	An eight-week course of *L. plantarum* significantly improved certain metaboli 2h-postprandial glucose levels) relative individuals and there were no reports o effects.
Constipation	*Lacticaseibacillus paracasei* strain Shirota	Daily consumption of this probiotic for alleviate constipation and improve the symptoms in patients with depression a IL-6 levels. Furthermore, this probiotic appeared to regulate the gut microbiota disease.
Rheumatoid arthritis	*Lactobacillus acidophilus* La-14, *Lactobacillus casei* Lc-11, *Lactococcus lactis* Ll-23, *Bifidobacterium lactis* Bl-04 and *Bifidobacterium bifidum* Bb-06	The mixture of these probiotic strains r biomarkers and enhanced oxidative/nitr with rheumatoid arthritis.
Stress	*Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, *Levilactobacillus brevis*	The intake of beverage fermented with may reduce the stress and modulate the (correcting dysbiosis) in university stud with their lifestyle or diet.
Allergic rhinitis	*Bifidobacterium animalis* subsp. *lactis* BB12 and *Enterococcus faecium* L3	When administered as a prophylactic tr these probiotic strains reduced signs an rhinitis and diminished the need of con
Atopic dermatitis and cow's milk protein allergy	*Lactobacillus rhamnosus* ŁOCK 0900, *Lactobacillus rhamnosus* ŁOCK 0908, and *Lactobacillus casei* ŁOCK 0918	Supplementation of the children's diet preparation for three months led to a sig atopic dermatitis symptom severity. Fu preparation is safe, and triggers benefit sensitised patients.
Fibromyalgia syndrome	*Lactobacillus rhamnosus* GG, *Lactobacillus paracasei*, *Lactobacillus acidophilus* and *Bifidobacterium bifidus*	Probiotic treatment produced an improv reducing errors on an attention task, bu memory, in patients with fibromyalgia
Irritable bowel syndrome	*Bacillus coagulans* LBSC	Probiotic intervention was safe for hum efficacious in relieving overall pathoph irritable bowel syndrome and thereby i quality of life.

Target condition	Probiotic	Key findings
Halitosis	*Weissella cibaria* CMU	Oral ingestion of *W. cibaria* CMU may subjective halitosis and improve oral-h life, being reported the possibility of or college students without any difficultie
Pancreatitis	*Bacillus subtilis* and *Enterococcus faecium*	Probiotic supplementation seemed to b reducing the duration of hospitalization acute pancreatitis.
Community-acquired common colds	*Lactiplantibacillus plantarum* HEAL9 and *Lacticaseibacillus paracasei* 8700:2	Intake of this probiotic product may be multiple colds in subjects prone to getti
Constipation, diarrhoea, acute respiratory infections and nutritional status	*Lactobacillus casei* strain Shirota	Daily intake of fermented milk contain bacterium strongly prevented the incide acute respiratory infections in Vietnam Furthermore, this probiotic drink may c prevention and nutritional status impro

; (Gyawali et al., 2019; Swanson et al., 2020)]. A case-by-case optimisation is expectedly required, considering not only the demands of the microorganisms to be administered or enhanced in the host's gut, but mainly because each individual has its unique gut microbiome and even its genetic predispositions can impact the viability and effectiveness of the administered probiotics, prebiotics and synbiotics (Peng et al., 2019). Additionally, diverse combinations of bacterial strains and substrates will work differently on diverse dysbiotic states, considering their specific characteristics and those of their resulting by-products and metabolites (Cunningham et al., 2021).

Table 1. Examples of clinical trials involving synbiotic mixtures in the amelioration of several conditions, run in the last 4 years (2018-2021). Only studies referring explicitly the term "synbiotic" were considered. When available, the CFU numbers and mass, for the microorganisms and substrate counterparts, respectively, are presented.

Microorganism(s) [concentration]	Substrate(s)	Conditions	Main conclusions	Type of study	Other info	Reference
Lactobacillus acidophilus, *Bifidobacterium lactis*, *Bifidobacterium bifidum*, *Bifidobacterium longum* [10^9 CFU each]	Inulin-based prebiotic	Type 2 diabetes	Significant decrease in glycemic biomarkers; decreased insulin resistance	Randomized, double-blind clinical trial	24 weeks; 120 participants from Iran	(Nasri et al., 2018)
Lactobacillus casei, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Streptococcus thermophilus* [10^9 CFU]	Fructo-oligosaccharide	Non-alcoholic fatty liver disease (NAFLD)	Significant improvement in liver enzymes (in combination with sitagliptin)	Randomized, double blind trial	16 weeks; 138 patients from Iran	(Sayari, Neishaboori, & Jameshorani, 2018)
Bifidobacterium animalis subsp. l*actis* (strain BB-12) [10^8 CFU/mL] + yogurt starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*)	Inulin (1.5g)	NAFLD	Synbiotic yogurt improved hepatic steatosis and liver enzyme concentrations	Open-label randomized controlled clinical trial	24 weeks; 90 patients from Iran	(Bakhshimoghaddam, Shateri, Sina, Hashemian, & Alizadeh, 2018)
Lactobacillus paracasei, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*,	Fructo-oligosaccharide (6 g)	Brain disorders	Weak effects in the management of	Secondary study from a broader randomized,	24 weeks; 49 elders from Brazil	(Louzada & Ribeiro, 2018)

Microorganism(s) [concentration]	Substrate(s)	Conditions	Main conclusions	Type of study	Other info	Reference
Bifidobacterium lactis [10^9 – 10^8 CFU each]			depressive symptoms or cognitive decline	double-blind controlled clinical trial		
Lactobacillus acidophilus (strain T16), *Lactobacillus casei* (strain T2), *Bifidobacterium bifidum* (strain T1) [2×10^9 CFU/g each]	Inulin (800mg)	Polycystic ovary syndrome (PCOS)	Improved PCOS-associated insulin resistance; demonstrated synbiotic activity higher than probiotics themselves	Randomized, double-blind, placebo-controlled clinical trial	12 weeks; 60 patients from Iran	(Samimi et al., 2019)
Lactobacillus rhamnosus, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus salivarius*, *Bifidobacterium bifidum*, *Bifidobacterium longum* [1×10^{13} CFU]	Fructo-oligosaccharide (175 mg), *Ulmus davidiana* (150 mg), *Geum urbanum* (10 mg), Inulin (100 mg)	Irritable bowel Syndrome (IBS)	Decrease the fatigue in IBS individuals; relief in abdominal pain/discomfort, bloating, stool patterns	Randomized, double-blind, placebo-controlled clinical trial	8 weeks; 28 patients from Korea	(S.-H. Lee et al., 2018)
Bifidobacterium lactis (DSMZ 32269), *Bifidobacterium longum* (DSMZ 32946), *Bifidobacterium bifidum* (DSMZ 32403), *Lactobacillus rhamnosus* (FloraActive 19070-2), *Lactobacillus acidophilus* (DSMZ 32418) [5.0×10^9 CFU]	Short chain Fructo-oligosaccharide (947 mg)	IBS	Attenuation of IBS symptoms such as pain, flatulence, stool pressure, feeling of incomplete bowel movements; decreased severity of IBS symptoms	Randomized, double-blind, placebo-controlled, parallel group trial	8 weeks; 68 patients from Poland	(Skrzydło-Radomańska et al., 2020)

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The synbiotic formulations can be delivered to the consumer as food products, dietary supplements or drugs (Andrade et al., 2020). Currently, there are several products in the market, sold as dietary supplements under the label "synbiotics". However, in many of them, the following disclaimer may be read: "Statements regarding dietary supplements have not been evaluated by the FDA and are not intended to diagnose, treat, cure, or prevent any disease or health condition." In fact, the FDA is the authority that regulates both the food and drug markets in the United States of America, whereas in the European Union, the EFSA establishes the rules for the introduction of novel foods, while drugs are regulated by the European Medicines Agency (Cordaillat-Simmons, Rouanet, & Pot, 2020; O'Toole, Marchesi, & Hill, 2017). There are clear and well-established definitions for both food supplements – which are intended to maintain or improve a healthy state in a healthy or at-risk population – and drugs, with an expected therapeutic or prophylactic effect in human diseases (Cordaillat-Simmons et al., 2020). In addition, together with a distinct definition, specific requirements are established for each product category. However, concerning the dietary supplements, other regulations apply. There is no global consensus on what should or should not be part of this category; a substance might be considered a dietary supplement in a certain region but fall into another class of products if considering other jurisdictions, with consequent changes in the legislation to be applied. Although the safety concerns must always be addressed, some of the claimed health benefits might result more from empirical observation than from exact scientific evidence (Dwyer, Coates, & Smith, 2018). It is also important to mention a novel product category, proposed by the FDA in 2012, and accepted in 2019 in Europe, designated as Live Biotherapeutic Product (LBP): "a biological product that: 1) contains live organisms, such as bacteria; 2) is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and 3) is not a vaccine" (European Pharmacopoeia Commission, 2019; FDA, 2016). This definition, and respective evaluation criteria, can be applied to probiotics and synbiotics – but not to prebiotics. Regardless of the final formulation and application, products with the claim "synbiotics" should be attentively considered, as some lack a proper confirmation of the substrate utilisation (either by the co-administered or the host's microorganisms), making them a simple mixture of probiotics and prebiotics, not falling within the real definition of synbiotic.

4. Conclusion and future perspectives

Probiotic microorganisms, prebiotic substances and synbiotic products have gained a high interest among the scientific community, clinicians, and food and pharmaceutical industries. In fact, several scientific reports, including some clinical trials, have supported the use of probiotics, prebiotics and synbiotics as an efficient health-promoting strategy. Despite these advances in knowledge, the molecular mechanisms underlying the beneficial effects of these products have not yet been fully elucidated. In this scope, future studies aiming a comprehensive and in-depth analysis regarding the mechanisms of action underlying their health benefits should be carried out. Furthermore, additional clinical trials concerning the "best" probiotic, prebiotic and synbiotic products, would be of extreme importance, in terms of efficacious dose, duration of treatment, efficiency, tolerance and safety, for a given health condition and considering the consumer's unique gut environment. Indeed, such data would facilitate the selection process of these products for both the industry and the consumers.

Note: Several *Lactobacillus* species mentioned throughout this chapter have been recently classified. To facilitate the reading, the species were referred here as in the original papers. The correspondent nomenclature is as follows (the former nomenclature appears first followed by the new name):

Lactobacillus casei = *Lacticaseibacillus casei*

Lactobacillus paracasei = *Lacticaseibacillus paracasei*

Lactobacillus rhamnosus = *Lacticaseibacillus rhamnosus*

Lactobacillus plantarum = *Lactiplantibacillus plantarum*

Lactobacillus brevis = *Levilactobacillus brevis*

Lactobacillus salivarius = *Ligilactobacillus salivarius*

Lactobacillus acidophilus = *Lactobacillus acidophilus*

Lactobacillus delbrueckii subsp. *bulgaricus* = *Lactobacillus delbrueckii* subsp. *bulgaricus*

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