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## Biochemical parameters and potential health benefits of functional probiotic chocolates: A systematic review

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### Abstract

Consumption of probiotic functional foods, i.e., foods with probiotic effects and special dietary effects with health impacts are getting an increased demand day by day. In this progression, it may be noted that chocolate. The most delicious desert to be known worldwide is also getting evolved and becoming well known for its functional values. Life microorganisms i.e. probiotics are being used in chocolate preparations along with other conventional ingredients. The main aim of this evolution in the dairy industry is to impart several healthcare benefits to local mass of population and to popularize this probiotic product for improvement of health. In this systematic review, the effectiveness of probiotics functional chocolate in the treatment of different diseases and disorders was evaluated using different double-blind studies. This systematic review will be presented with multiple healthful effects of chocolates containing probiotics and functional foods.

**Keywords:** Algae-based probiotics, antibiotic associated diarrhoea, colorectal cancer, gut microbiota, irritable bowel disorder, and microencapsulation

### Introduction

Chocolate is one of the most delicious food products which is hugely popular among all age groups. A variety of chocolates has evolved over the years in satisfying taste, odour, colour, texture, and appearance of various consumer groups. In 1900 BC, chocolate yielding was first started by the Inca civilisation people in Mesoamerica which is modern day South America (Picher, 2023) [39]. Unlike today's era, chocolate was prepared in the form of fermented beverages during ancient times (Predan *et al.*, 2019) [23].

Evolution of making chocolate on an industrial scale has led to the use of various materials such as cocoa seeds, cocoa butter, iced sugar, roasted kernels, dairy products such as milk, dried fruits, and nuts as ingredients in modern day production of chocolate. Chocolate is generally sweet in taste despite the presence of materials such as bitter cocoa seeds, thus the flavour found in chocolate is produced by a fermentation process (Bussy *et al.*, 2019) [6]. There are various types of chocolates in the market such as white milk chocolate, dark chocolate, baked chocolate etc. (Selvasekaran & Chidambaram, 2021) [29]. However, chocolate has several disadvantages too. The chocolates easily available in the market contain a high amount of fat, sugar as well as preservatives thus regular consumption of these products can lead to various health complications such as increased blood sugar levels, increased cholesterol, and an increase in triglyceride levels; which in the long run causes obesity and other ailments such as 'Kidney Stone' development (Tan *et al.*, 2021) [33]. Keeping these in mind, researchers have invented chocolates with functional enhancements by adding beneficial ingredients such as probiotics, i.e., favourable live bacteria. This article will be presented with multiple positive physiological effect of chocolates and their biochemical aspects which contain probiotics with different functional foods.

### Functional Probiotic Chocolates and human health

FAO of the United Nations and the World Health Organization, have defined probiotics as the microorganisms which are live with ability of withstanding with acidic nature of stomach due to presence of several gastric & pancreatic juices, also capable of colonising inside the Gastro Intestinal tract of the host body and potentially favourable for improving intestinal health, hence improving overall health status of the person (Binda *et al.*, 2020) [5].

Human intestine is the harbour of several microorganisms, they may be delivering beneficial effects and some of these may be pathogenic too. When the pathogenic microorganisms get increased in number several health complications start arising, e.g. gastric disturbances, diarrhoea, constipation, Colic in infants, candida infections, *H. pylori* bacterial infections, Colorectal Cancer, Diabetes mellitus type II and many more (Alveraz *et al.*, 2021) [3]. The root cause of these is malabsorption of nutrients through the intestinal inner lining, due to increased pathogenicity of small intestine, termed as dysbiosis (Singh *et al.*, 2021) [31].

Administration of probiotics is the new age dietary treatment, which has been proved to be successful to combat against dysbiosis and to balance the flora of human guts. Probiotic enriched chocolate is one of the delicious yet healthy dietary healing product to improve and balancing the gut health and to cure other health related complications (Savitha & Sankaranarayanan, 2021) [28].

The food industries around the globe are dedicating their time and passion for the invention of these functional chocolates to deliver the health benefits by using the strains of pre-detected probiotics. Some the probiotic strains with intention to impart positive healthful effects are- *Lactobacillus rhamnosus GG*, *Lactobacillus reuteri*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Lactobacillus acidophilus* etc. (Sanders *et al.*, 2019) [26].

Microencapsulation, is one of the technologies, invented to induce these microbial strains into food product developments (Yao *et al.*, 2020) [38]. These health benefits are very much specific with selective microbial strains, will be discussed below.

### Health Benefits of chocolate containing probiotics and functional foods

Several studies reveal the potential positive effects associated with the ingestion of probiotics in capsule form or food products developed enriched with probiotics bacteria. With the increasing consumer's demand, the commercial yielding of probiotic enriched foods also getting a noticeable productivity. As this probiotic chocolate production is showing a wide range of beneficial effect on

human health, so that it gains its pharmaceutical interests too (Ramlucken *et al.*, 2021) [24]. In case of patients with gastroenterological disorders, probiotic shows a healing effect over it by acting over gut microbiota. Intestinal ecosystem shows significant improvements by consumption of probiotic enrich chocolate or food products (Forssten *et al.*, 2020) [11]. Also, in case of irritable bowel syndrome probiotic chocolates show improvement by decreasing the colonic contraction. Probiotic chocolates are meant to be healthful impacts over gut disorders, colorectal cancer, diarrhoea etc. (Sharifi-Rad *et al.*, 2020) [30].

Prospects of Probiotic chocolates would turn functional foods to a new dimension, those products are even fortified with omega-3 fatty acids, which may deliver quite positive benefits for combating cardiovascular disease related to high level of triglycerides (Faccinetto-Beltrán *et al.*, 2021) [10]. These health benefits of probiotic functional chocolates are being enlisted in this paper.

Intestinal ecosystem shows significant improvements by consumption of probiotic enrich chocolate or food products.

### Against Lactose intolerance

A physiological condition known as lactose intolerance occurs in people who are unable to produce the enzyme lactase, also known as  $\beta$ -galactosidases (Ibrahim *et al.*, 2013) [13]. This lactase must be divided into glucose and galactose in order to assimilate the disaccharide in milk. Lactase deficiency prevents people from digesting milk, which frequently causes issues for newborns. Individuals who suffer from lactose intolerance may experience symptoms such as nausea, vomiting, cramps, diarrhoea, flatulence, and abdominal pain (Tewari *et al.*, 2019) [35]. Low levels of lactase enzyme activity contribute to the reduced lactose digestion of over 60% of the human population. Probiotics are live microorganisms that augment the natural microbiota in the gastrointestinal tract. Research has demonstrated that probiotics have a number of advantageous health effects, including bettering immune function, lowering blood cholesterol, and enhancing intestinal health. Probiotic bacteria found in fermented and unfermented milk products have been linked to a reduction in the clinical symptoms of Lactose Intolerance (LI), according to mounting data (Oak *et al.*, 2019) [21].

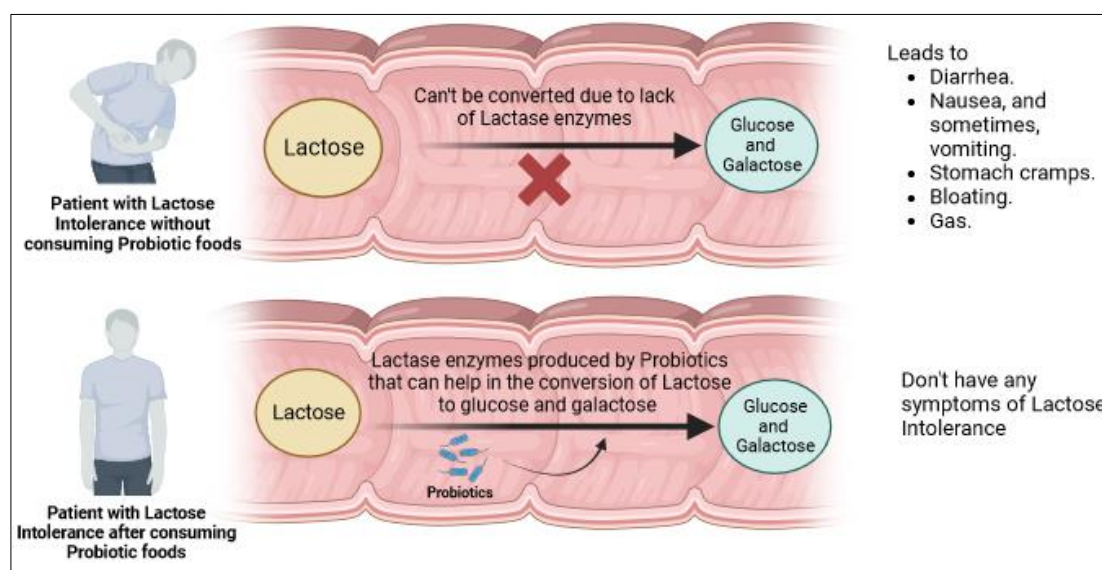
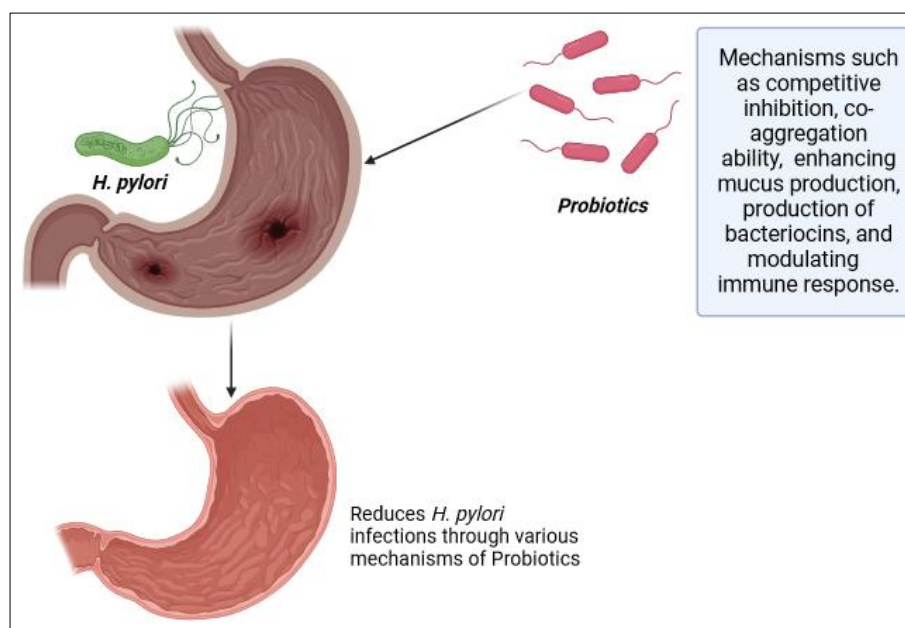


Fig 1: Probiotics against Lactose intolerance

### Against gastric disturbances

Several clinical researches and trails show that probiotics have extensive benefit over the curing of gastric disturbances. *Helicobacter pylori* is one of the leading causes of gastric ulcer and peptic ulcer, including antibiotic associated diarrhea, gastroenteritis. Studies show the result about complete eradication can be possible by consumption

of probiotics chocolate containing the strains, such as *Lactobacillus*, *Bifidobacterium* and *L. johnsonii* (Koga, 2022) [17]. By dietary treatments with aforementioned strains of probiotics, mitigation of the negative effects of *H. pylori* through the favourable colony formation in epithelial or mucosal cells and production of organic acids, is possible for long term health benefits (Chakravarty & Gaur, 2019) [7].



**Fig 2:** Mechanism of probiotics against gastric disturbances

### Against Baby Colic

Colic is extensive crying and fussiness normally can be seen in the infants, for several hours for no obvious reason. Colicky babies often cry from 6 p.m. to midnight. Colicky crying is louder, more high-pitched, and more urgent sounding than regular crying. Colicky babies can be very hard to calm down.

As chocolates are already popular and appealing food item among children, so probiotic chocolates will be a good choice for treatment approach, carrying the strains of *Lactobacillus casei*, *L. rhamnosus*, *S. thermophilus*, *B. breve*, *L. acidophilus*, *B. infantis*. Chocolates containing these probiotics are proven to show effective results on reducing colic in infants and children (Ong *et al.*, 2019) [22].

### Against Diabetes and Obesity

Type II Diabetes mellitus and obesity both are metabolic disorders, can be associated directly or indirectly on the absorption rate of the nutrients we consume, on the metabolic rate and on the insulin, resistance develops due to various causative features.

So here the nutrient absorption plays an important key role for the treatment and healing and to recovering these disorders. Probiotic act as a healing agent by improving the small intestinal microbiota, by destroying the pathogenic microbes and by increasing the colonies of epithelium and mucosal lining in the small intestine (Tao *et al.*, 2020) [34]. Once the intestinal health improves, carbohydrate absorption and assimilation will get a potentially good effect, which ultimately helps in increased secretion of insulin, which shows a change of blood glucose level in diagnostic investigation reports of Oral glucose tolerance

tests (OGTT), post prandial glucose testing and, in haemoglobin A1c (HbA1c) (Wang *et al.*, 2021) [36s].

Probiotic bacteria enriched chocolates containing the strains such as *Lactobacillus acidophilus* NCFM, *Lactobacillus gasseri* SBT2055, *Lactobacillus rhamnosus* chocolate shows significant differences in improvement of obesity and diabetes (Abenavoli *et al.*, 2019) [1].

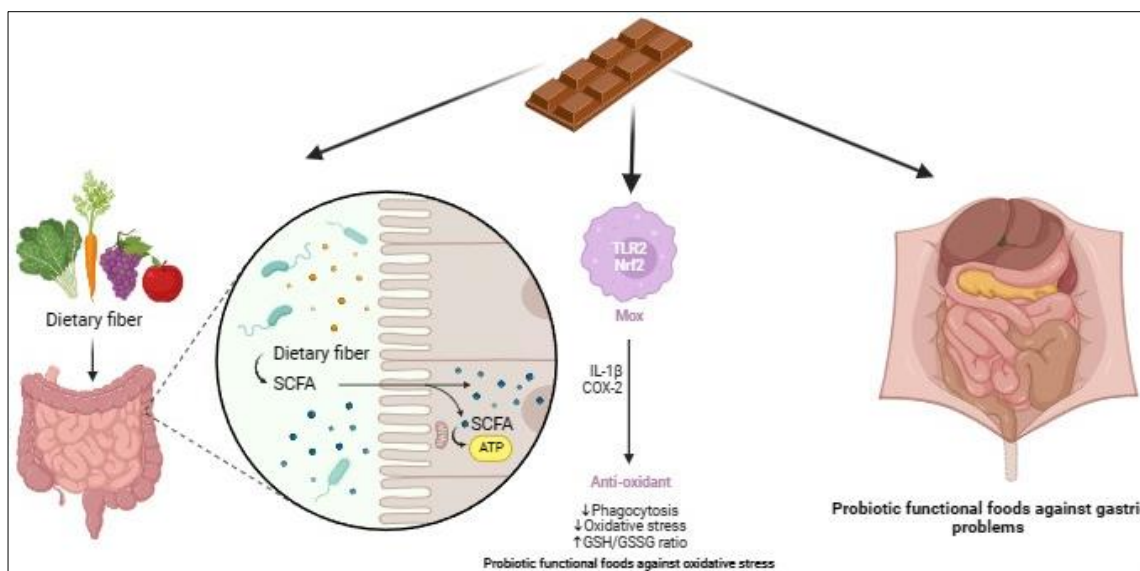
### Against Inflammatory Bowel Disorder/Irritable Bowel disorder

Inflammatory bowel disorder is associated with invasion of pathogenic bacteria in guts, production of antimicrobial substances, disorders in immunoregulations results in local inflammatory reaction in large intestine and colon (Actis *et al.*, 2019) [2]. Introduction of probiotics enrich chocolate shows significant beneficial effect by building up an immunological barrier in the mucosal lining of guts. Resulting in reduction of the symptoms of Irritable Bowel Syndrome (IBS). *L. acidophilus*, *L. plantarum*, *L. casei* are the potential strains of probiotics, incorporation of these in chocolate development and consumption and show remarkable improvements (Jakubczyk *et al.*, 2020) [15].

### Against Colorectal Cancer

Colorectal cancer can be developed from non-cancerous polyps in the initial stages and later presents its metastasis. Location of colorectal cancer, as name suggests, at colon or rectum. Successful dietary treatments with probiotics & probiotics enriched chocolate can modify the metabolic activities of intestinal micro flora and alteration of physicochemical conditions in the colon (Sivamaruthi *et al.*, 2020) [32].





**Fig 3:** Health Benefits of chocolate containing probiotics and functional foods against different diseases and disorders

**Table 1:** Probiotic Strains used in Chocolates to prevent different diseases

Sl. No.	Probiotic Strains used in Chocolates	Diseases to be treated	Sources
1	<i>Lactobacillus rhamnosus</i> , <i>L. rhamnosus</i>	Diarrhoea, antibiotic associated	(Kopacz & Phadtare, 2022) [18]
2	<i>Lactobacillus rhamnosus</i> , <i>Lactobacillus reuteri</i>	Candidiasis	(Andrade <i>et al.</i> , 2022) [4]
3	<i>Lactobacillus</i> and <i>Bifidobacterium</i> species, ( <i>Lactobacillus plantarum</i> PBS067, <i>Lactobacillus acidophilus</i> LA-14, <i>Bifidobacterium breve</i> PBS077)	Renal Calculi/Urolithiasis	(Wigner <i>et al.</i> , 2022) [37]
4	<i>Bifidobacterium</i> species, <i>Bifidobacterium lactis</i> , <i>Bifidobacterium longum</i> , <i>Bifidobacterium breve</i> , <i>Bifidobacterium infantis</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus rhamnosus</i> ,	Chronic constipation	(Dimidi <i>et al.</i> , 2020) [9]
5	<i>Lactobacillus casei</i> Shirota, <i>L. acidophilus</i> , <i>B. bifidum</i> , <i>L. rhamnosus</i> , <i>L. plantarum</i>	Pharyngitis & Laryngotracheitis	(Lehtoranta <i>et al.</i> , 2020) [19]
6	<i>Lactobacillus</i> , <i>Bifidobacterium</i> and <i>L. johnsonii</i>	<i>Helicobacter pylori</i> infection	(Saracino <i>et al.</i> , 2020) [27]
7	<i>Lactobacillus</i> GG, <i>L. rhamnosus</i> Lactis, <i>Lactobacillus fermentum</i> , <i>Bifidobacterium bifidum</i> , <i>Bifidobacterium lactis</i>	Infantile atopic eczema	(Rusu <i>et al.</i> , 2019) [25]

The table presents some of other disease which can be treated potentially by introducing Probiotic enriched

chocolates. Here the specific potential strains of probiotics are enlisted with the disease to be treated.

**Table 2:** Biochemical parameters in probiotic functional chocolates

Sl. No.	Ingredients used in Probiotic functional chocolates	Biochemical parameters		Sources
		Nutrients	%/gm	
1	Ragi Malt, Camal milk powder, Honey, <i>Streptococcus thermophilus</i> , <i>Lactobacillus bulgari</i> , <i>Lactobacillus delbrueckii</i>	Carbohydrates	59.45 gm	(Kenwala <i>et al.</i> , 2023) [16]
		Protein	7.81 gm	
		Fat	26.90 gm	
		Ash	1.84%	
		Moisture	4.0%	
		Nutrients	%/gm	
2	Yogurt powder (50%), Cocoa butter, Cocoa powder, <i>Lactobacillus acidophilus</i> , <i>Bifidobacteria</i> .	Carbohydrates	32.21 gm	(Chetana <i>et al.</i> , 2013) [8]
		Protein	9.21 gm	
		Fat	47.20 gm	
		Ash	2.43%	
		Moisture	2.55%	
		Nutrients	%/gm	
3	<i>Lactobacillus acidophilus</i> LDMB-01, cocoa powder, cocoa butter, Sucrose powder, Skim milk powder, soy lecithin, ethyl vanillin, porcine pepsin, plate count agar, bile salts.	Carbohydrates	35.23 gm	(Islam <i>et al.</i> , 2022) [14]
		Protein	6.51 gm	
		Fat	53.00 gm	
		Ash	1.64%	
		Moisture	1.29%	
		Nutrients	%/gm	

The Table presents more than one functional chocolate with has been developed with different ingredients with their nutritional compositions.

The ingredients of different probiotic chocolate, show slight differences in nutritional compositions during proximate

analysis. Kenwala *et al.* (2023) [16] revealed in their study, high quantity carbohydrate content in their formulated chocolate due to presence of ragi malt but slightly lower in fat content. While presence of cocoa butter in different studies conducted by Chetana *et al.* (2013) [8] & Islam *et al.*

(2022) <sup>[14]</sup> have shown higher amount of total fat in their formulated chocolate.

### Probiotic functional chocolates and Consumers' acceptability

The global market of functional foods is expanding day by day, especially those supplements enriched with probiotics. Consumers are showing great interest to those products, which are enhanced with adequate active & viable probiotics. As chocolate is one of the delicious food products so it is expected to be more popularized as other probiotic enriched foods. The economy of one country depends on the industrial turn overs (Muhammad *et al.*, 2021) <sup>[20]</sup>. So, researchers are hopeful that, this product with enrich the economic growth of country and globe as well.

### Conclusion

Chocolates, the mouth-watering food product can deliver the richness and beneficial effects of probiotics as this is very attractive for all the age groups. The popularity of chocolate can make the production successful with enhancement of global economy as well via consumer's acceptance throughout the globe. The extensive researches are needed to isolate more sources of probiotic strains to make the chocolates in cheaper cost and available for all the strata of society. Milk and dairy products are common sources to isolate probiotic strains. Other sources are till under research and with the intension of deliver good physiological benefits the investigations have to be continued. The combination of probiotic with different functional foods will be healthier. From this review article it was concluded that uses of different functional foods increase the potential health benefits of the final developed chocolate.

### Future Prospect

The lifestyle of today's generation is going towards a faster livelihood, where maintaining proper regimes of exercises and diets are really difficult. As a result, a number of health issues related to gastritis, *H. pylori* bacterial infections, Colon cancers, IBS are arising. For combating these with the faster lifestyle, probiotic chocolate will be a good treatment option for ensuring a balanced intestinal health which may help to fight against aforementioned diseases and to deliver a sound gut microbiota. We can discuss the future prospects of probiotic chocolate under two sections; impact of probiotic chocolate over health and commercial viability.

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