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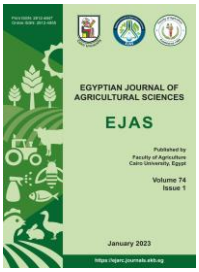

REVIEW ARTICLE

Probiotics in Dairy Foods: Advantages and Disadvantages

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ABSTRACT

Probiotics are beneficial microbes when they colonized well in the gut of the host. A great interest with probiotic dairy foods is increasing worldwide because of their health claims for consumers. Probiotics have two main genera, *Lactobacillus* and *Bifidobacterium*. Recently, *Akkermansia muciniphila* represents the next generation of probiotics. Modulations of gut microbiota and lowering the risk of metabolic syndrome by probiotics have taken a great attention in research. Survival of probiotics in different dairy products illustrates a struggle for these microorganisms, especially during the cold storage of these products. Cheddar cheese and ice cream are a good carrier for different probiotic species. Microencapsulation or adding prebiotics are the main strategies for enhancing the viability of different probiotic species during the cold storage of dairy products. Application of red laser technology could also enhance production of organic acids and flavor compounds of *L. casei* during the cold storage of Labneh. This review focuses on the evolution of probiotic definitions, health benefits and adverse effects of probiotics and challenges of probiotics in dairy foods.

***Keywords**: Probiotics, A. muciniphila, gut microbiota, probiotic dairy foods, omics*

1. INTRODUCTION

Functional foods have a positive impact on health of consumers with economic benefits worldwide. They include probiotics, prebiotics, vitamins and minerals, which could be used as a food supplement in fermented milks, sports drinks, baby foods, and chewing gum (Yang, 2008). Functional food is defined as a "food that has special health benefits. The term "functional food" was first introduced in Japan in mid-1980s (Swinbanks and O'Brien, 1993). It is suitable for the consumption by special group of people and has the function of regulating human body functions and not used for therapeutic purposes" (MOH, 1996). Probiotic is defined as "a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract" (Mc Farland, 2000 and Salaminen, 2001). This definition was intended for utilization of probiotics in animal feed products. Two main genera of gram-positive bacteria *Lactobacillus* (*L.*) and *Bifidobacterium* (*Bif.*) are used extensively as probiotics (Holzapfel *et al*., 2001). However, other genera like *Enterococcus*, *Streptococcus*, and *Saccharomyces* have also been marketed as probiotics. Viable lactic acid bacteria (LAB) of probiotic dairy products have several clinically proved health effects, such as reduction severity of diarrhea, improvement the balance of the intestinal microbiota by antimicrobial activity, alleviation of lactose intolerance symptoms, prevention of food allergy, enhancement of immune system, antioxidant anti-tumor activities

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(Anderson *et al.,* 2001). A probiotic strain should well survive during the manufacturing process and without negative effect on the sensory properties of the food product. The strain and the claimed properties should maintain stability in the food product during processing and subsequent storage (Saarela *et al.,* 2000). In general, the food industry has applied the recommended level of 10^6 CFU/g at the time of consumption for *Lactobacillus acidophilus*, bifidobacteria, and other probiotic bacteria (Boylston *et al.,* 2004).

Probiotics and prebiotics (non-digestible oligosaccharides with health impact on the host) have been used in production of different dairy products such as fermented milks and cheeses, fruit juices, cereals, and chocolate. Very little research has been done on symbiotic foods. Hence, there is a need to develop diverse probiotic and symbiotic foods, which can be used as nutrient supplements to promote health (Saro and Arora, 2017). This review gives insights into (1) the evolution of the definition of probiotics, (2) recent mechanisms for health benefits of probiotics, (3) side effects that associated with over consumption of probiotics (4) advantages and disadvantages of probiotics in dairy products.

2. History and definition of probiotics

The Greek root of probiotics is "Pro bios" meaning for life. A Nobel Prize Winner Ellie Metchnikoff (father of natural immunity) was the first to introduce the concept of probiotics (Metchenikoff, 1907). The definition of probiotic evolved during the last few years as shown in Table (1). The evolution of the definition of probiotics started from beneficial substances secreted by microbial species (Lilly and Stillwell, 1965) to live microorganisms or product containing viable defined microbial strains that stimulate different health benefits to the host with keeping in our consideration dose of consumption for the host. The main genera of probiotic microorganisms are presented in Table (2).

Table (1): Development of definition of probiotics during the last years.

Author(s)	Definition
Lilly and Stillwell (1965)	"Growth promoting factors produced by microorganisms."
Parker (1974)	"Organisms and substances with beneficial effects for animals by influencing the intestinal microflora."
Fuller (1989)	"A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance."
Havenaar and Huis Int Veld (1992)	"A mono- or mixed culture of live microorganisms which, applied to animal roman, affect beneficially the host by improving the properties of the indigenous microflora."
Salminen *et al.* (1998)	"A viable microbial food supplement which beneficially influences the health of the host."
Diplock *et al.* (1999)	"Probiotic food is functional if they have been satisfactorily demonstrated to beneficially affect one or more target functions in the body beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction in the risk of diseases."
Naidu *et al.* (1999)	"A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract."
Schrezenmeir and de Vrese (2001)	"A preparation of a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host."
FAO/WHO (2001) and Reid *et al.* (2003)	"Live microorganisms which when administered in adequate amounts confer a health benefit on the host."
Elshaghabee (2017)	"Live microbial strains with health impact on host when being consumed daily with enough amounts (not less than 10^6 to 10^8 CFU/g) and incorporated into gut micro-biome"

probiotics are present in food matrix referring to

Table (2): Microbial cultures used as probiotics.

Culture	Example(s)
Escherichia	*E. coli* Nissle 1917
LAB*	*Lactobacillus* (*L.*) *acidophilus, L. casei, L. plantarum, L. rhamnosus, L. gasseri, Lactococcus* (*Lc.*) *lactis, Pediococcus* (*Pedio.*) *acidilactici, Enterococcus* (*Ent.*) *faecalis*
Bifidobacterium	*Bif. longum, Bif. lactis, Bif. bifidum* … etc.
Propionibacterium	*P. shermenii*
Bacillus	*B. coagulans*
Akkermansia	*Akkermansia muciniphila*
Saccharomyces	*Sacch. boulardii*

***=** Lactic acid bacteria

One of the most applied strategies to enhance the viability of different probiotic strains is applying prebiotics that are useful to manipulate the intestinal microbiota. Human milk, e.g., "Mama Milk" contains components like *N*-acetyle glucoseamine that promote the growth of bifidobacteria (Gyorgy, 1954). Yazawa *et al*., 1978 and 1982) discovered that a number of different non-digestible oligosaccharides were "bifidus" factors.

In 1995, Gibson and Roberfroid defined prebiotics as "non-digestible but fermentable oligosaccharides (NDFOs) by beneficial gut microbiota". They link definition of prebiotics with probiotics when both prebiotics and "symbiotic" (Flesch *et al.,* 2014).

3. Criteria for selection of probiotics

In order to ensure that probiotic cultures substances have therapeutic effects, certain characteristics are needed especially that probiotics are strain specific (Ibnou-Zekri *et al*., 2003) and preferably, probiotic strains used for humans should be isolated from human origin (Ouwehand *et al.,* 2002). Data in Table (3) summarize the potential criteria for selection of probiotic cultures.

A guideline for standard criteria for defining probiotics was established by FAO/WHO (2002). It contains two major's axes for

Table (3): The four criteria for selection of probiotics

Criteria	Description
Safety (non-toxic and non-pathogenic)	It must be generally regarded as safe (GRAS).
Survival during food processing conditions	Probiotics have to survive during different manufacture and storage conditions till they reach to the consumer whereas the log viable count should retain higher that 6 Log CFU/g.
Survival during gastrointestinal tract (GIT) transit	In order to enhance the health of the host, probiotics have to survive under different GIT conditions. These include: lysozyme resistance, stomach acidity and bile tolerance and their ability to adhesion to mucus.
Health benefits towards the host	After adhesion to mucus, probiotics have to exert different therapeutic effect such as modulate the immune system and gut microbiota toward health composition, reduce the risk of infection and mutagens and reduce levels of cholesterol.

Adopted from Tuomola *et al* **(2001)**

evaluating the probiotic criteria. These are safety and authenticity. These guidelines did not recommend any *in vitro* or *in vivo* technique for the evaluation. The Indian council of medical research (ICMR) and department of biotechnology (DBT) designed the guidelines for evaluating probiotics starting from strain identification to final product. ICMR-DBT guidelines for evaluating the probiotic properties of any strain consist of three main stages including safety, efficacy and effectiveness then labeling of final product. The key difference between the two previous guidelines is the effectiveness of probiotics, which is optional in the joint FAO/WHO (Mahasneh *et al.*, 2010).

Several models (*in vitro*, *in vivo*, omics and human cell lines) had been utilized for evaluating the probiotic criteria of different microbial strains. In this respect, Salminen *et al.* (1998) found that orally administrated *Bif. longum* to germ-free mice resulted in increased the bacterial colonization in the intestinal tract to a concentration of 10^9- 10^{10} CFU/g intestinal content in 2-3 days, and this translocation of the colonized *Bif. longum* was not associated with causes, neither infection nor any harmful effect. These results indicate that *Bif. longum* is safe and survive well in the colon. Therefore, it could be used as probiotic starter culture.

In vitro models for evaluating probiotic criteria at first stage are more preferable than *In vivo* models because of the cost and ethical issues (Byakika *et al*., 2019). In this manner, *L. acidophilus* groups were more tolerant to low pH than strains of *L. paracasei* and *L. rhamnosus* after exposure to the simulated gastric juice containing pepsin. Milk as a medium, showed a protective effect on strains less resistant to the gastric acidity in tube test model (Schillinger *et al.*, 2005). Bile salts (sodium cholate and sodium deoxcholate) caused a rapid decline in the viability of *Enterococcus faecalis* ATCC 19433. The detergent effect of bile salts resulted in decreasing the viability of *Enterococcus faecalis* (Begley *et al*., 2005).

EL-Deib *et al.* (2010) evaluated the health benefits of commercial starter culture using test tube model. They found that *L. casei* 01 and *Bif. bifidum* Bb-12 had the highest ability to assimilate cholesterol from broth medium and the highest antioxidant activity. Recently, El-Shafai *et al.* (2018) used *in vitro* model, tube-based test, for evaluating the probiotic criteria of *L. casei* FEGY9973 including acid and bile tolerance and the fermentation profile of fructose as steatogen. No ethanol could be detected from fermentation of fructose in medium for colonic microbiota by tested strain.

Omics methods were recently used for evaluating the probiotic criteria of different microbial strains. Omics techniques basically used universal detection of genes (transcriptomics), mRNA translated proteins (proteomics) and metabolites (metabolomics) (Horgan *et al*., 2011). Koskenniemi *et al*. (2011) found an increase in transcript levels of 316 genes and 42 intracellular and surface exposed proteins in bile stressed *L. rhamnosus* GG.

Human cell line (*ex vivo*), mainly Human colon cell lines, Cancer coli-2 (Caco-2) and HT29, is widely used for evaluating the adhesion efficiency of probiotics (Aissi, 2001). It was noticed that probiotic strains that adhere with high efficiency to human cell line usually have the same trend *in vivo* (Papadimitriou *et al*., 2015).

Animal intestines, e.g., pig ileum, have used to investigate the adhesion properties of probiotics (Kos *et al*., 2003). Furthermore, Wistar rats were used in different studies to evaluate the probiotic criteria of different microbial strains because the gastric transit in these rats is close to natural gastric transit in human (Saxami *et al*., 2012).

Probiotic spore forming *Bacillus* spp. has a high survival rate against acid tolerance and a high stability during heat treatment and cold storage condition (Bader *et al*., 2012). Furthermore, they have also been observed to reduce levels of pathogens exclusion, antimicrobial effects, antioxidant activity and immune-modulatory (Shobharani *et al*., 2015 and Ripert *et al*., 2016). *Bacillus* sp. as a potential probiotic with future concern has been reviewed by Elshaghabee *et al*. (2017).

Akkermansia muciniphila represents the next generation of probiotics, which was first isolated by Derrien *et al.* (2004) from mammalian gut. It is oval shaped, gram negative, non-motile and strict anaerobic, can use mucin as sole carbon, nitrogen and energy source and it releases sulfur, and the main two metabolites are acetate and propionate. A large proportion (approximately 85%) of *A. muciniphila* is vancomycin-resistance (Hansen *et al*., 2012). Therefore, it is used for patients treated with multiple antibiotics (Dubourg *et al*., 2013). Finally, all methods used for evaluating the probiotic criteria were well reviewed by Byakika *et al.* (2019).

4. Therapeutic effects of probiotics

The recent major five health benefits for different probiotics include a.) probiotics promote healthy gut microbiota; b.) stimulation of immune System; c.) anti-carcinogenic effects; d.) Reducing the risk of non-alcoholic fatty liver disease (NAFLD); and e.) Management of cardiovascular disease (CVDs)

4.1. Probiotics promote healthy gut microbiota

Gut microbiota plays an important role in human health. The human gut microbiota contains 10^{14} bacterial species (Kunz *et al*., 2009). Several anaerobic genera are dominant in human gut mainly *Bacteroidetes*, *Firmicutes*, *Ruminococcus*, *Eubacterium*, *Lactobacillus* and *Actinobacteria* (Neish, 2009; Roberfroid *et al.*, 2010). The major metabolites profile of different gut microbiota was present in organic acids (mainly lactic, succinic and formic acids), short chain fatty acids (acetic, propionic and butyric acids), indoles and ethanol. Gut microbiota is a key player for many host physiological process *e.g.*, energy intake, production of vitamins, maturation and stimulation of the immune system (Derrien *et al*., 2011). Host's age, disease, stress and diet have a high impact on modulation of the composition of gut microbiota (Wang *et al*., 2011; Sonnenburg *et al*., 2016). Imbalance of gut microbiota (dybosis) triggers different metabolic diseases such as obesity, inflammatory bowel disease, diabetes, fatty liver and hypercholesterolemia (Vespasiani-Gentilucci *et al*., 2018).

The pioneer human study by Simenhoff *et al*. (1996) was the first research to demonstrate the over-growth of pathogenic bacteria "Small Bowel Bacterial Overgrowth" (SBBO) in small intestine. They also found that *L. acidophilus* NCFM could reduce levels of dimethylamine and nitro-dimethylamine levels and SBBO in patients. Different probiotic strains play a vital role in enhancing the balance of gut microbiota. Probiotics produce antimicrobial substances in colon where they act that could reduce levels of pathogens including opportunistic pathogen *e.g.*, *Clostridium difficile* that is implicated in antibiotic-associated diarrhea (Ouwehand *et al*., 2016). The antimicrobial activity of different probiotic strains is due to production of wide range of antimicrobial substances. These include bacteriocins, organic acids (mainly lactic and acetic acids) and hydrogen peroxide (Zihler, 2010). Furthermore, probiotic *L. paracasei* could also promote specific changes in fatty acids uptake (Aronsson *et al*., 2010).

A. muciniphila could regulate some metabolic functions of the host including positive shifts in gut microbiota, body and fat mass gain, declined serum triglyceride and fasting glucose levels and enhanced insulin sensitivity in mice (Everard *et al*., 2013 and Everard *et al*., 2014).

Fermentation of soluble fibres (prebiotics) in the colon by gut microbiota including different probiotic species resulted in production of short chain fatty acids (SCFAs), i.e., mainly acetate, butyrate and propionate and enhancement the growth of bifidobacteria. Furthermore, the intestinal pH would be decreased and subsequently inhibit various pathogens (Macfarlane *et al.*, 2006). Lactate and acetate play important roles in cross-feeding metabolic pathways of other bacterial species whereas lactate-producing *Bif. adolescentis* works together with lactate-consuming and butyrate-producing bacteria like *Anaerostipes caccae* / *Eubacterium hallii* (Belenguer *et al*., 2006). Dietary fructans could stimulate hormone glucagon like peptide-1 and peptide YY (Uris-Silvas *et al*., 2008). Results from *in vitro* fermentation model showed that different gut microbiota could ferment short chain fructo-oligosaccharides (FOS), *i.e.,* Probiotic *Bifidobacterium, Bacteroides, Faecalibacterium, Lactobacillus, and Roseburia* can ferment oligofructose, however, few gut microbiotas can utilize long-chain fructans (Ramirez-Farias *et al*., 2009; De Vuyst and Leroy, 2011).

4.2. Stimulation of immune System

The relationship between intestinal microbiota, immune system, inflammation and intestinal barrier is well recognized. The prophylactic and therapeutic effects of probiotics against dysbiosis and inflammation have also been established and proven clinically (Ducatelle *et al*., 2015). The therapeutic efficacy of probiotics has been found to be triggered by affecting gene expression of tight junction proteins, as well as increasing the secretion of mucus, modulating mucosal and systematic immunity, stimulating host antimicrobial peptides (AMPs) and IgA secretion and by improving the functionality of epithelial cells (Tsai *et al.*, 2012 and Wang *et al.*, 2014). The major advantage of probiotic interventions lies in their therapeutic action without provoking the

host innate immunity that makes them more reliable among medical practitioners for the treatment of gut barrier related disorders.

Probiotics could enhance the immune response of host at different levels. Probiotics could regulate host innate immune response by activating the toll-like receptors (TLR), dendritic cells, macrophages, and T and B-lymphocytes (Vander pool *et al*., 2008 and Yan and Polk, 2010). Long-term effect of yoghurt consumption (200 g/d of yoghurt for 1 year) could decrease allergic symptoms and had little effect on levels of interferon gamma in young and senior adults (Van de Water *et al.*, 1999). Kwon *et al.* (2010) demonstrated that a probiotic mixture consisting of *L. acidophilus, L. casei, L. reuteri, B. bifidium*, and *St. thermophilus* stimulated regulatory dendritic cells and reduced the intestinal inflammation cascade. *B. breve* AH1205, *B. longum* and *L. salivarius* AH102 were shown to reduce airway allergy (Lyons *et al.*, 2010). *L. acidophilus* Lafti L10 regulates genes involving in immune response including IL-23 signaling pathway (Van Baarlen *et al*., 2011).

Microarray analysis of whole genome of *L. acidophilus* NCFM resulted in regulating different genes related to viral defense in murine bone marrow-derived dendritic cells including TLR-2, IFN-β, IL-12, and IL-10. However, these effects were not seen for *Bif. bifidum* Z9 and *E. coli* Nissle 1917 (Weiss *et al*., 2010).

Some active components of probiotic could regulate the immune system of the host. In this manner, *L. rhamnosus GG*-derived soluble protein, p40, was noticed to reduce levels of tumor necrosis factor (TNF), IL-6, keratinocyte chemo-attractant, and interferon (IFN)-γ production indicating that p40 regulates innate immunity and Th1 immune responses (Yan *et al*., 2011).

Spore forming probiotic (SFP) like *Bacillus coagulans* containing 1×10^9 CFU/g living bacteria could enhance the immune functions, spleen index and secretory immunoglobulin A (SIgA) of intestinal mucosa in Broilers (Xu *et al*., 2017). Also, inactivated *B. coagulans* (*Weizmannia coagulans*) GBI-30 could enhance immune response by increasing levels of cytokines e.g., IL-1, TNF-alpha and IFN-gamma using *in vitro* model (Jensen *et al*., 2017). There are different mechanisms by which SFP could enhance immune responses, and other beneficial effects was reviewed by Elshaghabee *et al*. (2017). Non-commensal and non-pathogenic yeast *Saccharomyces boulardii* could inhibit the enterotoxigenic *E. coli* (ETEC)-induced expression of pro-inflammatory cytokines and chemokines at both transcriptional and protein expression levels, including IL-6, IL-8 and IL-10 (Zanello *et al*., 2011).

Probiotics are weak inducers for pro-inflammatory cytokines and contribute to reduction of inflammation by induction of T_{reg} in the crypts that down-regulate proinflammatory signaling through production of IL-10 and transforming growth factor-β (TGF- β) (Izcue *et al*., 2006). A wide range of probiotics has been reported to inhibit Th1 polarizing by activation of TLR2 mediated pathways (Evrard *et al*., 2011). The redirection of Th1/Th2 balance towards Th2 in inflamed gut encourages the expression of anti-inflammetory/ regulatory cytokines like IL-10 and TGF- β associated with T_{reg} tolerance and Il-6, Il-12, IFN-γ and TNF-α suppression (Jeon *et al.*, 2012).

4.3. Anti-carcinogenic effects

The numbers of colon cancer patients increased during this decade, particularly in industrialized countries. Healthy diet could reduce the incidence of colon cancer (Goldin and Gorbach, 1992). Moreover, a study by Goldin *et al.* (1992) showed the anti-carcinogenic effect of probiotic *L. acidophilus* in rat model. A possible mechanism for this effect was related to the ability of *L. acidophilus* to inhibit β-glucuronidase, nitroreductase, azoreductase, and β-glycosidase that convert procarcinogenes to proximal carcinogens in colon. Furthermore, Abd El-Gawad *et al.* (2005) found that yoghurt and soya yoghurt containing bifidobacterial could inhibit the proliferation of Ehrlich ascites tumor cells using both *in vitro* and *in vivo* models. A clinical study was conducted by Davis and Milner (2009), which demonstrated that symbiotic therapy (*L. rhamnosus* and *Bif. lactis* plus inulin) could reduce the risk of colorectal cancer.

Butyrate as main metabolites of fermentation of prebiotics oligosaccharides or polysaccharides produced by probiotics or other gut microbiota had inhibitory effect against proliferation of tumor cells in colon (Candela *et al*., 2011). Recently, supplementation of fermented cow or soy milk with water soluble curcumin enhanced the anti-carcinogenic activity of both types of fermented milks (Abdelrazik and Elshaghabee, 2021).

4.4. Reducing the risk of non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) became a world health concern in both adolescents and adults as a result of obesity. NAFLD is defined as an increase of accumulation of fat in hepatocytes exceeding 5 to 10 % of total liver weight (Neuschwander-Tetri and Caldwell, 2003). Until now, no medical therapy is approved for NAFLD treatment (Day *et al*., 1998; Byrne *et al*., 2009).

In a clinical study by Zhu *et al*. (2013), they observed that the obese and fatty liver patients were observed to have different ratios of firmicutes, bacteroidetes, actinobacteria and alcohol-producing bacteria compared to the healthy patients. Recently, Vespasiani-Gentilucci *et al*. (2018) established a link between changes of gut microbiota and pathogenicity of NAFLD, where levels of fecal *Anaerococcus, Bradyrhizobium, Dorea, Peptoniphilus, Propionibacterium acnes*, and *Ruminococcus* were reduced, while proportions of *Oscillospira* and *Rikenellaceae* increased in NAFLD patients.

The main metabolites of fermentation accumulated sugars like fructose in intestine by different clusters of gut microbiotas, organic acids e.g., lactic and succinic acids, SCFAs such as acetate, propionate, butyrate and ethanol (Salminen *et al.,* 1998). Lactate and acetate play important roles in cross-feeding metabolic pathways of other bacterial species, where lactate-producing *Bifidobacterium adolescentis* works together with lactate-consuming and butyrate-producing bacteria like *Anaerostipes caccae*/*Eubacterium hallii* (Belenguer *et al*., 2006), and between acetate-feeding bifidobacteria and butyrate-producing *A. caccae* and *Roseburia* sp. (Falony *et al*., 2006). In addition, SCFAs can attenuate accumulation of triglycerides in liver cells (hepatocytes) via increasing the ratio of propionate to acetate that could inhibit lipogenesis (Daubioul *et al*., 2002).

Ethanol and acetaldehyde are metabolites of sugar fermentation by heterolactic intestinal bacteria. Ethanol can be metabolized to acetate and acetaldehyde. Increasing blood circulation of acetaldehyde might lead to formation of reactive oxygen species (ROS), thus increasing oxidative stress inducing liver injury (Medina *et al*., 2004). Baker *et al*. (2010) reported that ethanol produced by gut microbiota in NAFLD patients contributed to development of NAFLD resulting in an increased levels of alcohol circulation in the blood, induced host alcohol dehydrogenase and activity of cytochrome P450 2E1 gene expression and elevated NADH levels. Elshaghabee *et al*. (2016b) found that the non-mannitol producing intestinal hetero-fermentative lactobacilli (*i.e*., *Weissella confusa*) was shown to produce high amounts of ethanol from fructose fermentation.

Probiotics represent one of the dietary strategies to reduce the risk of NAFLD. *L. casei* Shirota strain could attenuate the severity of fatty liver in mice fed high fructose diet (Wagnerberger *et al.*, 2013). A probiotic cocktail containing *L. paracasei* CNCM I-4270, *L. rhamnosus* I-3690 and *Bif. animalis* subsp. *lactis* I-2494 had the ability to modulate the abundances of forty-nine of operational taxonomic units (OTUs) *i.e.*, *Desulfovibrionaceae*, *Oscillibacter* and *Clostridium* XIV in mice fed high fat diet. Supplementation of acidophilus milk with calcium pyruvate could reduce levels of interleukin-6 and pro-inflammatory cascade in rats fed high fat diet (Elshaghabee, 2019).

A. muciniphila, as a next generation oprobiotics, regulate different metabolism pathways of the host either in living or pasteurized form, whereas both forms could reduce the body and fat mass gain as well as reduce serum triglycerides and fasting glucose, and enhance insulin sensitivity in mice (Evered *et al*., 2013). Furthermore, *A. muciniphila* could improve the gut barrier through enhancement of the expression of tight junction protein including occludin, claudins, and ZO-1, ZO-2 and ZO-3 and it could reduce the circulation of endotoxemia like LPS levels and inhibition of inflammatory response resulting in enhancement of glucose and lipid metabolism (Grander *et al*., 2018). Recently Ahn *et al.* (2019) demonstrated that a probiotic cocktail could reduce levels of triglycerides in liver of sixty-eight NAFLD patients.

4.5. Management of cardiovascular diseases

Cardiovascular diseases (CVDs) are the leading cause of mortality overall the world. According to WHO, approximately 31% of global death caused by CVDs during the year 2016 and by 2030, CVDs will be affecting approximately 23.6 million people around the world (WHO, 2009). CVD is a metabolic syndrome associated with increased low-density lipoprotein (LDL) cholesterol, increased

triglyceride-rich lipoproteins, low levels of high-density lipoprotein (HDL) cholesterol and blood pressure (BP) (Vasquez *et al*., 2019). Most cardiovascular diseases can be prevented by addressing unhealthy diet and obesity, sedentary life style and behavioral risk factors. Modulation of gut microbiota using healthy diet could be used as a strategy to prevent and/or treat CVDs.

Several studies suggested that probiotic have positive impact in improving functionality of endothelial layer-related CVDs. Rashid *et al*. (2015) reported that application of probiotics to rats suffering endothelial dysfunction, improved the pathological condition of the rats. The oral administration of probiotic drink (Kefir) for eight weeks could repair the vascular endothelial architecture and reduce oxidative stress along with an increase in nitric oxide (NO) bioavailability and reduce levels of hypertensive rats (Friques *et al*., 2015). This effect of probiotics was further confirmed in a study by Yap *et al.* (2016) which proved that lactic acid bacteria could partially reverse the relaxation deficit of the aorta and increased the NO level. Gomez-Guzman *et al.* (2015) reported that oral administration of the probiotic consortium (*L. fermentum* or *L. coryniformis* plus *L. gasseri)* re-established gut microbiome and improved endothelial dysfunction leading to reduced vascular proinflammatory and prooxidative status.

Some studies on humans and human cells have also shown an improvement in endothelial function due to probiotic treatment. Cheng *et al.* (2013) reported that *L. plantarum* or *St. thermophilus* fermented soy milk stimulated NO production and eNOS activity, signifying their impact in improving endothelial function. A 6-week *L. plantarum* supplementation to stable coronary artery patient, increased NO bioavailability along with reduced systemic inflammation thus leading to improved endothelial function, as measured by brachial artery flow-mediated dilation. Probiotic supplement improved both functional and biochemical parameters of endothelial dysfunction, including systolic BP, vascular endothelial growth factor, pulse wave velocity (PWV), interleukin-6, tumor necrosis factor alpha (TNFα), and thrombomodulin in obese postmenopausal women (Szulińska *et al*., 2018).

Probiotics in hyper-cholestromia-Cholesterol provides an important basic block for body tissues. Elevated blood cholesterol is a well-known major risk factor for coronary heart diseases (Abd El-Gawad *et al*., 2005).

Hypertension also plays a major role in the development of CVDs and heart failure. Recent studies suggested that probiotics and their metabolomics could be used for hypertension management through lowering levels of total cholesterol, LDL-cholesterol, blood glucose levels, insulin resistance and regulating the renin–angiotensin system (Khalesi *et al*., 2014). Furthermore, angiotensin II can cause vasoconstriction and elevate BP. Therefore, ACE inhibition is a key clinical target for BP control (Thushara *et al*., 2016).

Several clinical studies in humans have also demonstrated the ability of probiotics to reduce abnormally high BP levels. Oral administration of *L. plantarum* significantly decreased systolic BP in heavy smokers (Naruazewicz *et al*., 2002). Brantsaeter *et al*. (2011) observed a decrease in preeclampsia incidence with chronic intake of probiotics, which is associated with hypertension and inflammation. Probiotics in Arterial Hypertension-Arterial hypertension constitutes a main risk factor for the development of severe pathologies, such as acute myocardial infarction, heart failure, stroke, and renal failure, as well as for premature death worldwide (Vasquez *et al*., 2019).

5. Disadvantages of probiotics

Generally, the side effects (disadvantages) of probiotics are uncommon. However, according to the national center for the complementary and alternative medicine (Yadav *et al*., 2013), increase consumption of probiotics or prebiotics results some common side effects including:

- Diarrhea, increased bowel movements
- Bloating and/or flatulence (gas)
- Abdominal cramping
- Recently, probiotics were found to react with certain drugs like sulfasalazine.
- In rarer cases, probiotics can cause infections, especially in immune-compromised people (Chad *et al*., 2010).
- Prebiotic FOS can increase level of plasma cholesterol in some individuals (Mortensen *et al*., 1988).
- There are reported risks associated with genetically engineered probiotic strains (David, 2008).

6. Probiotics in dairy products: Challenges and opportunities

Dairy products represent a good matrix for different probiotics. Furthermore, dairy products

are a good environment for growth of different probiotic strains (Phillips, 2006). Probiotic dairy drinks were first commercialized by Danisco (Mäkeläinen *et al*., 2009). Yoghurt has long been characterized as a healthy product with many beneficial effects for consumers (Hamann and Marth, 1983). The bacteria used in traditional yoghurt fermentation, *L. bulgaricus* and *S. thermophilus*, do not belong to the indigenous intestinal flora, are not bile acid resistant and do not survive passage through the gut (Gilliland, 1979). These traditional yoghurt bacteria may, nevertheless, have positive effects as a result of fermentation metabolites, either by an inhibitory action towards pathogens or improvement of lactose digestion (Hoier, 1992).

The survival of probiotic bacteria in fermented dairy bio-products depends on various factors such as, the strains used, interaction between species present, culture conditions, chemical composition of the fermentation medium, final acidity, milk solids content, availability of nutrients, growth promoters and inhibitors, concentration of sugars (osmotic pressure), dissolved oxygen (especially for *Bifidobacterium* sp.), level of inoculation, incubation temperature, fermentation time as well as storage temperature (Kneifel *et al*., 1993).

Low pH of the environment of fermented milks has a negative effect on the viability of probiotics. Hood and Zottola (1988) reported that *L. acidophilus* (strain BG2FO4) showed a rapid decline in numbers at pH 2.0, but the number of viable cells did not decrease significantly at pH 4.0. These results were confirmed by Lankaputhra and Shah (1995), who concluded that six strains of *L. acidophilus* studied, survived well at pH 3.0, or above. Kehagias *et al*. (2006) showed that the viability of bifidobacteria growing as single species was very good. During the cold storage, the increase of acidity was more pronounced in yoghurt as compared with fermented milks.

Generally, *Lactobacillus* strains showed a good cellular stability throughout storage period. On the other hand, the cell counts of *Bif. lactis* LAFTI® B94 decreased by one log cycle at the end of storage (Donkor *et al*., 2007). El Dieb *et al.* (2012) showed that co-culture of yoghurt culture with low acid production and *L. casei* -01 and *Bifid* bacteria Bb-12 did not negatively effect on the growth of both probiotic strains.

Labnah (concentrated yoghurts) is a popular fermented milk product consumed in the Middle East. The nutritional and therapeutic properties of it are considered better than yoghurt (Nsabimana *et al*., 2005). Labneh as probiotic carrier foods because it contains higher total solids than yoghurt, therefore it may be considered a suitable matrix for probiotics (Abd EL-Salam *et al*., 2011). Different process factors have adversely effect on the viability of probiotics in labneh including pH, redox potential, and amount of probiotic inoculation and flavoring agents (Rocha *et al*., 2014 and Castro *et al*., 2015).

Cheese represents the top product of many dairy marketing in recent years. Furthermore, it has several positive impacts as a carrier of probiotic microorganisms compared with fermented milks because the buffer capacity in opposition to the high acidic environment in the GIT. Moreover, the dense matrix; high-fat content of different types of cheese might provide an additional guard to probiotics in the stomach (Dantas *et al*., 2016). Both fresh and ripened cheese can be used as probiotic carriers, but due to its manufacturing procedure, fresh cheese proved to be a more suitable carrier for probiotic microorganisms. Fresh cheese, *e.g*., cottage cheese, is un-ripened cheese and its storage takes place at refrigerator temperatures. Further, its shelf life is somewhat limited, and no extended time of ripening is required (Masuda *et al*., 2005).

There are various studies probing into the viability of different kinds of probiotic microorganisms in different kinds of fresh cheese. Vinderola *et al*. (2000) investigated the survival of some probiotic bacteria (*Bif. bifidum*, *Bif. longum*, *L. acidophilus*, and *L. casei*) in Argentinean fresco cheese as a type of fresh cheese. The results of their study revealed that even though viable counts of probiotics were reduced in 16 days at about one log, last counts were still adequate and acceptable. In another study on cottage cheese, the enhancement of *Bif. infantis* in the initial day was noticed. Nevertheless, huge decreases in survival rates were observed after 15 days (Farnworth, 2003).

Domiati cheese is the most popular soft white pickled cheese in Egypt and in other Middle East countries. It is made mainly from

buffalos' milk, cow's milk, or a mixture of both, but it is also made from sheep's or goat's milk (Mehaia, 2002). Supplementation of Domiati cheese with *L. acidophilus* La-5 and *Bif. longum* ATCC15707 enhanced the microbiological quality of Domiati cheese during cold storage (El-kholy *et al.,* 2014). Supplementation of Tallaga cheese with 3 % Mashroum could enhance the viability of *L. reuteri* NRRL-B-14171 during thirty days of cold storage (El-kholy *et al.*, 2016).

Ice cream is the most popular dairy products and it also presents a good carrier of different probiotic strains because the pH of ice cream is near to 7 and it contains high levels of fat and solids not fat which protect probiotics form the adverse effect of freezing (Akin *et al.,* 2007). At -20 °C of storage, counts of *L. acidophilus* and *L. rhamnosus* were stable during two months of cold storage. The viability of different probiotic stains in dairy frozen yoghurt and fermented ice cream with pH less than 4.5 was less than their viability in ice cream (Hamayouni *et al.*, 2012).

Microencapsulation of different probiotic strains represent a wonderful strategy for enhancing their viability in different dairy products especially bio-yoghurt whereas the pH is less than 4.5 (Sarao and Arora, 2017). The encapsulation of the probiotic cells is usually carried out to protect of the probiotic living cells against and unfavorable or adverse environment either in product of during GIT transit (Champagne and Kailasapathy, 2008; Zuidam and Shimoni, 2009). Inulin has been used as a thermal protective agent in the form of symbiotic microcapsules to improve the thermal sensitivity of prebiotics on probiotic to *L. casei* MTCC 1423 (Babu and Nithyalakshmi, 2011).

El Dieb *et al.* (2012) found that encapsulated of *L. casei* 01 and bifidobacteria Bb-12 had higher viability than free cell during storage of bio-yoghurt. Alginate, k-carragenan and guar gum were better than Arabic gum as capsule material for *Bif. longum* under similar condition of GIT (Elshaghabee, 2016a). Labneh samples with encapsulated *L. casei* had higher viable count and sensory scores than the control. In addition, labneh samples with *L. casei* in milk-alginate microcapsules showed a high viability during cold storage and under simulated GIT conditions (El Shafei *et al*., 2018). The observations made were that encapsulation is not a necessity for the survival of the cells in the case of Cheddar cheese and ice cream but while considering yoghurt, labneh and Karish cheese, it was important. Hence, this laid stress on the importance of the food carrier for the probiotic survival. As per the study of Sharp *et al*. (2008), both Cheddar cheese and yoghurt are able to protect probiotic cells by providing a suitable environment while manufacturing as well as during storage. Cheddar cheese was observed to be better than yoghurt for delivering the probiotics to GIT.

The viability of the probiotics in ice cream is enhanced by encapsulation and there was no effect on the products' sensory parameters after three months of storage (Homayouni *et al*., 2008). The survival of both *L. acidophilus* and *Bif. bifidum* increased when they were co-encapsulated (Sarao and Arora, 2017). Recently, exposure of red laser to *L. casei* strain enhanced its viability during the cold storage of Labneh (Elshaghabee *et al*., 2022). The prospective effect of laser technology on the viability of different probiotics needs further investigation on molecular basis.

Conclusion

Genera *Lactobacillus* and *Bifidobacterium* have been used as probiotics. Probiotic bacteria had beneficial effect on the host basically by improving the gut microbiota balance and stimulation of the immune system. On the other hand, over consumption of probiotics could cause different side effects including diarrhea and bloating and/or flatulence (gas). A probiotic strain has to survive well during manufacturing process without the loss of viability or negative effect on the sensory properties of dairy products. The stability and viability of the probiotic cultures can be increased by a recent technology known as microencapsulation, addition of prebiotics and laser technology as a recent technique. However, extensive research is required to be conducted on the efficacy of micro-encapsulation to deliver probiotics for their controlled and targeted release in the gastrointestinal tract. Also, changes at proteomic level need more investigation for laser treated probiotics.

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الميكروبات الداعمة للحيوية في الأغذية اللبنية: المزايا و العيوب (بحث مرجعي)

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ملخص

تعتبر الميكروبات الداعمة للحيوية (البروبيوتك) ميكروبات نافعة تستوطن أمعاء العائل. وفي الآونة الأخيرة تزايد الاهتمام بالميكروبات الداعمة للحيوية في المنتجات اللبنية على المستوى العالمي بسبب تأثيراتها الصحية الإيجابية. وتشمل الميكروبات الداعمة للحيوية بصفة رئيسية بكتيريا تتبع جنسين رئيسيين هما جنس اللاكتوباسيلس وجنس البيفيدوبكتريا. حديثا، يمثل ميكروب *Akkermansia muciniphila* جيلاً جديداً من الميكروبات الداعمة الحيوية. وفي الأونة الأخيرة، نالت هذه الميكروبات الاهتمام في البحث لقدرة هذه الميكروبات علي تحوير ميكروفلورا أمعاء العائل لتكون أكثر صحية وإتزانا وكذلك خفض مخاطر الإصابة بمتلازمة الأيض. يمثل تحمل الميكروبات الداعمة للحيوية لظروف التصنيع وخلال مدة التخزين وكذلك خلال مرورها في القناة الهضمية تحدياً هاماً لإحداث تأثيرات صحية للعائل. تعتبر الأجبان وبخاصة جبن التشدر والمثلوجات القشدية من أفضل الأغذية اللبنية كناقل لعديد من الميكروبات الداعمة للحيوية وذلك لانخفاض حموضتها مقارنة باليوغورت. وتعتبر طريقة الكبسلة الدقيقة والمدعمات الحيوية (البريبيوتيك) من الاستراتيجيات الرئيسية لتحسين حيوية الميكروبات الداعمة للحيوية خلال فترة التخزين البارد. أدى استخدام التشعيع بالليزر الأحمر إلي تحسين إنتاج نواتج التمثيل الغذائي مثل الأحماض العضوية ومركبات النكهة خلال فترة تخزين اللبنة. يتناول هذا البحث المرجعي تطور تعريف الميكروبات الداعمة للحيوية، التأثيرات الصحية لها، التأثيرات الضارة عند تناول جرعات مرتفعة منها و كذلك التحديات التي تواجهها في منتجات الألبان المختلفة.

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