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Review

In vitro probiotic and industrial properties of bacteria isolated from fermented food products

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Abstract

Probiotics are live microorganisms present in naturally fermented food products, and also added to other products as supplements to improve the hosts' health and microbial balance. Bacteria are considered as probiotics based on selection criteria that include the ability to survive the transition through the gastrointestinal tract conditions (pH and bile salt concentration), adhesion to the intestinal epithelium, auto-aggregation, and antibiotic resistance. The industrial properties of probiotic bacteria associated with their incorporation into food products are essential for the application of probiotic cultures in the development of functional foods. Probiotic bacteria must survive industrial applications, grow adequately in food products during their shelf life, and be technologically suitable for their incorporation into foods products so that they retain viability and efficacy. The antimicrobial activity of probiotic bacterial strains against foodborne pathogenic bacteria may also be a characteristic parameter for probiotics to be included in the composition of probiotic preparations and probiotic foods. This review discusses the *in vitro* and industrial properties of bacteria isolated from a variety of fermented food products.

Introduction

Probiotics are non-pathogenic live microorganisms that improve the microbial balance in the gastrointestinal tract (GIT), and confer health benefits to the hosts (Colombo *et al.,* 2018). Additionally, they are able to stimulate the growth of beneficial microorganisms, thus strengthening the body’s natural defence mechanisms (Sharma *et al.,* 2014). The defensive mechanisms of probiotics include their adherence to the intestinal epithelium, and the permanent colonisation of the GIT by synthesising significant levels of extracellular components (Nemska *et al.,* 2019). These ensure their survival against adverse environmental agents (Grigoryan *et al.,* 2018) and adaptation to different physiological stresses such as stomach acidity and bile salt secretions (Kim *et al.,* 2019). Probiotics have also been associated with nutritional utilisation efficiency, the production of short-chain fatty acids and essential vitamins, and competitive exclusion of pathogenic organisms. Isolates of certain strains have been evaluated for the potential use of feed additives (Kim *et al.,* 2019), in food and feed fermentations (Edalati *et al.,* 2018), and as starter cultures (Azat *et al.,* 2016).

Probiotic products, most of which contain lactic acid bacteria (LAB), have become globally desirable (Klimko *et al.,* 2020). Among these, dairy products are widely used for human consumption as they contain probiotic bacteria of selected strains belongings to genera *Lactobacillus* and *Bifidobacterium* which are considered desirable and beneficial (Gupta and Prakash, 2017; Prabhurajeshwar and Chandrakanth, 2019). Human milk is also a source of microorganisms used for the development and initial composition of the intestinal microbiota of newborns since LAB isolated from nursing mothers have exhibited probiotic properties and health benefits (Reis *et al.,* 2016). Additionally, food products such as sap extract of the coconut palm inflorescence, *neera*, which is a naturally fermented drink (Somashekaraiah *et al.,* 2019), traditional pickles (Tokatli *et al.,* 2015), and naturally fermented olives (Argyri *et al.,* 2013), together with a number of other food types have been used as a source of probiotic bacteria. Selection criteria, including acid and bile salt tolerance, intestinal cell adhesion, antimicrobial activity against pathogens (Maragkoudakis *et al.,* 2006; Ryu and Chang, 2013; Tokatli *et al.,* 2015), auto-aggregation activity, cell surface hydrophobicity, and biofilm formation (Feng *et al.,* 2017; Trunk *et al.,* 2018; Klimko *et al.,* 2020) determine whether a microorganism can be considered as probiotic.

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probiotics have been used as potential alternative biocontrol agents against the potential threat of pathogens (Hossain *et al.,* 2017). These organisms have developed antimicrobial activity against a set of food spoilage and foodborne pathogens (Madureira *et al.,* 2011) by producing antimicrobial metabolites such as organic acids, hydrogen peroxide, and bacteriocins (Khaneghah *et al.,* 2020), all of which contribute to microbiological safety through the prevention/decrease in the growth and risk of contaminating pathogens. Therefore, the consumption of functional probiotic products increases the demands from and wellbeing of the consumers (Colombo *et al.,* 2018)as well as the commercial scale of the products. However, the stability of probiotic bacteria in various types of fermented food products is a concern in food technology. This affects the endurance of probiotic bacteria in the GIT and their significance as health-promoting agents (Afzaal *et al.,* 2019). Various intrinsic and extrinsic factors including oxygen, storage temperature, pH, processing conditions, and post-acidification in fermented food products can affect the viability and stability of probiotics. Studies have revealed that several protection methods including encapsulation (Afzaal *et al.,* 2019; Moghanjougi *et al.,* 2020), microencapsulation (Corona-Hernandez *et al.,* 2013), and microparticles (e Silva *et al.,* 2013) are available which can improve the viability and stability of probiotic bacteria and their endurance in the food products as well as in the GIT. Therefore, this review mainly focuses on the characteristic probiotic and industrial properties of bacteria isolated from different fermented food products.

Fermented food products containing probiotic bacteria

There has been a demand for functional foods that are therapeutic, curative, and supplemented with probiotics (Afzaal *et al.,* 2019). Probiotics are ingested in adequate amounts in functional foods that have potentially positive health effects and provide optimal health benefits in addition to their basic nutritional value. Furthermore, they contribute by reducing the risk of diseases and health-related problems (*i.e.,* lowering blood cholesterol levels). As a result, people could greatly incorporate functional foods into their diet (Vella *et al.,* 2014). Fermented foods are an integral part of the human diet due to their nutritional composition (Tamang *et al.,* 2020), and a predominant carrier (source) of probiotic bacteria that must survive in sufficient numbers in the products to secure their physical and genetic stability during the storage of the products. Some literature define the term fermented foods as "foods or beverages produced through controlled microbial growth, and the conversion of food components through enzymatic action" (Dimidi *et al.,* 2019). This is similar to the definition given by the International Scientific Association for Probiotics and Prebiotics (ISAPP) which is "foods made through desired microbial growth and enzymatic conversions of food components" (Marco *et al.,* 2019).

Fermented foods are also considered as functional foods, some of which are listed in Table 1. Tamang *et al.* (2020) provides a detailed description regarding fermented foods. The presence of probiotic bacteria in these food products and their adaptation to the physiological stresses in the GIT (acidic and bile salt secretions) considerably improve the GIT health through their adherence and colonisation ability (Rezac *et al.,* 2018; Kim *et al.,* 2019). However, with relation to their health benefits apart from the previously known terminology of probiotics, the recently published literature has proposed the probiotics to have three main classes; (1) 'true probiotic' referring to a viable and active probiotic cell, (2) 'pseudo-probiotic' referring to a viable and inactive cell, and (3) 'ghost probiotic' referring to non-viable/dead cell, further classifying each of them into two groups based on their site of action, either *in vivo* or *in vitro* (Zendeboodi *et al.,* 2020). This is to provide a new efficient approach and conceptualisation for all aspects of probiotic health benefits. Probiotic-enriched food products are associated with the provision of health benefits to the consumers (Figure 1), and protect GIT from the colonisation by pathogenic microorganisms.

Possible side effects of fermented foods containing probiotics on human

Probiotic food products are known to exert health benefits, particularly in the prevention and treatment of various conditions and related risks, including inflammatory and oxidative stress in lungs, gut, and liver (Vasconcelos *et al.,* 2019), together with metabolic disorders such as postprandial glycaemic conditions (Grom *et al.,* 2020a), metabolic bone disease (osteoporosis) (Lee *et al.,* 2020), and neurological conditions (Tamtaji *et al.,* 2020). However, in certain conditions, they may develop possible adverse effects include systemic and those localised in the intestinal region (Pace *et al.,* 2020). The intestinal side effects may involve small intestine bacterial overgrowth and resistant gene transfer. Additionally, the joint FAO and WHO (2002) guidelines drafted for the evaluation of probiotics in

Table 1. Selected fermented food products containing probiotic bacteria.

Food product	Source	Reference
Parmigiano Reggiano cheese	Dairy milk	Bertani *et al*. (2020)
Kalari cheese	Buffalo buttermilk	Mushtaq *et al*. (2019)
Artisanal cheeses	Dairy, ewe, and buffalo milk	Kamimura *et al*. (2019)
Kefir	Kefir grain and milk	Nielsen *et al*. (2014)
Kombucha	Beverages, tea, and sugar	Talebi *et al*. (2017)
Pickles	Fermented cucumbers	Tokatli *et al*. (2015)
Tempeh	Fermented soybeans	Soka *et al*. (2015)
Idli batter	Ground rice	Shivangi *et al.* (2020)
Teff dough (*Eragrostic tef*)	Fermentation of dough	Mulaw *et al*. (2019)
Koumiss	Mare's milk	Rong *et al*. (2015)
Utonga-kupsu	Fermented fish	Singh *et al*. (2018)
Miso	Soybeans, rice, and barley	Inoue *et al*. (2016)
Kimchi	Fermented vegetables	Ryu and Chang (2013); Won *et al*. (2020)

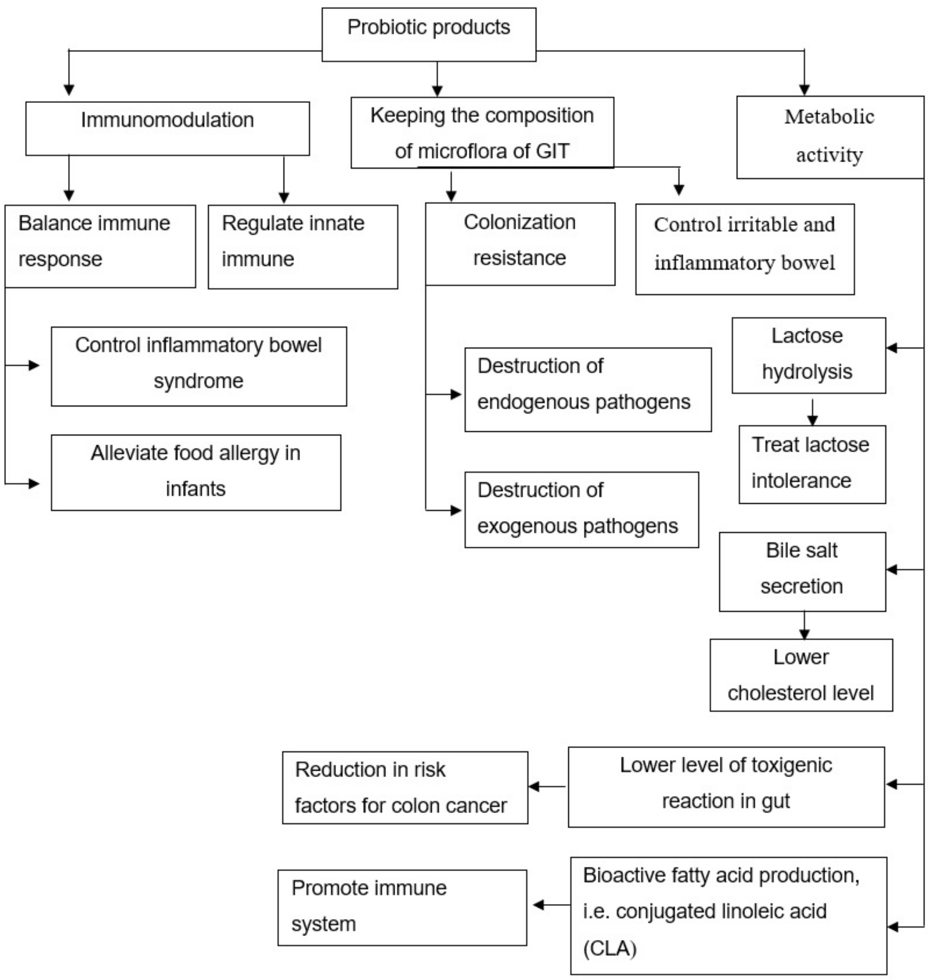

Figure 1. Benefits of consuming probiotic products.

foods stated that probiotics may theoretically be responsible for four categories of side effects which include (1) systemic infections, (2) harmful metabolic activities, (3) excessive immune stimulation, and (4) gene transfer in patients with underlying medical conditions.

Most people consume fermented food products containing probiotics for their beneficial effects (Kariyawasam *et al.,* 2020), and not many focus on their possible adverse effects. The current most serious issue associated with probiotic use, particularly in the gut, is the genetic transfer of antibiotic resistance from probiotic strains to other strains of bacteria, including harmful strains that gradually develop antibiotic resistance, and become difficult to treat (Pace *et al.,* 2020; Li *et al.,* 2020). The presence of transferable resistant genes in large numbers within the intestinal microbiota may impose a potential risk when the pathogens develop resistance, and consequently cause antibiotic treatment failure. Small intestine bacterial overgrowth is another possible adverse effect that can happen as a result of probiotic food consumption. The association between small intestine bacterial overgrowth and probiotic supplementation in patients with brain fogginess has been reported by Rao *et al.* (2018). Some may experience problems of the digestive system including flatulence, bloatedness, and diarrhoea (Pace *et al.,* 2020).

In vitro properties of probiotic bacteria isolated from fermented food products

In vitro investigations have been conducted for the selection of bacteria that have potential probiotic properties including low pH and bile salt tolerance, adhesion to intestinal epithelial cells, production of antimicrobial substances, antibiotic resistance, cell surface hydrophobicity, auto-aggregation, colonisation of intestinal cells, and prevention of pathogens (Jeronymo-Ceneviva *et al.,* 2014; Somashekaraiah *et al.,* 2019; Mulaw *et al.,* 2019; Xu *et al.,* 2020; Shivangi *et al.,* 2020). These properties can be strain-specific, thus differing within the same bacterial species (Klimko *et al.,* 2020).

Tolerance to physiological stresses (acidic and bile salt conditions)

For probiotic bacteria, the most significant characteristic is their survival/tolerance to the adverse conditions in the GIT, including gastric acidity (Succi *et al.,* 2005) and bile salts (Chen *et al.,* 2019) to maintain their population (Lakra *et al.,* 2020), and also their viability within the food. These have been commonly studied under simulated physiological conditions (simulated gastric environment) *in vitro* (Zielińska *et al.,* 2015; Mulaw *et al.,* 2019; Sakandar *et al.,* 2019; Shivangi *et al.,* 2020). According to the draft guidelines for the evaluation of probiotic bacteria in foods which was reported by a joint FAO and WHO working group, resistance to gastric acidity and bile salts are among the currently used *in vitro* tests for the study of probiotic strains (FAO and WHO, 2002). Most probiotic strains isolated from various fermented food products can resist the acid and bile salt conditions, and thus gain a probiotic potential for the development of functional foods. Azat *et al.* (2016) suggested that the acid and bile resistant strains of LAB isolated from traditional Xinjiang fermented cheese and those isolated from traditional Mongolian dairy products have high probiotic potentials (Takeda *et al.,* 2011). Similarly, strains of LAB such as *Lactobacillus plantarum* NO1, *Pediococcus pentosaceus* MP1, *L. plantarum* AF1 (Ryu and Chang, 2013), and *P. acidilactici* P7 (Liu *et al.,* 2020) have been shown as having high degree of acid and bile tolerance, thus showing the potential use as novel probiotics.

The findings of several *in vitro* tests that were performed for the selection of potential acid and bile tolerant probiotic strains showed that the strains can survive at different pH values and bile salt concentrations (Melgar-Lananne *et al.,* 2013; Hashemi *et al.,* 2014; Mulaw *et al.,* 2019; Klimko *et al.,* 2020; Won *et al.,* 2020; Xu *et al.,* 2020) as indicated in Table 2, and those strains may differ in survival rates even under the same pH and bile salt conditions (Azat *et al.,* 2016). These tests can also be performed to ensure the quality of probiotic cultures during manufacturing, storage, and throughout the shelf life of the products. Good probiotics should survive passage through the GIT, and arrive at their site of action in order to function properly, and should be able to be manufactured and incorporated into food products without losing viability (Mishra and Mishra, 2012).

Antibiotic resistance of probiotic bacteria

Several probiotic bacteria are considered as biological therapeutics, host immune-modulating agents, and can resolve the dysbiosis posed by external factors such as antibiotics and other substances (Chen *et al.,* 2019; Das *et al.,* 2020). However, most of them naturally carry antibiotic resistance genes, and may contribute to the transfer of these genes to other commensal bacteria or pathogens present in the GIT (Botina *et al.,* 2011; Sharma *et al.,* 2016; D'Aimmo *et al.,* 2007). This can be due to the intrinsic and mobile genetic elements of probiotics

Table 2. Survival of different probiotic strains (isolated from various foods and other products) to physiological conditions.

Species	Strain	Source	Survival (%) to physiological condition					Reference
			pH			Bile salt		
			2	**2.5**	**3**	**0.2%**	**0.3%**	
Lactobacillus plantarum	JCM 1149	Ergo (yoghurt)	57.36	80.31	93.02	-	93.62	Mulaw *et al*. (2019)
Enterococcus faecalis	R4	Artisanal cheese	0	0	-	-	89.62	Hermanns *et al*. (2014)
Lactobacillus rhamnosus	WEI 30	IM* of healthy piglets	51.23	-	89.29		84.34	Feng *et al*. (2017)
Lactobacillus plantarum	ATCC14917	Fruits and flowers	91.20	93.70	94.10	94.70	-	Sakandar *et al*. (2019)
Bacillus mycoides	AK-01	Indian Ayurveda	58.91	92.33	66.24	-	91.95	Vinothkanna and Sekar (2019)
Bacillus licheniformis	AG-06		72	85.95	63.63	-	84.01	
Lactobacillus plantarum	Y5	Artisan cheese	0	28.57	-	-	85.71	Hermanns *et al*. (2014)
Lactobacillus kunkeei	JNGBKS6	Fruits and flowers	84.74	86.20	88.00	83.40	-	Sakandar *et al*. (2019)
Enterococcus hirae	R5	Xinjiang cheese	-	-	85.3	-	85.1	Azat *et al*. (2016)
Lactobacillus plantarum	CIP 103151	Teff enjera dough	60.15	77.98	90.28	-	91.37	Mulaw *et al*. (2019)
Lactobacillus kunkeei	JNGBKS7	Yellow rose	85.80	87.40	87.45	85.50	-	Sakandar *et al*. (2019)
	JNGBKS8	Pink rose	81.35	84.65	85.36	83.40	-	

Lactobacillus brevis	WEI 22	IM* of healthy piglets	53.28	-	91.94	-	85.57	Feng *et al*. (2017)
Pediococcus pentosaceus	77F	Pig faeces	87.8	-	93.31	-	83.74	Sirichokchatchawan *et al*. (2018)
Pediococcus acidilactici	72N		86.45	-	91.97	-	83.94	
Lactobacillus rhamnosus	WEI 41	IM* of healthy piglets	46.08	-	91.05	-	84.14	Feng *et al*. (2017)
Lactobacillus pentosus	ZFM94	Healthy infant faeces	30.59	43.99	57.43	74.00	63.20	Ye *et al*. (2020)
Lactobacillus fermentum	MA7	Human breast milk	73.82	-	80.20	-	100	Asan-Ozusaglam and Gunyakti (2019)
	MA8		77.77	-	89.49	-	100	
Weissella confusa	OF126	Ogi-funfun	82.4	91.2	-	-	90.9	Adesulu-Dahunsi *et al*. (2018)
	WS90	Ogi-baba	65.3	75.3	-	-	93.8	
Lactobacillus paracasei	CP133	Rice straw silage	-	97.10	-	-	0	Kim *et al*. (2019)
Lactobacillus plantarum	CP134	Kimchi	-	98.42	-	-	95.65	

IM* = Intestinal mucosa, and “-” = data not available.

that confer resistance to various antibiotics (Zheng *et al.,* 2017). The intrinsic / innate / natural antibiotic resistance mechanism of probiotic bacteria ensures their survival in the internal environment (Das *et al.,* 2020). This is a desirable characteristic of the probiotics so that they can re-establish in the host GIT during or after antibiotic treatment (Gueimonde *et al.,* 2013). Egervärn *et al.* (2010) have demonstrated the potential of probiotic strain *L. reuteri* ATCC 55730 to transfer tetracycline resistance gene *tet*(w) to faecal *Enterococcus, Bifidobacteria*, and *Lactobacilli*.

The probiotic resistance of LAB strains isolated from Armada cheese (Spanish goats' milk cheese) against cefoxitin, oxacillin, vancomycin, teicoplanin, nitrofurantoin, and trimethoprim (Herreros *et al.,* 2005) is a good example. Additionally, Mulaw *et al.* (2019) reported that all the isolates of LAB they tested (E052, E031, T035, and K011) were resistant to kanamycin and streptomycin. Likewise, Oussaief *et al.* (2020) also reported that all the examined strains were resistant to erythromycin and kanamycin, while only the strain LEFS 41 was resistant to ampicillin. The antibiotic resistance of nine probiotic strains; three *L. bulgaricus* and six *Streptococcus thermophilus* isolated from commercial yoghurt and cheese in Tianjin, China, was investigated by Wang *et al.* (2019) who found that eight isolates were resistant to at least one antibiotic, and six isolates displayed multidrug resistance.

Probiotics with antibiotic resistance genes can also disseminate the resistance through food products, thus becoming a food safety concern (Sharma *et al.,* 2016). The presence of probiotics in dietary supplements in large amounts promotes them to become a reservoir of antibiotic resistance genes in the host gut (Zheng *et al.,* 2017) where the resistant genes can be transferred to pathogens (particularly during horizontal resistance gene transfer) that cause serious clinical complications and public health problems.

Bacterial cell surface hydrophobicity and auto-aggregation activity

Bacterial cell surface hydrophobicity determines the adhesion capacity of bacteria to various surfaces including animal or human epithelial cells (Krausova *et al.,* 2019). Auto-aggregation in probiotics is necessary for adhesion to intestinal epithelial cells, and prevents the colonisation by other intestinal pathogens (Feng *et al.,* 2017). Both auto-aggregation and hydrophobicity are among the criteria used for the selection of probiotic bacteria (Collado *et al.,* 2008). *In vitro* analyses on certain strains of *Lactobacillus* and *Bifidobacterium* isolated from humans and animals indicated that surface hydrophobicity and auto-aggregation were observed with considerable variations among the strains (Krausova *et al.,* 2019; Feng *et al.,* 2017).

Cell surface hydrophobicity can be determined using the technique of microbial adherence to hydrocarbon assay (xylene) as reported in *L. acidophilus* M92 (Kos *et al.,* 2003), and *L. rhamnosus*, and *Enterococcus hirae* (Azat *et al.,* 2016). According to Kos *et al.* (2003), *L. acidophilus* M92 was subjected to treatments of pH at 2.8, 4.5, and 7.2 to characterise its cell surface hydrophobicity and auto-aggregation. Their results showed that both hydrophobicity and auto-aggregation of the strain slightly decreased at pH 2.8 (55.85 and 62.14%, respectively) as compared to pH 4.5 (73.59 and 68.89%, respectively) and pH 7.2 (70.96 and 71.30%, respectively). Meanwhile, the isolates of *Bifidobacterium bifidum*, *B. merycicum*, and *Lactobacillus* strains (FS2, PS11, MF5, CM1, and PM8) showed good auto-aggregation activity (Vlková *et al.,* 2008), and *L. rhamnosus* strains (SP13, PS2, and FM22) showed the most hydrophobic characteristics (Caggia *et al.,* 2015). Table 3 shows the hydrophobicity and auto-aggregation activities of different probiotic strains isolated from different food products and other sources.

Industrial properties of probiotic bacteria

The probiotic characteristics of bacteria have been the area of focus for scientists from various disciplines such as medical, pharmaceutical, and industrial (Sharifi *et al.,* 2017). The industrial sector has taken a leading role in the development and/or production of novel probiotic foods. This resulted from the increase in consumer awareness towards their capacity to maintain health benefits. Additionally, researchers have also directed considerable focus at improving the existing methodologies for probiotic delivery in addition to investigating new applications (Pavli *et al.,* 2018). In the food fermentation processes, probiotics such as *L. acidophilus* can be added as an adjunct to contribute to the organoleptic of foods as well as extension of the products' shelf life. Moreover, their strong inhibitory action against food spoilage and foodborne pathogens promotes their use as biopreservatives (Anjum *et al.,* 2014; Abbasiliasi *et al.,* 2017). Probiotics have also been used in technological applications (*e.g.,* acidification and milk coagulation) due to their quality and diversity, as well as their usefulness as starter in the dairy industry (Bujnakova and Strakova, 2017). However, the main challenge associated with the application of probiotic cultures in the development

Table 3. The hydrophobicity and auto-aggregation activities of some probiotic strains.

Species	Strain	Source	Parameter		Reference
			Hydrophobicity	Auto-aggregation	
Streptococcus sp.	LEFS55	Dromedary milk	62.70%	76.52%	Oussaief *et al*. (2020)
Pediococcus acidilactici	K98	Wheat flour dough Babroo	42.0%	31.0%	Sharma *et al*. (2019)
Fructobacillus fructosus	JNGBKS2	Narcissus	30.48%	30.89%	Sakandar *et al*. (2019)
Lactobacillus kunkeei	JNGBKS8	Pink rose	37.89%	42.37%	
Weissella confusa	MD1	Fermented batter	29.51%	100%	Lakra *et al*. (2020)
Weissella cibaria	MD2		31.72%	100%	
Staphylococcus epidermidis	E2	Human infant faeces	30.00%	40.00%	Devi *et al*. (2015)
	F2a		30.00%	35.00%	
Bifidobacterium adolescentis	IF1-11	Breast-fed infant faeces	18.62%	82.52%	Zuo *et al*. (2016)
Lactobacillus brevis	CCMA1284	Cauim	95.33%	22.09%	Fonseca *et al*. (2020)
Lactobacillus plantarum	CCMA0359	Cocoa	80.02%	20.10%	
Lactobacillus plantarum	C10	Cured beef	40.36%	93.82%	Wang *et al*. (2018)
Enterococcus hirae	R5	Xinjiang cheese	70.98%	33.65%	Azat *et al*. (2016)
Pediococcus pentosaceus	MLEV8	Cow milk	97.1%	96.3%	Colombo *et al*. (2018)
	MSI7	Silage	98.4%	78.9%	
Bifidobacterium bifidum	A8	Commercial dairy culture	98.3%	-	Gueimonde *et al*. (2005)
Enterococcus durans	F10a	Human infant faeces	ND	ND	Devi *et al*. (2015)
Lactobacillus mucosae	CM21	Cow milk	48.0%	61.7%	

ND = not determined.

of functional foods is their viability maintenance during processing (da Cruz *et al.,* 2009). They are technologically required to be suitable for the incorporation into food products, able to maintain their viability and efficacy in the food products, and able to endure industrial applications and survive adequately in the products during their shelf life. The followings are some of the technological or industrial properties of probiotic bacteria.

Antimicrobial activity

Food spoilers and foodborne pathogens are detrimental microorganisms that spoil / deteriorate food products and pose serious health hazards. Mitigation / prevention strategies often involve the use of beneficial microorganisms such as probiotics. Competitive niche exclusion of the pathogens in the host GIT and in the foods by the probiotics is one of the possible mechanisms employed in the mitigation strategy (Khaneghah *et al.,* 2020). This shows that the fermented food products that contain probiotics are not only a source of nutrition but also possess functional health benefits to protect the consumers from the risk of foodborne pathogens (Mulaw *et al.,* 2019). Abbasiliasi *et al.* (2017) reported that probiotic strain (*P. acidilactici* KP10) isolated from traditional dried curd was found to have inhibitory activity against some of the common foodborne pathogens including *Listeria monocytogenes* ATCC 15313, *Salmonella enterica* ATCC 13311, *Shigella sonnei* ATCC 9290, *Klebsiella oxytoca* ATCC 13182, *Enterobacter cloaca* ATCC 35030, and *Streptococcus pyogenes* ATCC 12378. Similarly, Wang *et al.* (2018) also reported the antagonistic effect of *L. plantarum* PIC33 and *L. plantarum* SK5 against *Staphylococcus aureus*, *Salmonella enterica, Shigella dysenteriae*, and *Escherichia coli*.

Similarly, other *in vitro* investigations have also reported that the acid-bile tolerant LAB isolates exhibited inhibitory activity against the growth of some foodborne pathogens (Klimko *et al.,* 2020; Ryu and Chang, 2013). Mulaw *et al.* (2019) demonstrated the inhibition of LAB strains against commonly known foodborne pathogens such as *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes*, *Salmonella enterica* Typhimurium, and *Escherichia coli* ATCC 25922. The inhibitory effect of other LAB strains against *Escherichia coli* CVCC1570, *Staphylococcus aureus* CVCC1882, and *Salmonella pullorum* AS1.1859 was reported by Feng *et al.* (2017). Additionally, a fermented dairy product containing optimised levels of prebiotic ingredients, namely fructooligosaccharide (FOS) and isomaltooligosaccharide (IMOS), was found to have antimicrobial potential against *Escherichia coli* and *Staphylococcus aureus* (Shafi *et al.,* 2019). They also demonstrated that a synbiotic fermented dairy product exhibited antidiabetic potential in diabetic rabbits. Similarly, a synbiotic yoghurt prepared by probiotic bacterium (*L, acidophilus*) and prebiotic ingredients (FOS and IMOS) was observed with significant hypolipidaemic potential in hyperlipidaemia-induced rabbits fed with the synbiotic yoghurt (Sarfraz *et al.,* 2019). The effect of Minas Frescal cheese, Prato cheese, and whey dairy beverages enriched with probiotic *L. casei* on *in vivo* and *in vitro* anti-hyperglycaemic activity has recently been reported (Grom *et al.,* 2020a). Besides, Grom *et al.* (2020b) suggested that ripened cheese, fermented dairy products, and whey-based products possess an effect on postprandial glycaemic regulation. This generally implicates that, in addition to their antimicrobial role and modulating the GIT microbiota, probiotic-enriched food products also possess potential effects on different health-related conditions, probably metabolic disorders and other conditions.

Generally, due to their antimicrobial role, probiotic-enriched food supplements might be a necessity to inhibit the growth of various spoilage and pathogenic microorganisms in fermented functional foods, particularly during packaging in order to improve food safety and extend the products shelf life (Mishra and Mishra, 2012). The health benefits, including prevention and treatment of various GIT disorders and pathogenic infections, will manifest when they are sufficiently consumed (Gupta and Prakash, 2017; Prabhurajeshwar and Chandrakanth, 2019). In functional food development, the application of probiotics is an essential approach to enhance food safety by controlling the growth of spoilage and pathogenic bacteria through the antimicrobial substances they produce (Mishra and Mishra, 2012; Espitia *et al.,* 2016; Pavli *et al.,* 2018).

Tolerance to salt and phenolic compounds

Probiotic tolerance to salt and phenolic compounds is another essential property of probiotics. LAB strains can tolerate high salt concentration which can be a common occurrence during food processing. Additionally, they may also initiate metabolism in which the bacteria can produce lactic acid and further inhibit the growth of undesirable microorganisms in food products (Divya *et al.,* 2012). Several probiotic strains have been evaluated *in vitro* for their potential applications in the food industry such as for the preparation of starter cultures, functional foods, and probiotic products. For example, a newly isolated bacteriocin-like inhibitory substance-producing

Lactococcus lactis Gh1 strain was evaluated for such applications (Jawan *et al.,* 2020). Their results demonstrated that the strain has a huge potential for application in the food industry, as it was able to tolerate different growth-inhibiting conditions including salt concentrations (0.1 to 4%). Prabhurajeshwar and Chandrakanth (2019) also reported that different isolates of *Lactobacillus* isolated from commercial yoghurt were found tolerant to salt concentrations of 1 - 6%. Similarly, isolates of *L. plantarum* and *Enterococcus faecalis* isolated from Italian Bella di Cerignola table olives showed potential probiotic salt tolerance in the growth media supplemented with 2.5 to 7.5% salt concentrations (Bevilacqua *et al.,* 2010).

Contrary to these findings, other researchers have reported that higher salt concentration inhibits or decreases the growth of some probiotic bacteria (Simonson *et al.,* 2003; Jawan *et al.,* 2020). Goswami *et al.* (2017) reported that species of LAB belongings to *Enterococcus durans, L. plantarum, L. fermentum*, and *L. casei* which were isolated from Kahudi, a traditional rapeseed fermented food product of Assam, India, showed no signs of significant growth at the salt concentrations of 7, 8, and 9%. Similarly, Mulaw *et al.* (2019) reported that one of the LAB isolates (E052) isolated from traditionally fermented Ethiopian food products was unable to grow at 6.5% salt concentrations. It is henceforth suggested that the tolerance of probiotic bacteria to different levels of salt concentrations and other growth-inhibiting factors may be species- and strain-dependent. Furthermore, heterofermentative LAB has also been found more tolerant to NaCl as compared to homofermentative LAB (Jawan *et al.,* 2020).

Phenol is a catabolic product that can be formed by deamination of some aromatic amino acids in the gut by bacteria, and has a characteristic bacteriostatic activity against some bacterial strains (Divya *et al.,* 2012; Abbasiliasi *et al.,* 2017). Studies have suggested that probiotic bacteria are tolerant to phenol which can also be one of the selection criteria for their consideration as probiotic bacteria (Hermanns *et al.,* 2014), and their phenol resistance indicates that they have potential application in the food industry (Jawan *et al.,* 2020). Some autochthonous LAB strains including *L. plantarum* (25F and 22F), *P. pentosaceus* 77F, and *P. acidilactici* 72N were found tolerant to 0.4% phenol concentration (Sirichokchatchawan *et al.,* 2018); while *L. plantarum* Y5, *Enterococcus faecium* U3, and *E. faecalis* R4 were also tolerant to the same concentration of phenol (Hermanns *et al.,* 2014). Similarly, *L. helveticus* LA2, *L. casei* LA1, *L. delbrueckii* LA4, and *L. bulgaricus* LA3 isolated from indigenously pickled vegetables and fermented beverages (Kumar *et al.,* 2012), *P. acidilactici* KP10 isolated from traditional dried curd (Abbasiliasi *et al.,* 2017), and *Lactococcus lactis* Gh1 (Jawan *et al.,* 2020) were all found to be tolerant to phenol compound.

Growth at different ranges of temperature

Temperature is a physical condition that determines the growth and metabolism of microorganisms including probiotic bacteria. Genera of commonly known LAB, including Lactobacillus, *Lactococcus, Leuconostoc, Streptococcus, Pediococcus,* and *Enterococcus* isolated from various food products can grow at various temperatures (Wassie and Wassie, 2016), and show different profiles of metabolites during fermentation. The growth of probiotic bacteria may vary considerably based on the growth media and incubation temperature used. Østlie *et al.* (2005) reported that probiotic strains studied in UHT milk such as *Lactobacillus* strains (*L. acidophilus* LA5, *L. acidophilus* 1748, *L. johnsonii* LA1, and *L. rhamnosus* GG) and *Bifidobacterium animalis* BB12 showed viable cell numbers at 30, 37 and 45°C following fermentation for 48 h. On the other hand, Mulaw *et al.* (2019) reported that acid and bile salt tolerant LAB isolate (E031) was not able to show any growth at 45°C; similar finding was reported for *Leuconostoc* isolated from raw cow milk (Wassie and Wassie, 2016).

LAB have wide industrial applications as starter cultures in the manufacturing of various fermented food products due to their ability to survive at different temperature ranges during processing and storage. The viability of lyophilised probiotic cells of *E. faecium* L3, *L. plantarum* L4, and *L. acidophilus* M92 during 75 days of storage at -20°C, 4°C, and 15°C indicated their potential application in the food industry (Kos *et al.,* 2008). Furthermore, all three strains exerted an antagonistic effect against some foodborne pathogens including *Listeria monocytogenes, Salmonella typhimurium*, and *Yersinia enterocolitica*. Goswami *et al.* (2017) demonstrated that probiotic species namely *E. durans, L. plantarum, L. fermentum*, and *L. casei* were able to survive between 15°C - 40°C, but none survived at 4°C, 45°C, and 50°C. In the case of *Lactococcus lactis* Gh1, Jawan *et al.* (2020) revealed its ability to survive at -30°C to 37°C. The tolerance of these strains at low temperatures may be helpful to control foodborne pathogens which remain a challenge to the food industry since they can survive most common food processing conditions, including extreme pH, high salt concentration, low water activity, and refrigerated temperatures.

Proteolytic activities

In LAB, the proteolytic system have various functions, and is widely used in fermented food manufacturing, especially in the dairy industry. The process of proteolysis (the breakdown of milk protein) which is performed by LAB is an important process to generate amino acids and peptides for bacterial growth and the formation of metabolites. The proteolytic system is also essential in flavour development in fermented milk products (Beganović *et al.,* 2013). Studies have revealed that various probiotic strains isolated from dairy products exhibit proteolytic activities, and are used as a functional starter culture for fermented dairy products (Liu *et al.,* 2010; Beganović *et al.,* 2013). Raveschot *et al.* (2020) demonstrated that two LAB (*L. helveticus* and *L. delbrueckii*) isolated from traditional Mongolian dairy products (yoghurt and fermented milk) were observed with the highest proteolytic activities among the examined strains. Similar findings were also observed for *L. lactis* LEFS 29 and *Streptococcus* sp. LEFS (Oussaief *et al.,* 2020), *L. helveticus* M92 (Beganović *et al.,* 2013), and *Bacillus subtilis* CP350 (Kim *et al.,* 2019).

Conclusions

It has been well explained that probiotic-enriched food products provide health benefits to the host, and modulate the balance of microbiota in the GIT. The characteristic properties that the probiotics exhibit along the passage through the GIT which include low pH and bile tolerance, adherence to the intestinal epithelium, and auto-aggregation determine that probiotic food products have beneficial effects on human health. From industrial perspectives, probiotics are able to survive industrial applications in the food processing facilities, thus ensuring the improvement of food safety and extending the products' shelf life. Therefore, probiotic strains can be applied in large-scale industrial production, and their stable properties will facilitate their ability to be cultured and incorporated into various food matrices without the loss of viability and functionality. In general, the technological issues associated with the development of foods containing probiotics with adequate numbers throughout shelf life, as well as their stability after ingestion are the principal concerns of the food industry.

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