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Probiotics and their applications: an insight on studies performed in Cameroon.

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Key words

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Abstract

Nowadays, there is a rise in the prevalence of cardiometabolic diseases and the phenomena of multiple antibiotics resistance in humans and animals. For these reasons, consumers' interest towards natural products for the management of health issues has increased worldwide. The sector of probiotics which stands as a safe and efficient example of these natural products is currently of growing interest. Indeed, probiotics are microorganisms with demonstrated safety issues for which consumption is associated to positive effects on the host's health. There are several studies dealing with probiotics in the literature. In this study, the focus was made on the state of art of research on probiotics in Cameroon. This review paper brings deep insights on the studies on probiotics performed in Cameroon. It highlights the reported sources of probiotics, and the different methods used for the isolation and identification of these health benefits microorganisms. It also provides information on the different techniques used for the functional characterization of the probiotic properties of the strains. Finally, it also highlights the different fields where the applications of probiotics were reported.

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1. Introduction

1.1. Definition

The definition of probiotics endorsed and assumed in the 4 corners of the world is that they are living microorganisms that, when taken in sufficient amounts, confer a positive benefit on the health of the host (Sanders, 2008). This definition is supported by international organizations such as the FAO (Food and Agriculture Organization) and the WHO (World Health Organization) which define probiotics as living microorganisms that provide health benefits to their hosts in appropriate doses (FAO/WHO, 2002). The discovery of probiotics is commonly attributed to Russian Nobel laureate Élie Metchnikoff, who postulated around 1907 that the longevity of Bulgarian rural people was associated to the regular consumption of fermented dairy products such as yoghurt (Gasbarrini et al., 2016). Since then, the mechanism of action of several probiotics has been demonstrated and several properties of probiotics are now well known. In general, probiotics can improve or stimulate the immune system (immunomodulatory properties) (Aghamohammad et al.., 2022), regulate and balance the intestinal microflora (Fossi et al., 2017b; Toukam et al., 2021), improve lactose metabolism (Rabot et al., 2010), reduce serum cholesterol (Ngongang et al., 2017; Bemmo et al., 2021; Fossi et al., 2022), and exhibit anti-inflammatory (Aghamohammad et al.., 2022), antagonist (Leska et al., 2022), antihypertensive and anti-stress effects (Blum- Menezes et *et al.*, 2019: Bobga et *et al.*, 2022).

1.2. commonly knowns probiotics

In the present work, it was assumed that the host could be a human being or an animal and thus, all microorganisms known for their probiotic abilities in humans or animals were considered. After a thorough investigation of the literature, it was found that the most known and most used probiotic strains belong to the genera Lactobacillus (i.e., *L. acidophilus, L. brevis, L. helveticus, L. bulgaricus, L. fermentum, L. casei, L. crispatus, L. gasseri, L. paracasei, L. plantarum, L. reuteri* and *L. rhamnosus*), Bifidobacterium (i.e., *B. adolescentis, B. lactis, B. animalis, B. pseudolongum, B. bifidum, B. pseudocatenulatum, B. brevis, B. longum,* and *B. thermophilum*), Saccharomyces (*S. cerevisiae* and *S. boulardii*), Enterococcus (some *E. faecium* and *E. faecalis*), Streptococcus (i.e., *S. sanguis, S. oralis, S. mitis, S. thermophilus*, and *S.*

salivarius), Pediococcus (*P. pentosaceus* and *P. acidilactici*), Leuconostoc (i.e., *L. kimchi, L. mesenteroides* and *L. pseudomesenteroides*), Bacillus (some *B. cereus*, *B. clausii*, *B. polyfermenticus*, *B. pumilus*, *B. subtilis*, *B. licheniformis*, *B. coagulans*, and *B. laterosporus*) and some *Escherichia coli* strains (*E. coli* M17 and *E. coli* Nissle 1917). It was also found that other lesser known probiotic strains belong to microbial genera such as Akkermansia (*A. muciniphila*), Bacteroides (*B. dorei, B. fragilis, B. ovatus, B. uniformis* and *B. xylanisolvens*), Carnobacterium (*C. divergens* and *C. maltaromaticum*), Fructobacillus (*F. tropaeoli* and *F. fructosus*), Kluyveromyces (*K. fragilis* and *K. marxianus*), Lactococcus (*L. cremoris* and *L. lactis*), Oenococcus (*O. oeni*) and Wessellia (*W. cibaria, W. confuse, W. hellenica, W. kimchi, W. koreensis* and *W. paramesenteroides*). Reference-based experiments for some of the above-mentioned strains may be found in Table 1. Despite the considerable number of probiotic strains that have been isolated and studied to date, it remains essential to underscore that some microbial species with probiotic potential are still subject to scientific controversy. This is due to the fact that certain strains, while exhibiting beneficial probiotic effects, have also been associated with food safety risks or pathogenicity in specific contexts. A well-known example concerns Enterococcus faecalis and Enterococcus avium, which have been reported multiple times for their probiotic properties (Jiao et al., 2022; Oruc et al., 2021), but which have also been implicated in foodborne illnesses and identified as opportunistic human pathogens (Câmara et al., 2020).

This paradox is not unique to Enterococcus species—it extends to several genera commonly explored for their probiotic functions. Therefore, it is always crucial to systematically assess the safety profile of any microbial strain, no matter how promising it may appear in terms of health effects. Before any strain can be recommended or commercialized as a probiotic, rigorous toxicological, genomic, and functional evaluations are necessary to rule out the possibility of adverse effects. This precaution ensures that the health benefits of probiotics do not come at the expense of consumer safety, especially in vulnerable populations.

1.3. Main sources of probiotics

The term "probiotic" requires clarification, as it can be used in two distinct but often overlapping contexts. On one hand, it refers to the living microorganisms themselves, and on the other, to foods or formulations that contain these microorganisms. This dual usage can lead to ambiguity and misunderstanding if not explicitly distinguished. As microbial entities, probiotics are ubiquitous in nature and can be isolated from a wide array of sources, including animals and animal-derived products, plants and their derivatives, as well as inert environments such as soils and aquatic systems (Tang et al., 2022; Marchwińska et al., 2022; Yaylacı et al., 2022). These diverse origins provide a vast microbial reservoir for the identification of strains with potential probiotic properties.

Once these microorganisms are isolated, they are subjected to identification, characterization, and safety evaluation to ensure they meet the criteria to be considered probiotics (Figure 1). Only strains proven to be non-pathogenic and functionally beneficial are selected for application. These validated strains can then be preserved in usable forms, often through encapsulation, or used to ferment various food matrices, thereby enhancing their health-promoting effects (Mariod & Fatima, 2022). Fermentation offers a dual advantage: it allows the growth of probiotics within a substrate and leads to the production of beneficial metabolites, which further support immune and digestive health (Mariod & Fatima, 2022).

Typical examples of probiotic-rich fermented foods include yoghurt, kefir, kombucha, miso, kimchi, tempeh, sauerkraut, sourdough bread, pickles, and certain cheeses. Beyond these traditional products, probiotics are also used to ferment plant-based foods, such as fruits, vegetables, legumes, and cereals, which expands their applicability and aligns with dietary preferences such as vegetarian or vegan diets (Goderska et al., 2022; Syiemlieh & Morya, 2022).

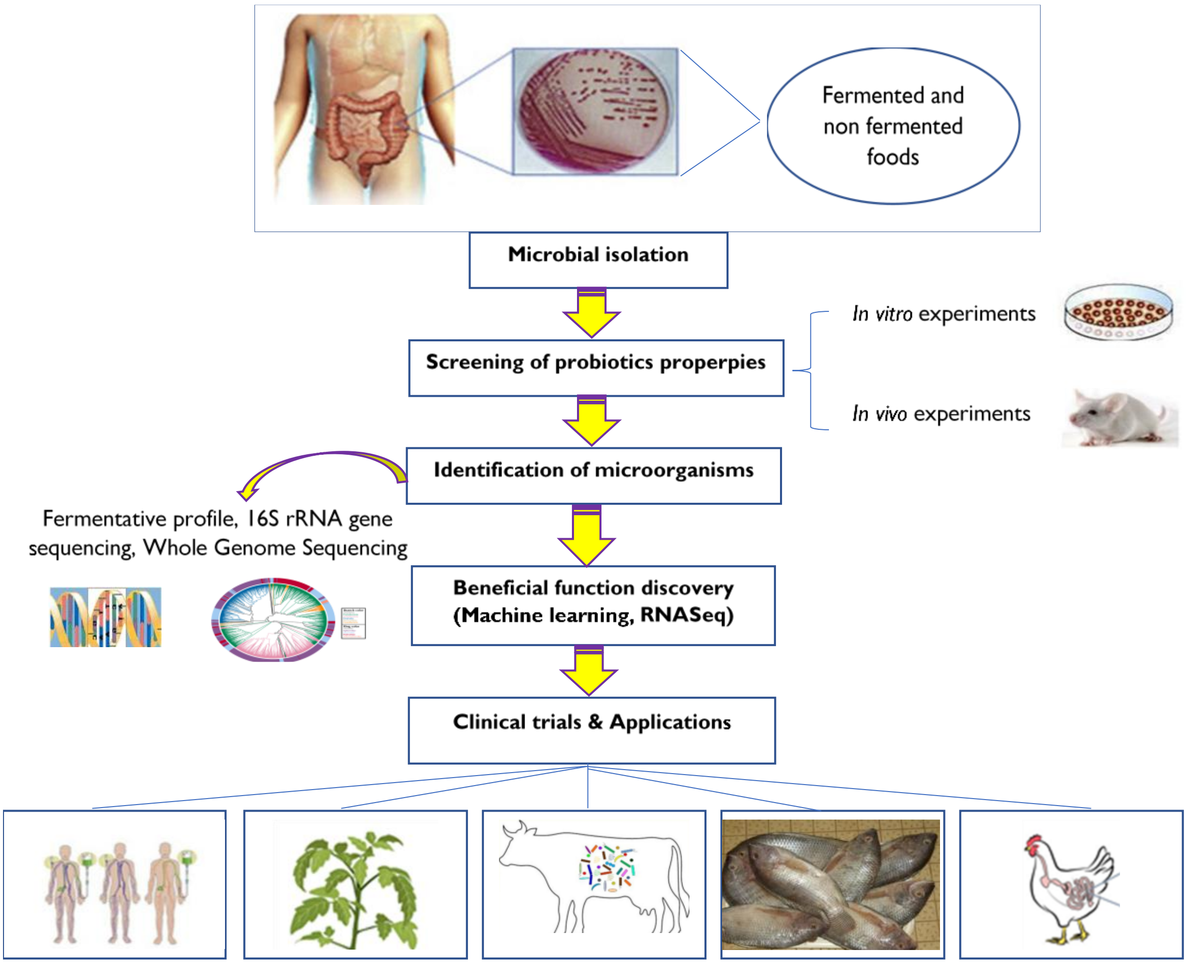

Fig. 1. Graphic summary of the development routine of probiotics before authorization for industrial application.

1.4. World production, regulation, and utilization of probiotics

Although research on probiotics has increased exponentially in recent years, the global production of probiotics is difficult to estimate. Indeed, very few of the probiotics discovered reach an exploitable level on an industrial scale given the complexity of the mandatory preliminary investigations. In general, as observed in Fig. 1, to develop a new probiotic strain with underlying benefits, the required steps include

bacterial isolation, screening of probiotic properties using *in vitro* as well as *in vivo* experiments, characterization of the strain (phenotype, genotype), function discoveries through genes expression (RNASeq) or machine learning, and safety assessments exploring the complete characterizations (clinical trials, *in situ* applications and manufacturing methods) (O'Toole et *al.*, 2017). Tan et al. (2020) argued that according to the regulations of the Food and Drug Administration (FDA), clinical trials for safety evaluation are mandatory to make sure no adverse effects would occur in the volunteers involved before final applications. Thus, to date, only probiotics that have passed all these steps should be considered as such, and more rigorously, only probiotics that have obtained approval from a national or international regulatory organization (FDA, EFSA (European Food Safety Authority) and other local legislations) should be considered as probiotics. Notwithstanding the absence of a universal standard for safety assessment and regulation on probiotics, they are generally used either as drugs or as dietary supplements (Venugopalan et al., 2010). In a recent interesting review by Zommiti et al. (2020), it was suggested that regardless of their use (in humans as well as in animals), the most important parameter is that probiotics present no health hazard.

1.5. Health benefits' effects of probiotics

Table 1 presents at least one reported positive effects/properties of some well-known probiotic strains belonging to a specific genus. Depending on the strain used, probiotics may exhibit several health benefits.

Table 1. Some properties and health benefits of probiotics from different genera.

Genus	Species	Properties or health benefits	References
Aspergillus	*A. orizae*	Suppression of pancreatic tumor	Konishi et al. (2021)
		Activation of the blood immune responses and TNF-α, IL-1β, and HSP70 gene expression.	Dawood et al. (2020)
Akkermansia	*A. muciniphila*	Antibacterial, anti-obesity, anti-tumor effects in different *in vivo* models	Ghotaslou et al. (2022)
Bacillus	*B. subtilis*	Secretion of amino coumacin A and inhibition of *Helicobacter pylori*	Pinchuk et al. (2001)
	B. licheniformis	Inhibition of biofilm formation by *Vibrio parahaemolyticus* Dahv2	Gobi et al. (2016)
Bacteroides	*B. dorei*	Antiviral activity	Song et al. (2022)
	B. uniformis	Downregulation of cholesterol and triglycerides; repression of the serum glucose, insulin, and leptin	Gauffin et al. (2012)
	B. xylanisolvens	Boosted the level of natural anti-TFα IgM antibodies in healthy adults	Ulsemer et al. (2016)
Bifidobacterium	*B. bifidum*	Reduction of the incidence of diarrhea and induction of better stool consistency.	Chitapanarux et al. (2010)
	B. lactis	Decrease the frequency, duration of diarrhea, and hospital stay in children with acute diarrhea.	Abou El-Soud et al. (2015)
	B. pseudolongum	Reduction of dextran sodium sulfate (DSS) responsible of body weight loss and the release of several pro-inflammatory factors (interleukin (IL)-6, IL-1β, and tumor necrosis factor-α (TNF-α)) and increase the activities of antioxidant enzymes (T-AOC, SOD, GSH) and the concentrations of tight junction proteins (occludin, claudin-1, and ZO-1).	Guo et al. (2022)
Carnobacterium	*C. divergens*	Reduction of the epithelial cell invasion caused by *L. monocytogenes* Scott A.	Pilchova et al. (2016)
Enterococcus	*E. feacium*	Regulation of the inflammation in human enterocyte-like HT-29 cells	Tian et al. (2016)
	E. faecalis	Assimilation of cholesterol by deconjugating glycine of bile salts	Mishra et al. (2019)
Escherichia	*E. coli* M17	Effective against inflammatory bowel disease and diarrhea in infants and children	Fitzpatrick et al. (2008)
Fructobacillus	*F. tropaeoli*	Inhibition of bacteria quorum sensing and antibacterial activity	Simsek et al. (2022)
Kluyveromyces	*K. marxianus*	Modulation of the immune response and decrease the level of pro-inflammatory cytokines	Lisotti et al. (2013)
Lactococcus	*L. lactis*	Antimicrobial activity	Akbar et al. (2019)
Lactobacillus	*L. acidophilus*	Anticancer activity	Nami et al. (2014)
Lactobacillus	*L. brevis*	Antimicrobial and antioxidant activities	Kunduhoglu et al. (2021)
	L. bulgaricus	Antimicrobial, anti-inflammatory, lipid-regulating activities, and improved blood biochemical parameters.	Evivie et al. (2020)
	L. fermentum	Enhance the immunologic response and prevent community-acquired gastrointestinal and upper respiratory infections.	Naghmouchi et al. (2020)
	L. gasseri	Immunomodulatory effects	Sun et al. (2020)
	L. paracasei	Immunomodulatory potential and induction of cytokine and chemokine expression in Caco-2 cells	Chondrou et al. (2020)
	L. plantarum	Antioxidant, anticancer, anti-inflammatory, antiproliferative, anti-obesity and antidiabetic properties.	Arasu et al. (2016)
	L. rhamnosus	Reduction of the diarrhea duration	Li et al. (2019)
Leuconostoc	*L. citreum*	Immunomodulatory effects	Kang et al. (2016)
	L. mesenteroides	Modulate the maternal metabolism	Castro-Rodríguez et al. (2020)
Oenococcus	*O. oeni*	Immunomodulatory activity, lowering colonic injury and alleviating colitis symptoms.	Foligné et al. (2010)
Saccharomyces	*S. cerevisiae*	Neuroprotective, antioxidant, antidiabetic, anti-inflammatory, immune booster, antimalarial, and antitumoral effects	Farid et al. (2019)
Streptococcus	*S. thermophilus*	Increase the expression of anti-inflammatory IL-4, IL-5, IL-10 cytokines, and decrease the secretion of pro-inflammatory IL-1β and IFN-γ.	Dargahi et al. (2020)
	S. salivarius	Antibacterial activity against *Streptococcus mutans* responsible of tooth decay and dental plaque accumulation and acidification.	Burton et al. (2013)
Weissella	*W. confusa*	Antimicrobial activity	Lakra et al. (2020)
	W. paramesenteroides	Antimicrobial activity	Pabari et al. (2020)

In recent years, several evidence-based experiments *in vitro* and *in vivo* have confirmed the beneficial effects claimed for probiotics, and research in this direction is growing. For example, Bifidobacteria were mostly reported for their antimicrobial, anticancer, anti-inflammatory and have been employed in the prevention and management of gastrointestinal disorders, facilitate nutrient absorption and promote bone health (Chen et al., 2021). In addition, bacteria many probiotics from the genus Lactobacillus are known for their ability to reduce cholesterol levels (Cho and Kim, 2015), reduce diarrhea (Sazawal et al., 2006), improve symptoms of irritable bowel syndrome (Sinn et al., 2008), treat and prevent vaginal infections (Ravel et al., 2011), promote weight loss (Million et al., 2012), help prevent and reduce cold and flu symptoms (Leyer et al., 2009), prevent and reduce allergy symptoms (Ishida et al., 2005), help prevent and reduce symptoms of eczema (Kim et al.,2014), and promote gut health (Baarlen et al., 2011).

2. Objectives and data extraction methodology

This review summarizes current key evidence on probiotics isolated, identified, or used in Cameroon. To identify the relevant data, numerous review articles, original articles, and related books from reputable databases, such as Web of Science, PubMed, and Scopus were investigated. No open access published papers have been made available using the facilities provided by the People's Friendship University of Russia, Moscow, Russia. The literature was investigated between October 2022 and December 2022. Given that English and French are the two languages used in Cameroon, the literature investigation was conducted using the keywords in both languages. The research simultaneously included 2 or more following keywords always associated to "Cameroun" or "Cameroon": "probiotics" or "probiotiques", "fermented food" or "aliments fermentés".

3. Probiotics' studies from Cameroon

3.1. Source of probiotics

Table 2. Studies performed in Cameroon showing the sources, identification methods, probiotic properties and applications of probiotics in different fields.

Sources	Identification	Probiotic properties	Strains	Application	References
Fermented sap from African oil palm	API 50 CHL kit	Antimicrobial activity, acid and bile salts tolerance	L. pentosus, L. plantarum, and L. brevis	-	Fossi et al. (2015)
Curded milk	API 50 CHL Kit	Antimicrobial activity, acid and bile salts tolerance	*L. casei* LS3 and *L. plantarum* LM4	Substitute of antibiotics in chicken farming. Food processing formulation.	Fossi et al. (2017a)
Fermented sap from African oil palm	-	-	*L. rhamnosus* IS9	Inhibition of pathogens in the intestine of infected chicks	Fossi et al. (2017b)
Fermented sap from African oil palm	API 50 CHL kit and 16S rRNA gene sequencing	Antimicrobial activity, production of bacteriocins	*L. plantarum, L. rhamnosus* and *L. brevis*	-	Fossi et al. (2017c)
Fermented sap from African oil palm and corn beer	API 50 CHL kit and 16s rRNA gene sequencing	Acid and bile salts tolerance, cholesterol assimilation	*Levilactobacillus brevis* PW4 and *E. faeccium* Cb5	Reduction of LDL-cholesterol and increase HDL- cholesterol in mices sera.	Fossi et al. (2022)
"Sha'a"	rRNA ITS1-5.8S-ITS4 and NL1-28S-NL4 genes sequencing	Survival in gastric juice, auto-aggregation, co-aggregation, hydrophobicity, hemolytic, bile salt hydrolase and gelatinase activities, biogenic amine production, antibiotics and antifungals	*S. cerevisiae* LT25, *S. cerevisiae* LT16 and *Nakaseomyces delphensis* LT80	-	Piame et al. (2022)

		susceptibility, scavenging of DPPH radical			
"Sha'a"	API 50CH kits, rep-PCR genomic fingerprinting	Acid and bile tolerance, deconjugation of bile salts, and cholesterol-lowering property,	*L. plantarum* Lp10S and *L. plantarum* Lp11S	Cholesterol-lowering properties and reduction of atherosclerosis index in hypercholesterolemia rabbits	Zambou et al. (2013b)
"Sha'a"	API 50CH kits, rep-PCR genomic fingerprinting	Acid and bile salts tolerance, production of bacteriocins, and antimicrobial activity.	*L. plantarum* 2S	Formulation of a probiotic stable honey.	Zambou et al. (2013a)
Fermented maize beverage	-	Colonization of rat intestine	*L. lactis* sp.	Formulation of probiotic fermented food that improve growth performance and reduce the risk of cardiometabolic diseases.	Dangang et al. (2018)
Fermented milk	16S rRNA gene sequencing	Acid and bile salts tolerance, hemolytic activity.	*L. sakei* DMS 20017	Antimalarial activity in BALB/c mice	Toukam et al. (2021)
Fermented cow milk	16S rRNA gene sequencing	Acid and bile salts tolerance, antibiotics susceptibility, and hemolytic activity.	*L. fermentum* PRI 29	Management of Type-1 diabetes mellitus and oxidative stress.	Bobga et al. (2022)
Kindirmou and *pendidam*	16S rRNA gene	Acid and bile salt tolerance, detection of biogenic amine genes, antibiotic susceptibility, antifungal activity.	*P. acidilactici*, *L. fermentum* and *L. plantarum*	-	Nodem Sohanang et al. (2021)
Artisanal fermented milk	API 50CH kits, 16S rRNA gene	Bile salts tolerance, bile-salt hydrolase (BSH) activity, detection of *Bsh* genes, and antimicrobial activity.	*L. plantarum* (strains GGU, GLA51 and GLP56)	-	Bemmo et al. (2017)
Commercial yogurts	-	-	*L. bulgaricus*, *S. thermophilus*, *Bifidobacteria*, *L. acidophilus* P106, and *L. acidophilus* P110	Reduction of cholesterol and lipid levels in the serum of rats	Ngongang et al. (2016b)
Raw cow milk	API 50 CHL kit, RAPD-PCR	Acid and bile salts tolerance, antibiotics susceptibility, gelatinase, bile-salt hydrolytic, antimicrobial and hemolytic activities, cholesterol-lowering property	*L. plantarum* 1RM, *L. plantarum* 11RM and *L. plantarum* 29V	-	Sieladie et al. (2011)
Raw cow milk	API 50CH kits, Rep-PCR genomic fingerprinting	antimicrobial activity, acid and bile salt tolerance, antibiotic sensitivity, cholesterol assimilation	*L. plantarum*	-	Zambou et al. (2012)
Raw cow milk	API 50CH kits, rep-PCR genomic fingerprinting	-	*L. plantarum* 29V	Survival in honey and management of induced cardiovascular diseases	Bemmo et al. (2021)

				and hypercholesterolemia in Wistar rats	
Inner walls of bottle gourds (calabashes)	API 50CH kits	Acid and bile salt tolerance, cholesterol hydrolysis, antimicrobial activity, antioxidant activity	*L. casei* (strains BL1, BL14, BL19 and BL20), *L. fermentum* (strains BL21 and BL24), *L. plantarum* (BL4 and BC4) and *L. mesenteroides* (strains BL11, BL18 and BC3)	-	Tchamba et al. (2021)
Commercial	-	-	*L. fermentum* and *B. bifidum*	Formulation with raphia sap of a probiotic beverage endowed of antimicrobial activity.	Mbarga et al. (2021)
Commercial	-	-	*L. fermentum* and *B. bifidum*	Fermented fresh sap beverage	Mbarga et al. (2019)
Raphia palm wine	API 50CH kits,	Acid and bile salts tolerance, and cholesterol assimilation.	*L. pentosus* 1R32	Hypocholesterolemic effect in Wistar rats	Ngongang et al. (2016a)
Raphia palm wine	API 20C AUX	Acid and bile salts tolerance, and cholesterol assimilation.	*S. cerevisiae* 1R27 and *Candida famata* 3W29	Management of hypercholesterolemia in Wistar albino rats	Ngongang et al. (2017)
Raphia palm wine	API 50 CHL	Antimicrobial activity, acid and bile salt tolerance, antibiotic sensitivity, hemolytic activity and enzymes production.	*L. lactis*, *L. plantarum* and *L. brevis*.	-	Tongwa et al. (2019)
Water from submerged cassava fermentation	16S rRNA gene sequencing	Antimicrobial activity, bacteriocin-like inhibitory substances, acid and bile salts tolerance, bile salt hydrolase activity, detection of genes involved in the hydrolytic activity of bile salts.	*L. plantarum*, *L. brevis*, *L. paracasei*, and *L. paraplantarum*.	-	Foko et al. (2018)
Water from submerged cassava fermentation	16S RNA gene sequencing	Acid and bile salts tolerance, cell surface hydrophobicity, auto-aggregation, co-aggregation, antibiotic susceptibility, bile salt hydrolase, hemolytic and gelatinase activities, detection of acid resistance, bile salts resistance and biogenic amines genes.	*L. paracasei* (strains 62L, 63L, 64L, 80L, 84L, 85L, 86L, and 106L)	-	Foko et al. (2017)
Intestines from tilapia and common carp as well as pond water	16S RNA gene sequencing	Acid and bile salts tolerance, detection of acid and bile salts resistance genes, tolerance to simulated gastric and intestinal juices, bile salt deconjugation, cell surface hydrophobicity, auto-aggregation, co-	*L. lactis* subsp. lactis 1FT, *L. lactis* subsp. lactis 1FW, *L. lactis* subsp. lactis 3FT, *L. plantarum* 1KMT, *L. plantarum* 4BC, *L. plantarum* 13BC and *L. brevis* 1BT	-	Kaktcham et al. (2017)

		aggregation, hemolytic and gelatinase activities, antibiotic susceptibility, production of biogenic amines, PCR detection of biogenic amines production genes			
Chicken crop and intestine	API 50CHL	Antibacterial activity, acid and bile salts tolerance, antibiotic sensitivity	*E. faecium* LC, *L. cremoris* NPL, *Pediococcus* sp. L4	-	Bakari et al. (2011)
Intestines of Nile tilapia and common carp	-	-	*L. plantarum* 1KMT	Fish farming to improve growth performance, innate immunity parameters and resistance to diseases.	Temgoua et al. (2020)
Feces of farmyard chickens and ducks	-	-	*Lactobacillus plantarum* MW-18CGZ and *Lactobacillus paracasei* MW-37CGZ	1. Chicken farming for growth performance and nutritional quality 2. Meat from chicken to Wistar rats for safety	Wadoum et al. (2019b)
Water from submerged cassava fermentation	16S rRNA gene sequencing	Antimicrobial activity, bacteriocin-like inhibitory substances, acid and bile salts tolerance, bile salt hydrolase activity, detection of genes involved in the hydrolytic activity of bile salts.	*L. plantarum*, *L. brevis*, *L. paracasei*, and *L. paraplantarum*.	-	Foko et al. (2018)
Water from submerged cassava fermentation	16S RNA gene sequencing	Acid and bile salts tolerance, cell surface hydrophobicity, auto-aggregation, co-aggregation, antibiotic susceptibility, bile salt hydrolase, hemolytic and gelatinase activities, detection of acid resistance, bile salts resistance and biogenic amines genes.	*L. paracasei* (strains 62L, 63L, 64L, 80L, 84L, 85L, 86L, and 106L)	-	Foko et al. (2017)
Intestines from tilapia and common carp as well as pond water	16S RNA gene sequencing	Acid and bile salts tolerance, detection of acid and bile salts resistance genes, tolerance to simulated gastric and intestinal juices, bile salt deconjugation, cell surface hydrophobicity, auto-aggregation, co-aggregation, hemolytic and gelatinase activities, antibiotic susceptibility, production of biogenic amines, PCR detection of biogenic amines production genes	*L. lactis* subsp. lactis 1FT, *L. lactis* subsp. lactis 1FW, *L. lactis* subsp. lactis 3FT, *L. plantarum* 1KMT, *L. plantarum* 4BC, *L. plantarum* 13BC and *L. brevis* 1BT	-	Kaktcham et al. (2017)

Digestive tract of honey bees	16S rRNA gene sequencing	Acid and bile salts tolerance, hydrolase, gelatinase and hemolytic activities, cell surface hydrophobicity, auto-aggregation, antibiotic susceptibility, antioxidant activity.	*L. plantarum* (strains H15, H21, H24, H28 and H47)	-	Kenfack et al. (2018b)
Digestive tract of honey bees	16S rRNA gene sequencing	-	*L. plantarum* H24	Cytotoxic activities against Caco-2 colon cancer cells	Momo et al. (2020)
Digestive tract of honey bees	16S RNA gene sequencing	Acid and bile salts tolerance, screening for genes involved in resistance to acidity and bile salts, bile salt hydrolase activity, detection of *Bsh* genes, antimicrobial activity.	*L. plantarum* (strains H6, H15, H21, H24, H28, H44, H46, H47, and H82) and *L. paraplantarum* (strains H63, H67, and H87)	-	Kenfack et al. (2018a)
Feces of farmyard chickens and ducks	API 50 CH kit, High-Resolution Melting (HRM) Analysis,	Antimicrobial activity, deconjugation of bile salts, acid and bile salts tolerance, cell surface hydrophobicity, auto-aggregation, co-aggregation, antibiotic susceptibility, hemolytic activity, gelatinase activity, AMP-like factor expression, proteolytic activity, inter-antagonism (co-existence) assay.	*L. paracasei* MW-37CGZ, *L. paracasei* MW-38CGZ, *L. plantarum* MW-18CGZ, *L. plantarum* MW-48CGZ	-	Wadoum et al. (2019a)

Using the web of science database (PubMed, Google Scholar, Google, EMBASE, Medline, Cochrane and Sino Med library), a total of 34 publications from Cameroon dealing with probiotic microorganisms were selected. As shown in Table 2, the sources of probiotics are diverse including food, feces, intestine, and environmental samples.

Fermented foods are the first well-documented sources of probiotic microorganisms in the past and also in several recent studies worldwide (Fossi et al., 2022). Amongst fermented foods, the most reported are milk products due to their acidic character which might confer resistance to the low pH of the stomach to these microorganisms. In Cameroon, there are several reports highlighting raw milk and milk products as sources of probiotic microorganisms. Traditional fermented milk (Bemmo et al., 2017; Toukam et al., 2021; Bobga et al., 2022; Ngwa et al., 2022), cow's raw milk (Sieladie et al., 2011; Zambou et al., 2012; Bemmo et al., 2021), *Kindirmou* and *Pendidam* (Nodem Sohanang et al., 2021), curdled milk (Fossi et al., 2017a) as well as some commercial yoghurts sold in Cameroonian markets (Ngongang et al., 2016a) were reported as sources of probiotics. Besides milk products, some traditional beverages and wines were also reported as sources of probiotics. Amongst these products, one can cite the fermented maize beverage (Dangang et al., 2018), fermented sap from African oil palm (Fossi et al., 2015; Fossi et al., 2017c; Fossi et al., 2022), "Sha'a" (Zambou et al., 2013a; Zambou et al., 2013b; Piame et al., 2022), fermented raphia sap (Ngongang et al., 2016b; Ngongang et al., 2017; Tongwa et al., 2019), palm wine (Ngwa et al., 2022) and corn beer (Fossi et al., 2022). Recently, probiotics were isolated from the inner walls of bottle gourds (calabashes) used locally as fermenters to ferment raw cow milk (Tchamba et al., 2021). Animal intestines were highlighted as a source of probiotics microorganisms. Kaktcham et al. (2017) isolated probiotics from Nile tilapia and common carp intestines. Bakari et al. (2011) isolated probiotics from chicken crops and intestines. In fact, probiotics are included in the microbiome of animals and humans where they played an important role in the well-being of the host. Moreover, they also constitute the microflora of insects. Kenfack et al. (2018a) and Momo et al. (2020) isolated probiotics from the stomach of honey bees.

Probiotics colonize the gastrointestinal tract of the host for which they procured health benefits. They interact with pathogens through several action mechanisms and some of them might be excreted together with pathogens in feces. Probiotics were found by Wadoum et al. (2019a) in the feces of farmyard chickens and ducks. Environmental samples such as pond water where fish are reared were highlighted by Kaktcham et al. (2017) as a source of probiotics that probably originated from feces of fish farmed therein. In the study conducted by Foko et al. (2017, 2018), the authors isolated probiotics from the water from submerged cassava fermentation. Indeed, several microorganisms are involved in the process of cassava fermentation in Cameroon amongst which lactic acid bacteria. Hence, amongst these lactic acid bacteria, some might display some probiotic features.

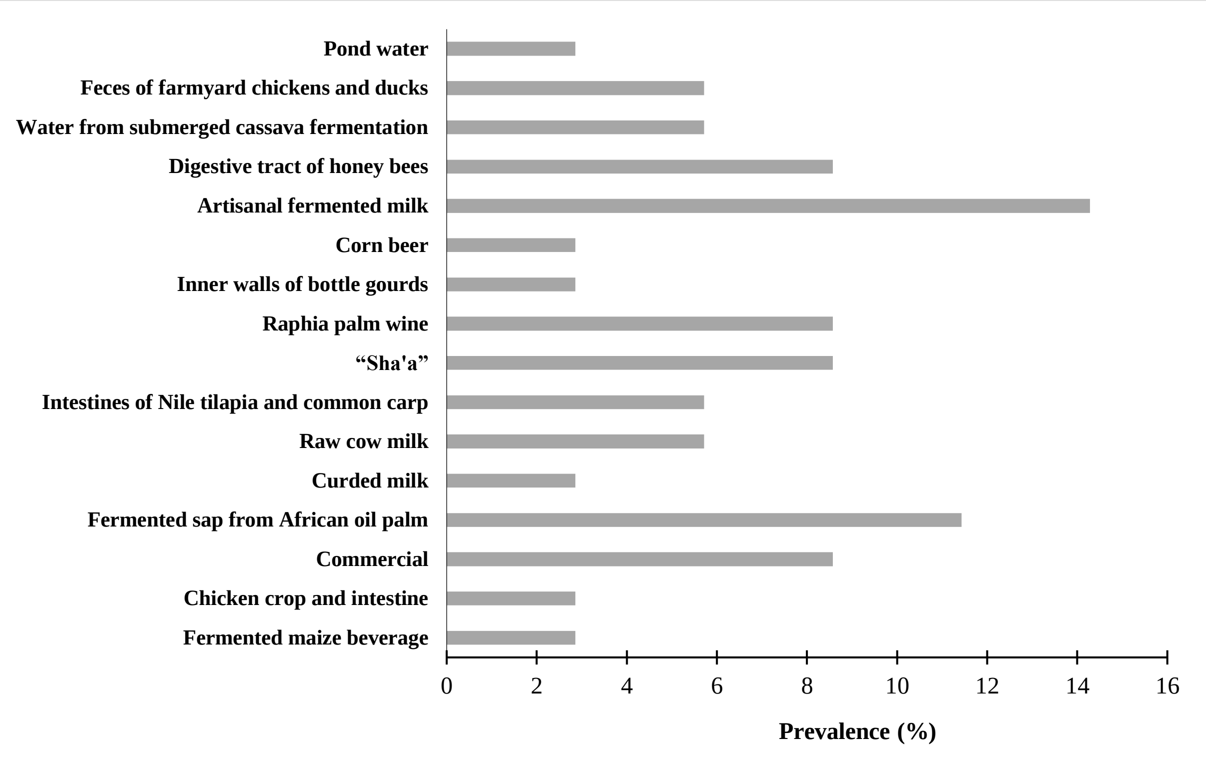

Fig. 2. Sources of probiotic microorganisms from studies performed in Cameroon

Globally, as observed in Fig. 2, the most important source of probiotics is artisanal fermented milk (14.29%). This might be ascribed to the well-known health benefit properties associated with the consumption of these indigenous foods amongst Cameroonians and particularly the Fulani ethnic group as highlighted in the literature (Mbawala et al., 2018). The second main sources were fermented sap from African oil palm (11.43%), a low-cost and ready-availability beverage widely consumed not only by Cameroonians but also by several African (Fossi et al., 2022). This is probably because of its simple sugars' content that favors the proliferation of microorganisms including probiotics.

3.2. Isolation of probiotics

In almost studies, probiotics belonging to lactic acid bacteria were isolated through culture on Man Rogosa and Sharpe agar (MRS) by the pour plate or the surface inoculation method. Incubation was almost performed under anaerobic conditions, at temperatures ranging from 30 to 37°C, and duration periods from 24 to 96 h. With regards to the pour plate method, it was used by Ngongang et al. (2016b), Fossi et al. (2017a), Bobga et al. (2022), Fossi et al. (2022) and Ngwa et al. (2022). The surface inoculation method reported in the publications was performed by adding 0.1 mL of the sample on MRS agar followed by spreading onto the surface of the culture media (Bakari et al., 2011; Tchamba et al., 2021). Some authors performed a preliminary pre-enrichment of the samples in MRS broth to activate the strains before their isolation. In those cases, the surface inoculation onto MRS agar was done by streaking (Sieladie et al., 2011; Foko et al., 2018). In some studies, differential growth factors were added to MRS media to improve its selectivity. These growth factors were L-cysteine-HCl 5%, w/v (Zambou et al., 2012; Kenfack et al., 2018; Wadoum et al., 2019a) and citric acid (10%, v/v) + pimaricin (0.1 g/L) (Nodem Sohanang et al., 2021).

However, some reported probiotics isolated from local sources were yeast strains. The isolation of these probiotic yeast strains was performed either by the pour plate method on Sabouraud Dextrose agar (Ngongang et al., 2017) or by the surface inoculation onto Potato Dextrose agar (PDA) supplemented with chloramphenicol (Piame et al., 2022).

3.3. Characterization of probiotics

The most reported preliminary characterization tests of isolates assigned to lactic acid bacteria in 100% of publications were catalase and Gram staining. Some authors associated to these two tests, oxidase (Bakari et al., 2011; Wadoum et al., 2019a; Nodem Sohanang et al., 2021; Tchamba et al., 2021; Fossi et al., 2022; Ngwa et al., 2022), growth at different temperatures (Bakari et al., 2011; Sieladie et al., 2011; Zambou et al., 2012; Foko et al., 2018; Kenfack et al., 2018; Tchamba et al., 2021; Bobga et al., 2022), growth under different NaCl concentrations (Bakari et al., 2011), production of CO_2 from glucose (Sieladie et al., 2011; Zambou et al., 2012; Foko et al., 2018; Kenfack et al., 2018; Wadoum et al., 2019a; Tchamba et al., 2021), motility (Wadoum et al., 2019a; Bobga et al., 2022), and spore formation (Wadoum et al., 2019). The isolates assigned to lactic acid bacteria were screened for their probiotic features and only these showing the properties were identified. The sugar fermentative profile of the selected isolates on API 50 CHL kit was mostly used for the phenotypic identification at the species level. Amongst the 34 publications on probiotics from Cameroon, the use of API 50 CHL kit was reported in 44.11% (15 publications) (Bakari et al., 2011; Sieladie et al., 2011; Zambou et al., 2012; Zambou et al., 2013b; Zambou et al., 2013a; Fossi et al., 2015; Ngongang et al., 2016a; Bemmo et al., 2017; Fossi et al., 2017a; Fossi et al., 2017c; Tongwa et al., 2019; Wadoum et al., 2019a; Bemmo et al., 2021; Tchamba et al., 2021; Fossi et al., 2022). Genomic identification of the isolates based on 16S rRNA gene sequencing was reported either associated with their fermentative profile on API 50 CHL kit or as alone identification technique (Foko et al., 2018; Kenfack et al., 2018; Nodem Sohanang et al., 2021; Toukam et al., 2021; Bobga et al., 2022; Fossi et al., 2022; Ngwa et al., 2022). Some molecular techniques such as high-resolution melting (HRM) analysis (Wadoum et al., 2019a), Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) analysis (Sieladie et al., 2011) and Rep-PCR genomic fingerprinting (Zambou et al., 2012; Zambou et al., 2013b; Zambou et al., 2013a; Bemmo et al., 2021) were often associated with the fermentative profile of the selected isolated on API 50 CHL kit to strengthen the results.

Opposite to bacteria, the identification at the species level of the probiotic yeast strains was based on their fermentative profile on API 20C AUX (Ngongang et al., 2017). Morphological criteria, production of CO_2, survival in presence of ethanol 8% (v/v), glucose 20% (w/v), and at pH 3, and the amplification of rRNA ITS1-5.8S-ITS4 and NL1-28S-NL4 genes following with sequencing of the PCR products were used by Piame et al. (2022) to identify probiotic yeast strains.

3.4. Assessment of probiotic properties of isolates

Probiotics are generally found in the oral cavity, gastrointestinal tract, upper respiratory tract, and vagina of human beings where they exert their health benefits effects. Amongst these sites, the most important one is the gastrointestinal tract. To reach that targeted site and exert their activity, there are some specific criteria which are required. They included their ability to survive in hostile conditions of gastric and intestinal juices and growth in the intestine. Moreover, their health benefits effects in the intestine are associated to their safety for the host, their ability to produce postbiotics which will inhibit pathogens and block their adhesion to human intestinal cell lines, and their ability to adhere and colonize the intestinal epithelial surfaces. These properties are therefore used to assess the probiotic feature of strains.

In studies conducted on probiotics in Cameroon, the tests performed were variable from one author to another. Amongst the publications on probiotics from Cameroon, only those for which studies were performed to assess the probiotic properties of isolates were selected. The rest of the studies wherein previously isolated and characterized probiotics were used for specific applications were excluded. Globally, the almost reported tests in 100% of the selected publications were acid and bile salts' tolerance. These tests were often associated with susceptibility to antibiotics, antimicrobial activity and/or hemolytic activity (Bakari et al., 2011; Fossi et al., 2015; Toukam et al., 2021; Bobga et al., 2022; Ngwa and *al*, 2022).

The production of enzymes of health and/or technological importance as well as the safety issue of the isolates were highlighted as probiotic features (Tongwa et al., 2019). Functional properties such as the regulation of cholesterol levels in the blood were also reported. They were the cholesterol assimilation (Zambou et al., 2012) and the cholesterol-lowering activity (Sieladie et al., 2011; Zambou et al., 2013b; Ngongang et al., 2016a; 2016b). Some studies reported as probiotic features the antioxidant activity of the isolates (Kenfack et al., 2018b; Tchamba et al., 2021, Piame et al., 2022), the cell surface hydrophobicity (Kenfack et al., 2018a; Wadoum et al., 2019a; Piame et al., 2022), the production of biogenic amines (Piame et al., 2022, Nodem Sohanang et al., 2021), their ability to aggregate and colonize the intestine and therefore exert antimicrobial activity through the inhibition adhesion of pathogens or production of postbiotics (Kenfack et al., 2018a; Wadoum et al., 2019a; Piame et al., 2022).

Besides these biochemical tests, molecular approaches based on the detection of genes involved in the expression of probiotic features of the isolates were also used. The genes detected were mainly those responsible for biogenic amines, acid and bile salts resistance, and bile salts hydrolysis (Bemmo et al., 2017; Foko et al., 2017; Kenfack et al., 2018a; Nodem Sohanang et al., 2021).

3.5. Potential application of probiotics

Fig. 3 depicts the different applications of probiotics in various fields from studies performed in Cameroon. They include the sector of animal feeding (23%), food formulations (14%), and the management and prevention of diseases like the cholesterol-lowering properties (27%), enhancement of antioxidant enzymes (9%), reduction of prooxidant (9%), management of gastric ulcer (5%), management of malaria (5%), management of diabetes (4%), and the management of gastrointestinal diseases (4%). The highest prevalence of studies on cholesterol-lowering properties of probiotics might arise from the silent-killer nature of diseases associated to high cholesterol levels in the body. According to the reports of Zhong et *al.* (2019), cardiovascular diseases for which billion of deaths are registered worldwide has as the main risk factors hypercholesterolemia. Cameroon with 15.8% of deaths related to cardiovascular diseases is not of rest (Nkoke et al., 2019). Thus, justifying the high proportion of studies on probiotics dealing with hypercholesterolemia.

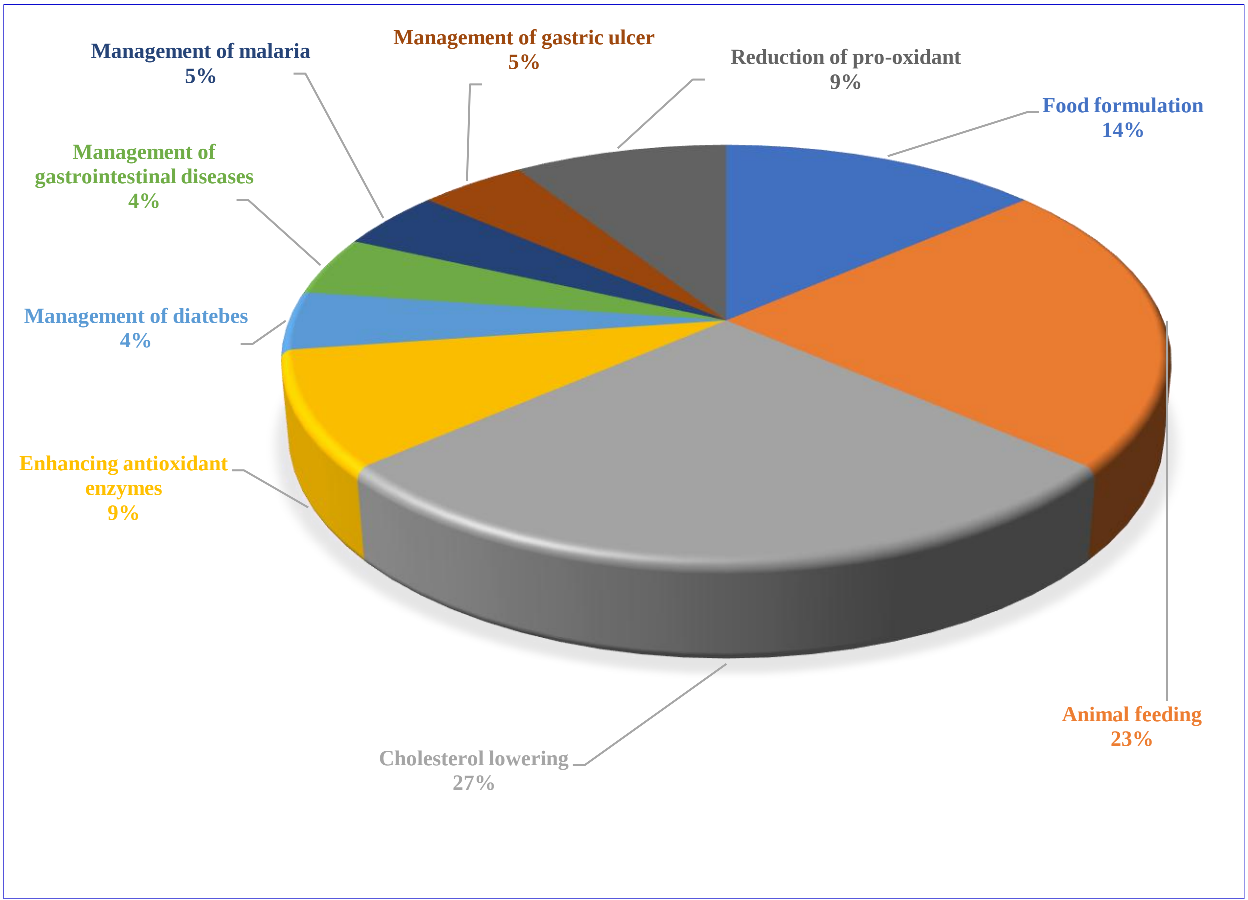

Fig. 3. Applications of probiotics from studies performed in Cameroon

3.5.1. In food formulations

In Cameroon, there are very few studies highlighting the application of probiotics in food formulations despite the large number of studies performed on probiotics from various matrices. Most of them deal with the isolation, characterization and assessment of the probiotic properties of the isolates. 14% of studies have reported the food application of probiotics (Fig. 3). Zambou et al. (2013a) tested the viability of the probiotic strain *L. plantarum* 2S in pasteurized honey samples stored at 4 and 25°C for 28 days. They found survival percentages of 64.47 and 90.66% after storage at 25 and 4°C, respectively. Besides, the physicochemical quality of the honey was not altered during the storage period independent of the temperature (Zambou et al., 2013a). Later, Bemmo et al. (2021) also tested the viability in pasteurized honey of the probiotic strain *L. plantarum* 29V isolated from raw cow milk. The authors found no change in the quality of honey after 28 days of storage (Bemmo et al., 2021). Thus, suggesting honey as a good matrix for carrying probiotics and ensure their delivery in their target site which is the gastrointestinal tract. Mbarga et al. (2019) formulated a beverage from the fresh sap of *Raffia farinifera* fermented by a mixture of two probiotic strains (*B. bifidum* and *L. fermentum*). The authors have determined the optimal fermentation conditions of fresh sap leading to a beverage containing 8.05 Log CFU/mL with good physicochemical quality and which was organoleptically acceptable (Mbarga et al., 2019).

3.5.2. In animal feeding

The sector of animal feeding is of growing interest nowadays as the demand for meat products has increased worldwide. To face that situation, antibiotics are abusively used in that sector leading to the presence of their residues in the final product intended for human consumption, and thus, the development of the phenomena of multi-drug resistance. Probiotics appear as an alternative as besides their antimicrobial activity, they possessed several other health benefits' properties. Their application in animal feeding including cattle, beef, buffalo, fish, and chicken have reported elsewhere in several studies. Although no *in vivo* or *in situ* test was performed, Kaktcham et al. (2017) have evoked the possible application in aquaculture of probiotics that were isolated from the intestines of common carp and Nile tilapia. Further future researches need to be performed in that field as the authors obtained probiotics strains with good properties. Temgoua et al. (2020) administrated the probiotic strain *L. plantarum* 1KMT isolated from the intestines of Nile tilapia and common carp to healthy fish and conducted the experiments for 60 days. They noticed a survival rate of more than 85%, and improvements in the growth performance, the intestinal microflora of fish, the innate immunity parameters, and resistance to disease induced by *Vibrio parahaemolyticus* 1T1.

In chicken farming, the probiotic strains *L. plantarum* (LM4) and *L. casei* (LS3) were administrated to chicks previously infected with *Salmonella enterica* sp. The authors noticed that the proliferation of the pathogen in the chicks' gastrointestinal tract was significantly ($p<0.05$) reduced by the probiotics (Fossi et al., 2017a). These observations suggest the potential use of probiotics as a substitute for antibiotics in chicken farming. They also highlighted the improvement of heat resistance of the probiotics in presence of some minerals like calcium and magnesium salts (Fossi et al., 2017a). Thus, suggesting their possible applications in food processing industries. Later, the authors demonstrated the ability of the probiotic strain *L. rhamnosus* (IS9) to significantly ($p<0.05$) reduce the proliferation of *S.* Enteritidis and *S.* Typhimurium in the small intestine of infected chicks, with a more pronounced action in 14-days old chicks (Fossi et al., 2017b). The probiotics (*L. paracasei* MW-37CGZ and *L. plantarum* MW-18CGZ) were formulated and administrated to five-day-old chicks for 42 days (Wadoum et al., 2019b). After this period the proximate composition of their meat was assessed. The authors noticed a significant improvement in the nutritional quality of the meat from chicken fed with probiotics compared to those with antibiotics (Wadoum et al., 2019b). These results suggest the use of probiotics in chicken farming rather than antibiotics. Later, the meat from chicken fed with probiotics and antibiotics was administrated to Wistar albino rats for 28 days. Although non-significant, the Protein Efficiency Ratio of rats fed with probiotics were higher than those with antibiotics. Moreover, no variation in the biochemical and hematology parameters of rats was noticed (Wadoum et al., 2019b). These results demonstrate the safety of meat from chicken fed with probiotics and the potential of these useful bacteria as biotherapeutic substitutes for antibiotics in chicken farming.

3.5.3. For the management and prevention of diseases

The potential application of probiotics from Cameroon in the formulation of foods which can be used to manage and prevent several diseases including cardiometabolic diseases was reported in some studies. Zambou et al. (2013b) have administrated to the white strain of male rabbits through oral gavage, the suspension of the probiotics *L. plantarum* Lp11S and *L. plantarum* Lp10S. The authors found that these probiotics when administrated together with cholesterol in the diet, led to a significant increase in HDL cholesterol while the (VLDL+IDL+LDL)-cholesterol levels and the atherosclerosis index significantly decrease. Ngongang et al. (2016a) induced hyperlipidemia in Wistar rats and tested the effect of oral gavage with the probiotic strain *L. pentosus* (8-10 Log CFU/ mL) for 4 weeks. The probiotic was not harmful and induced a significant reduction of total cholesterol, triglycerides, LDL and VLDL cholesterols in the rats' blood serum. However, it has also increased the HDL cholesterol level. The same experiments were repeated later by the authors in the same conditions with probiotics yeast strains *S. cerevisiae* 1R27 and *Candida famata* 3W29 instead of *L. pentosus*. They also found an increase in HDL cholesterol and a significant decrease in total cholesterol, triglycerides and LDL cholesterol (Ngongang et al., 2017). Bemmo et al. (2021) used pasteurized honey as a matrix to deliver probiotic strain *L. plantarum* 29V in the gastro-intestinal tract of adult male Wistar rats in order to manage induced cardiovascular diseases and hypercholesterolemia. The authors reported a lowering of VLDL-cholesterol, LDL-cholesterol, total cholesterol, triglycerides as well as the atherosclerosis index. In counterpart, the level of HDL-cholesterol was significantly increased. Momo et al. (2020) identified plantarone, kojic acid and methyl dodecanoate from the probiotic strain *L. plantarum* H24 isolated from honey bee tract with demonstrated DPPH free radicals scavenging and cytotoxic activities against Caco-2 colon cancer cells. They suggest the potential use of the strain in the formulation as a preventive and curative measure against the risks of colon cancer, and to prevent oxidative stress. More recently, Fossi et al. (2022) demonstrated the ability of probiotics (*Levilactobacillus brevis* PW4 and *Enterococcus faeccium* Cb5) to reduce LDL-cholesterol and increase HDL-cholesterol following their administration to Wistar albino rats fed with hyperlipidemic diet. Thus, suggesting their potential use for the management of cardiovascular diseases. Globally, these results suggest the potential use of these probiotic strains as functional foods for patients suffering from cardiometabolic disorders such as hypercholesterolemia and hyperlipidemia.

Besides, Mbarga et al. (2021) assessed the antagonistic effect of raphia sap fermented with probiotics against several pathogenic bacteria. They found that the fermented beverage was active against both Gram negative and positive bacteria. These results demonstrated that the consumption of that beverage might manage and/or prevent several diseases including sepsis, intoxination, gastroenteritis, and urinary tract infections. Toukam et al. (2021) assessed the schizontocidal activity of the probiotic strain *L. sakei* in BALB/c mice which were infected with the malaria agent *Plasmodium berghei*. The authors found that probiotics administration led to a significant ($p<0.05$) inhibition of the parasite growth, prevented body weight loss and decreased the hematological parameters. This observation suggests the potential use of probiotics in the treatment of malaria as a substitute for chemical drugs for which the phenomena of resistance is currently increased.

The ability of probiotics to inhibit gastric ulcers was also reported. After oral administration of probiotics (*Limosilactobacillus fermentum* strain BB101 (F1) and *L. casei* strain 02 (F2)) for 14 days, Ngwa et al. (2022) have induced gastric lesions on BALB/c mice using absolute ethanol. The authors noticed a significant ($p<0.05$) attenuation of the gastric ulcer, and the prevention from oedema, desquamation of epithelial lining, and hemorrhagic damages in mice pretreated with probiotics. Moreover, the gastric level of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) was significantly increased while its level of nitric oxide as well as lipid peroxidation products such as malondialdehyde, were significantly ($p<0.05$) reduced (Ngwa *et al.*, 2022).

The cell suspensions of the probiotic strain *L. fermentum* PRI 29 were administrated to BALB-c mice for which Type-1 diabetes mellitus was induced (Bobga et al., 2022). The authors noticed an improvement in oral glucose tolerance, polydipsia and polyphagia in mice treated with probiotics. The oxidative imbalance was in favor of antioxidants as the levels of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) were significantly ($p<0.05$) increased while those of prooxidants (nitric oxide and malondialdehyde) were significantly ($p<0.05$) reduced. Thus, demonstrating the potential use of probiotics for the management of Type-1 diabetes mellitus as well as cardiometabolic diseases.

4. Conclusion and future challenges

This review demonstrates that there are studies performed on probiotic microorganisms in Cameroon. It highlights shows that probiotics can be isolated from various sources including food, feces, gastrointestinal tract as well as environmental samples. These probiotics possess interesting health benefits' properties which can be exploited in food formulations and animal feeding. There are very few studies dealing with the applications of probiotics in foods and feeds.

However, the concept of probiotics in foods intended for human consumption and in animal feeding is not yet well developed in Cameroon and does not enter into the food habit of a great part of the population. Another important issue that should be addressed is the formulation of probiotics in a form that can be easily handled. This will ease its dissemination amongst local as well as traditional food producers. For that, there is a need for studies on the incorporation of probiotics in several products made traditionally which are consumed by the large majority of the population taking into consideration the context of the low income of the majority of local inhabitants. For future studies, next-generation sequencing and machine learning should also be considered as they will reduce the time consumption and costs associated to traditional biochemical tests used for the functional characterization of probiotics as well as their safety.

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Data Availability Statement

Upon reasonable request, the data used in this study will be made available from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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