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Review

Probiotic Potential of Lactic Acid Bacteria and Yeast Isolated from Cocoa and Coffee Bean Fermentation: A Review

Aylin López-Palestino [1,†], Regina Gómez-Vargas [1,†], Mirna Suárez-Quiroz [1,*], Oscar González-Ríos [1], Zorba Josué Hernández-Estrada [1], Olaya Pirene Castellanos-Onorio [1], Rodrigo Alonso-Villegas [2], Aztrid Elena Estrada-Beltrán [3] and Claudia Yuritzi Figueroa-Hernández [1,*]

[1] Tecnológico Nacional de México/Instituto Tecnológico de Veracruz, Unidad de Investigación y Desarrollo en Alimentos, M. A. de Quevedo 2779, Veracruz 91897, Mexico; m21020029@veracruz.tecnm.mx (A.L.-P.); m20020021@veracruz.tecnm.mx (R.G.-V.); oscar.gr@veracruz.tecnm.mx (O.G.-R.); zorba.he@veracruz.tecnm.mx (Z.J.H.-E.); olaya.co@veracruz.tecnm.mx (O.P.C.-O.)
[2] Facultad de Ciencias Agrotecnológicas, Universidad Autónoma de Chihuahua, Av. Pascual Orozco s/n, Campus 1, Santo Niño, Chihuahua 31350, Chihuahua, Mexico; ralonso@uach.mx
[3] Facultad de Enfermería y Nutriología, Universidad Autónoma de Chihuahua, Circuito Universitario s/n, Campus 2, Chihuahua 31125, Chihuahua, Mexico; aeestrada@uach.mx
* Correspondence: mirna.sq@veracruz.tecnm.mx (M.S.-Q.); claudia.fh@veracruz.tecnm.mx (C.Y.F.-H.)
† These authors contributed equally to this work.

Abstract: The market for probiotic foods has grown significantly in recent years. Some microorganisms isolated from food fermentations, mainly lactic acid bacteria (LAB) and yeasts, may have probiotic potential. During the fermentation of cocoa and coffee, a plethora of microorganisms are involved, including yeasts and lactic acid bacteria (LAB), several of which may have probiotic potential. For this reason, this study aimed to overview the probiotic potential of some LAB and yeasts isolated from these fermentation processes. For this purpose, a search was conducted in several specialized databases (Google Scholar, PubMed, ScienceDirect, and Scopus). As a result of this search, some strains of LAB and yeasts from cocoa were found to be potentially probiotic, with characteristics like those of commercial probiotic strains. The LAB genera that showed the most substantial probiotic potential were *Lactiplantibacillus*, *Limosilactobacillus*, and *Lactococcus*, while for yeasts, it was *Saccharomyces* and *Pichia*.

Keywords: probiotics; lactic acid bacteria; yeast; cocoa; coffee; fermentation

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1. Introduction

There has been a growing awareness among consumers in the last two decades that their state of well-being and their health are closely related. Therefore, they are looking for foods that provide health benefits besides fulfilling the function of nourishing. These foods are known as functional foods [1,2]. Among these foods are probiotics, which can be defined as live microorganisms that can improve the host's health when administered in a certain amount [2,3]. This term, derived from the Latin pro (in favor) and the Greek bios (life), was first used in 1954 [4]. Probiotics are a significant food industry segment; the global probiotics market, including dietary supplements, exceeded USD 71.2 billion by 2024. Additionally, this market is continuously expanding and is expected to reach USD 105.7 billion by 2029, showing a compound annual growth rate (CAGR) [5]. The expansion in this market may be related to the significant increase in consumers' interest in health and lifestyle issues or because they can use probiotic microorganisms to treat or prevent certain digestive or metabolic disorders [6].

Indeed, it is well known that probiotic microorganisms can colonize intestinal epithelial cells, resist unfavorable conditions of the gastrointestinal tract, even regulate the intestinal microbiota of the host, and exert various biological functions [7,8]. Some bacteria, such as lactic acid bacteria (LAB) and bifidobacteria, have been shown in several studies to have probiotic characteristics [7,9,10]. Additionally, some yeast strains, such as *Saccharomyces boulardii* and *Kluyveromyces marxianus* [11–14], and some strains of the genus *Bacillus*, such as *B. clausii* or *B. coagulans* [15,16], have been shown to have probiotic capacity. However, to be considered a probiotic, a microorganism should meet specific characteristics such as the following: being correctly identified (genus, species, and strain), not producing metabolites toxic to the host or any virulence factors, the ability to survive through the gastrointestinal tract (pH, enzymes, and bile salts), and the ability to colonize the epithelial cells of the intestine (adherence to epithelial cells, auto-aggregation, hydrophobicity). Furthermore, it should exert potential host health benefits, which should be validated in vitro, in vivo, and in clinical trials [7,16,17].

There is evidence that probiotic microorganisms can exert their benefits through the direct involvement of four mechanisms, which are listed below: (i) microbial antagonism through the production of antimicrobial compounds, (ii) direct competition for nutrients and adhesion to the epithelium with pathogenic bacteria, (iii) immunomodulation of the host, and (iv) inhibition of bacterial toxin production [18]. Principal sources of probiotic microorganisms are the human intestine (large and small), human milk, animal intestines, and various food products. Among foods, dairy products and fermented foods are the main source of probiotic microorganisms [18–21]. Recently, there has been a growing interest in microorganisms and bioactive compounds isolated from plant sources [22–24]. Considering this growth in demand for these products, there is a need to find alternative sources for isolating microorganisms with probiotic potential. Notably, fermented foods of plant origin are among them. Several yeasts and lactic acid bacteria are involved in cocoa and coffee fermentations, which could have potential probiotic characteristics [25–31]. Nevertheless, this has not been exhaustively discussed and reviewed. For this reason, this review article aims to provide an overview of the probiotic potential of the indigenous microorganisms of these fermentations, their status, and future research in this field.

2. Historical Evolution of the Concept of Probiotic Microorganisms

In 2014, an expert panel (International Scientific Association for Probiotics and Prebiotics-ISAPP) defined the most accurate term for probiotics: live microorganisms that, when administered in adequate amounts, confer a health benefit to the host [3]. However, extensive prior knowledge was necessary to reach this agreement on the term probiotic. Pasteur discovered lactic acid-producing bacteria, later known as lactic acid bacteria, in 1857. Later, at the end of the 19th century, scientists from the Pasteur Institute isolated LAB strains from the intestinal tract [32]. In the early 20th century, Elie Metchnikoff correlated the longevity of some populations in Bulgaria with the regular consumption of milk fermented with lactobacilli [18]. Tissier and Metchnikoff were the first researchers to demonstrate the beneficial potential of certain microorganisms (lactobacilli and bifidobacteria), suggesting that the intake of these microorganisms could restore the intestinal microbiota of patients with diarrhea [2]. In 1935, fermented milk containing a probiotic microorganism, *Lacticaseibacillus casei* Shirota, was launched, previously isolated by Dr. Minoru Shirota. Later, in 1965, Lilly and Stillwell first coined the term probiotic [2]. Subsequently, Fuller, FAO/WHO, and Hill et al. 2014 redefined the term probiotic. The main contributions to the evolution of the term probiotic are shown in Figure 1.

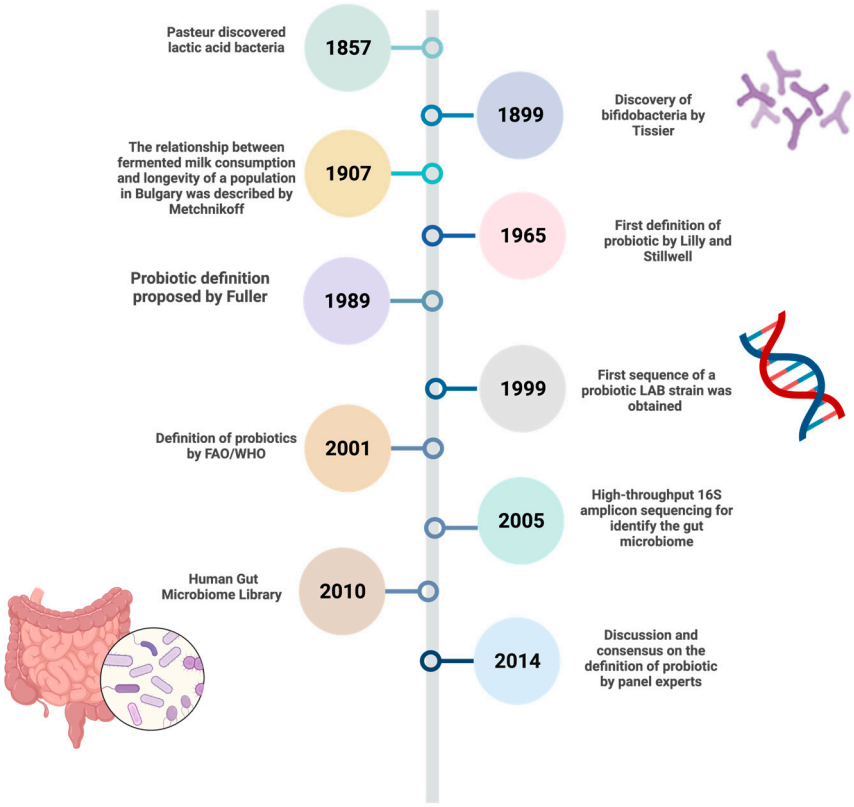


Figure 1. Timeline of main discoveries on the probiotic term. Created in Biorender.

All these scientific contributions served to enrich and strengthen the term probiotic. However, this also enriched other terms related to probiotics, such as prebiotics, synbiotics, and postbiotics [2]. ISAPP defines prebiotics as a substrate that is selectively utilized by host microorganisms, conferring a health benefit [33]. At the same time, symbiotics were defined by ISAPP as a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms, conferring a health benefit to the host. Host microorganisms include autochthonous (resident or colonizing) and allochthonous (externally incorporated, such as probiotics) microorganisms [34]. Finally, postbiotics, a term that has gained much prominence in recent years, were defined by ISAPP as a preparation of inanimate microorganisms and/or their components that confer a health benefit to the host. An effective postbiotic should contain inactivated microbial cells or cellular components that contribute to the observed health benefits, whether in the presence or absence of their metabolites [35].

3. Main Microbial Genera with Potential Probiotic Characteristics

Most microorganisms validated as probiotics belong to the genera *Bifidobacteria* and *Lactobacillus*. However, the genus *Lactobacillus* was reclassified in 2020 into 25 genera, including the modified genus *Lactobacillus* and *Leuconostoc*, as well as 23 new genera within which stand out: *Lacticaseibacillus*, *Lactiplantibacillus*, *Latilactobacillus*, *Lentilactobacillus*, *Levilactobacillus*, *Ligilactobacillus*, and *Limosilactobacillus*, which may have probiotic characteristics [36]. Other bacterial genera with probiotic species are *Lactococcus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, and *Bacillus*. In the case of yeasts, only *Saccharomyces boulardii* and *Kluyveromyces marxianus* have been accepted as probiotic microorganisms [13,37]. Table 1 shows the main microorganisms commonly recognized as probiotics. However, not all strains of an accepted probiotic species exhibit these characteristics.

Table 1. The main microorganisms' genera and species are reported as potential probiotics.

Microbial Genus	Main Species	Probiotic Strains Validated Examples	References
Lactobacillus	*Lb. acidophilus*, *Lb. helveticus*, *Lb. crispatus*, *Lb. johnsonii*, *Lb. delbrueckii*	*Lb. johnsonii* NCC 533, *Lb. helveticus* R0052, *Lb. acidophilus* LA-05	[38,39]
Limosilactobacillus	*Lim. fermentum*, *Lim. reuteri*	*Lim. fermentum* ME-3, *Lim. reuteri DSM17938*, ATCC PTA 4659, and ATCC PTA 6475	[40–43]
Lacticaseibacillus	*Lcb. casei*, *Lcb. paracasei*, *Lcb. rhamnosus*	*Lcb. casei* Shirota, *Lcb. casei* DN-114001, *Lcb. rhamnosus* GG *Lcb. rhamnosus* SD4 and SD11	[44–48]
Lactiplantibacillus	*Lpb. plantarum*	*Lpb. plantarum* 299v	[49–55]
Levilactobacillus	*Lev. brevis*	*Lev. brevis* MK05	[49,56–59]
Ligilactobacillus	*Lig. salivarius*	*Lig. salivarius* UCC118	[60–64]
Lactococcus	*L. lactis*	*L. lactis* subsp. *lactis* CAB701, *L. lactis* MKL8	[65–72]
Streptococcus	*S. salivarius*	*S. salivarius* LAB813	[73,74]
Enterococcus	*E. faecium* *E. faecalis*	*E. faecium* SF68	[75–78]
Pediococcus	*P. acidilactici*	*P. acidilactici* DSM 16342, GR1 and LB-3	[79–82]
Bacillus	*B. clausii* *B. coagulans*	*B. coagulans* BC30, *B. coagulans* Unique IS-2	[83–86]
Bifidobacterium	*B. lactis*, *B. longum*, *B. infantis*, *B. bifidum*, *B. breve*, *B. adolescentis*, *B. animalis*, *B. thermophilum*	*B. infantis* 35624, *B. lactis* Bi-07, *B. lactis* Bl-04, *B. lactis* HN019, *B. lactis* BB-12, *B. breve* M-16V	[87–96]
Saccharomyces	*S. boulardii*	*S. boulardii* CNCM I-745	[97–102]
Kluyveromyces	*K. marxianus fragilis*	*K. marxianicus* CBS 6936	[102–106]

4. Beneficial Effects on Host Health Associated with Probiotic Consumption

Probiotic microorganisms exhibit different mechanisms of action, although the exact way they exert their effects has not yet been fully elucidated. These mechanisms range from producing short-chain fatty acids and bacteriocins, lowering intestinal pH and nutrient competition, and stimulating mucosal barrier function and immunomodulation. Especially the latter has been the subject of numerous studies, and there is sufficient evidence that probiotics affect various aspects of the acquired and innate immune response by inducing phagocytosis and IgA secretion, modifying T cell responses, enhancing Th1 responses, and attenuating Th2 responses [107,108]. Some mechanisms by which probiotics exert their beneficial effects on the host are shown in Figure 2.

There is evidence that probiotic microorganisms can help prevent certain inflammatory and allergic diseases, such as rhinitis and atopic dermatitis. They also help reduce the incidence of diarrhea and may even protect against some types of cancer, such as bladder and colon cancer. Other beneficial effects of probiotics include controlling blood lipid levels in patients with mild hypercholesterolemia and preventing infection due to their antimicrobial activity against certain pathogenic microorganisms [109–111].

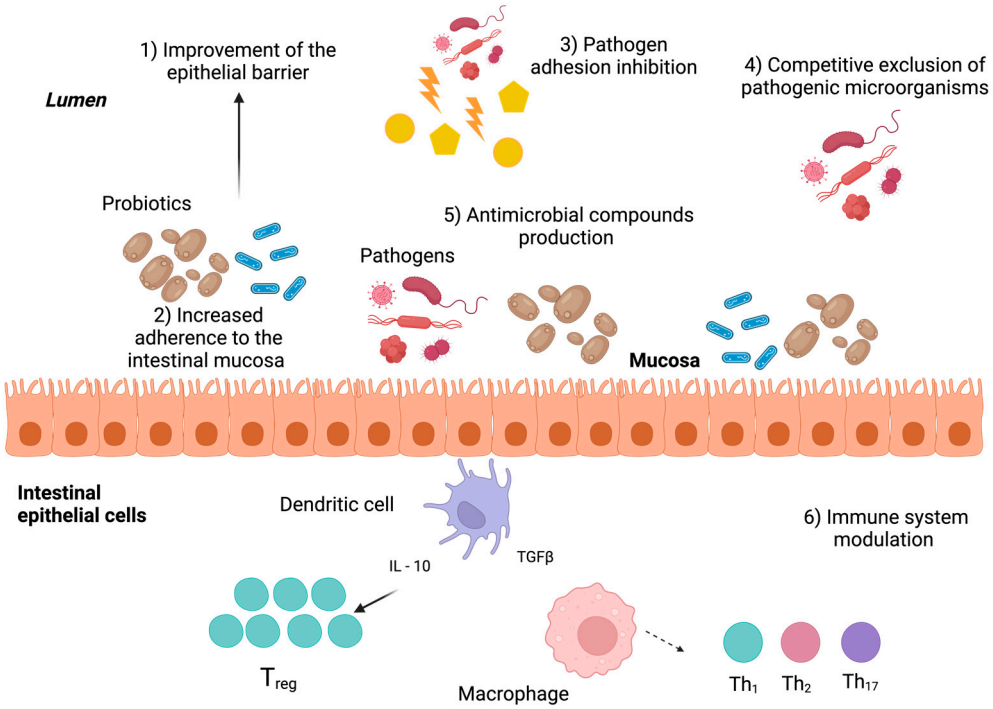


Figure 2. Main mechanisms of action attributed to probiotic microorganisms. Adapted from [108]. Created in Biorender.

Furthermore, it has been documented that probiotics can be used for the restoration of the intestinal microbiota (eubiosis), which is of utmost importance because the intestinal microbiota performs several essential functions in the body, such as the metabolism of xenobiotics, the regulation of the immune system, and the digestion of food. Proteins, peptides, and metabolites promote various cellular pathways and signals. Microbial homeostasis (eubiosis) of the host is mediated by a series of cellular signals stimulated by multiple proteins, peptides, and metabolites [112]. Several factors can promote an imbalance in the composition of the microbiota (dysbiosis), causing changes in physicochemical properties (pH, temperature, peristalsis, bile acids, secretions, immune responses, and motility). Some factors that cause dysbiosis in the microbiota are dietary patterns, infections, and antibiotic use [113]. In this regard, probiotic microorganisms can restore microbial homeostasis mainly by two mechanisms: (i) by colonizing functional niches left by the endogenous community, thus making it impossible for pathogenic or opportunistic microorganisms to occupy that space (competitive exclusion), and (ii) by actively reducing the invasion of opportunistic microorganisms through the production of specific compound metabolites, such as organic acids, short-chain fatty acids (SCFAs), reactive oxygen species, and bacteriocins [114].

In addition, probiotic microorganisms are commonly used to prevent or treat specific diseases, such as antibiotic-associated diarrhea, necrotizing enterocolitis, acute respiratory tract infections, and infantile colic [115]. Probiotics have also been indicated to decrease the effects of lactose intolerance, ulcerative colitis, Crohn's disease, and infections caused by *Helicobacter pylori* [116]. Significant scientific evidence supports these beneficial effects of probiotics; however, additional studies are required to validate their efficacy in some cases. It should be noted that these beneficial effects depend on the probiotic strain used [2,117]. Some documented adverse effects of probiotic microorganisms are diarrhea, sepsis, subacute bacterial endocarditis, and meningitis, although rare [117].

5. Selection Criteria and Recommended Dosage of Probiotic Microorganisms

5.1. Selection Criteria for Potential Probiotic Microorganisms

According to the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the European Food Safety Authority (EFSA), for a microorganism to be approved as a probiotic, it must meet several safety, functional, and technological criteria, as shown in Figure 3 [118]. In this figure, probiotics must undergo clinical trials after in vitro and in vivo studies to validate their safety and efficacy. These randomized placebo-controlled clinical trials (RCTs) are the gold standard for evaluating probiotics [119]. In addition, if these microorganisms are to be used in food or supplements, prior confirmation by human studies, such as randomized clinical trials or robust evidence from observational studies, is necessary [3,120].

It is essential to highlight that strains with probiotic potential must demonstrate a lack of pathogenicity or toxic compound production [121]. Therefore, the production of enzymes considered virulence factors, such as extracellular DNase, phospholipase, urease, extracellular proteases, coagulase, and hemolysin, must not be present in microorganisms with probiotic potential. Consequently, for the identification of virulence factors in strains with probiotic potential, in vitro tests are recommended to determine their production by using specific supplemented media that allow for the detection of the production of these harmful enzymes [121]. Another important test for the characterization of probiotic microorganisms is the presence of antibiotic-resistance genes. This resistance can be related to chromosomes, transposons, plasmids, insertion sequences, and prophages. Therefore, different tests are considered to determine antibiotic resistance, such as the disk diffusion method, Stokes method, minimum inhibitory concentration of antibiotics (agar and broth), the E test, and, recently, molecular screening, such as 16S rRNA gene sequencing analysis [7].

Another characteristic that probiotic strains must present is bile tolerance. Bile tolerance is a multifactorial phenomenon, including efflux pumps, the bile salt hydrolase (BSH) enzyme, the intrinsic capacity of cells to keep intracellular homeostasis, and changes in the cell membrane composition [122,123]. To measure this, the strain to be evaluated is grown in media with different levels of bile salts, and its viability can be determined by colony counting or absorbance at different time intervals [7,124].

Microbial adhesion to epithelial cells is an essential property of probiotics. This property involves a complex contact process between two membranes (microbial and human), which depends on the physicochemical composition of the cell surface of the probiotic microorganism. The adhesion of probiotics can be measured by the capacity for auto-aggregation and by determining the hydrophobic properties of the cell surface (hydrophobicity). The test of auto-aggregation capacity ensures that the cells of probiotic microorganisms have a high cell density in the intestine, thus contributing to the adhesion mechanism, while the hydrophobicity of the cell surface allows for better interaction between the probiotic and human epithelial cells. The test used to quantify auto-aggregation involves measuring the absorbance of a suspension of the probiotic strain in phosphate-buffered saline (PBS) measured at various time intervals [7,125]. The hydrophobicity of the cell surface can be measured using the Microbial Adhesion to Hydrocarbons Test (MATH), which consists of mixing water, a suspension of the probiotic strain, and a hydrocarbon. The two-phase solutions are mixed, and the hydrophobicity of the strain is measured via absorbance. Mammalian cells, such as Caco-2, HT-29, and fetal I-407, are widely used [7].

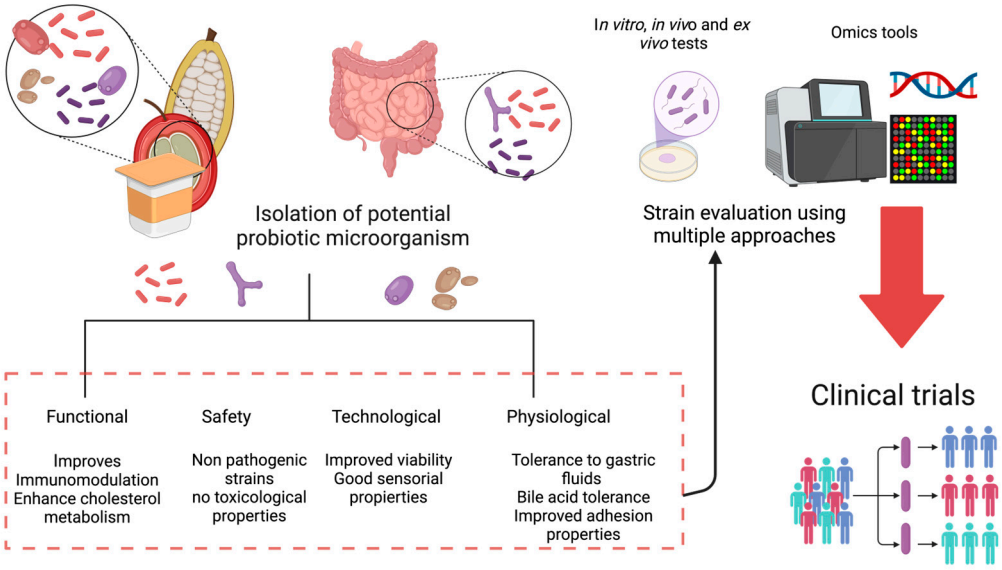


Figure 3. Methodology to approve a microorganism as a probiotic. Adapted from [125,126]. Created in Biorender.

The ability of probiotic strains to bind to other microorganisms is called co-aggregation. This mechanism can lead to the formation of a protective barrier that prevents pathogenic bacteria from colonizing the GIT epithelium. However, probiotics can also exhibit co-aggregation with other probiotics, which could increase their colonization potential, mainly when used as probiotic co-cultures [126]. This agglomeration of pathogens with probiotic microorganisms facilitates their elimination through feces. The property can be evaluated by testing through aggregation tests of probiotics with different pathogens, such as *S. aureus*, *E. coli*, *L. monocytogenes*, and *Salmonella* [7].

5.2. Recommended Dose of Probiotic Microorganisms

The efficacy and safety of probiotic microorganisms depend not only on the strain but also on the dose. It is necessary to have a specific dose for a probiotic microorganism to exert beneficial properties in the host. This dose depends on the microbial strain and the type of product in which the probiotic is found [127]. Many commercially distributed probiotic products contain between 1000 and 10,000 million colony-forming units (CFU) per dose. It is recommended that probiotic products have at least a bacterial concentration of 10^6–10^7 CFU/g or 10^8–10^9 CFU in 100 g or 100 mL of food to have their therapeutic effects [35,120]. However, it has been seen that some probiotic strains have shown efficacy at lower levels, while others require higher amounts [10].

6. Sources of Probiotic Microorganisms

Foods, mainly fermented foods, can be a source of microorganisms with probiotic potential [18,19,27]. One example is strains of *Lpb. paraplantarum* [128] and *S. cerevisiae* [129] isolated from a traditional Korean fermented food, Jangajji. These probiotic strains showed antioxidant and immunostimulatory activity, which could be used to formulate functional foods and pharmaceuticals. Another strain with probiotic properties isolated from fermented plant-based foods was a strain of *Weissella cibaria*, indigenous to kimchi (a traditional Korean fermented vegetable-based dish [130,131]. Nevertheless, this has not been exhaustively discussed and reviewed. In sour cabbage fermentations, LAB strains with probiotic potentiality belonging to the genera *Lactobacillus* and *Leuconostoc* were found. These LAB strains showed antimicrobial resistance tolerance to bile and acidic conditions. In addition, some of the *Lactobacillus* strains showed antimicrobial activity against Listeria

monocytogenes [132]. Finally, *Lpb. plantarum* strains were isolated from spontaneously fermented tangerine vinegar. These LAB strains showed potentially probiotic characteristics by displaying resistance to bile salts, gastrointestinal tract conditions, antioxidant capacity, bile salt hydrolysis, and cholesterol-lowering [133]. Other food sources from which microorganisms with probiotic potential can be isolated are shown in Table 2.

Table 2. Some food sources of potentially probiotic microorganisms.

Food Source of Isolation	Potentially Probiotic Microorganism	References
Fermented chili and pear	*Lpb. pentosus*	[134]
Pickles, milk, curd, wheat dough	*Lb. acidophilus*, *Lb. delbrueckii*	[135]
Artisanal Serpa cheese	*Lpb. plantarum*, *Lpb. pentosus*, *Lev. brevis*	[136]
Green olives from Morocco		[137]
Edible cultivated snail *Cornu aspersum maxima*	*Lpb. plantarum*	[138]
Fermented milk products		[139]
Pickled Chinese cabbage, carrots, and cowpea		[140]
Tangerine vinegar		[133]
Cocoa fermentation		[141,142]
Soybean, pickles, plant-based meat, soy sausage		[143]
Fermented quinoa drink		[144]
Traditional fermented cereal foods from China		[145]
Rotten fruits and vegetables such as apples, grapes, strawberries, tomatoes, cauliflower, and cucumber		[146]
Duimaj-traditional snack food from Iran	*Lpb. plantarum*, *Lpb. pentosus*	[147]
Harbin sausages	*Lev. brevis*, *Lat. curvatus*, *Lim. Fermentum*, *Pediococus pentosaceus*	[148]
Jangajji-traditional Korean fermented food	*Lpb. paraplantarum*	[128]
Kimchi-traditional Korean fermented food	*Weissella cibaria*	[131]
Chanakh cheese from Armenia	*Lcb. rhamnosus*, *Lb. helveticus*, *Lb. acidophilus*	[149]
Teff dough, Kocho, and Ergo- traditional Ethiopian fermented food	*Lcb. paracasei*, *Lpb. plantarum*	[150]
Vegetable and fruit juices	*Leuconostoc mesenteroides*, *Pediococcus pentosaceus*	[151]
Traditional Ethiopian foods and beverages (Bulla, Kotcho, Ergo, Bukuri)	*Pediococcus pentosaceus*, *P. acidilactici*, *L. lactis*	[152]
Fermented cereal-based foods, fermented pulses, and fermented milk products	*Enterococcus durans*, *Enterobacter faecium*, *Lpb. plantarum*, *Lim. fermentum*	[153]
Saudi Arabia's raw and fermented milk	*Lcb. casei*, *Lpb. plantarum and E. faecium*	[154]
Cocoa fermentation	*Lim. fermentum*, *L. lactis subsp. lactis*	[28]
Traditional fermented foods: rice-based ethnic fermented beverage, chhurpi, Khambir (wheat-based leavened bread)	*S. cerevisiae*	[155]
Shanklish (dried Labanah fermented by fungi), Jordanian green olives	*Pichia kudriavzevii*, *Pichia* sp. *S cerevisiae*	[156]
Kefir	*Saccharomyces unisporus*, *Kluyveromyces lactis*	[157]
Italian virgin olive oil	*Candida adriatica*, *Candida diddensiae*, *Nakazawaea molendiniolei*, *N. wickerhamii*, *Wickerhamomyces anomalus*, *Yamadazyma terventina*	[158]

Table 2. *Cont.*

Food Source of Isolation	Potentially Probiotic Microorganism	References
Buffalo Karish cheese	*S. cerevisiae. W. anomalus*, *P. kudriavzevii*	[159]
Fermented Brazilian table olives	*S. cerevisiae*, *P. guillermondii*, *C. tropicalis*, *Meyerozyma caribbica*, *Debaromyces hansenii*	[160]
Caxiri (Brazilian indigenous beverage), kefir, and cacao fermentation	*S. cerevisiae*, *Pichia kluyeri*	[161]

7. Cocoa Fermentation as a Source of Potentially Probiotic Microorganisms

Fermented foods have a high microbial biodiversity and are ideal sources for studying microorganisms with probiotic properties. Many microorganisms, mainly LAB and yeasts, are involved in cocoa [25,26,29,162].

7.1. Cocoa Fermentation Process

The fermentation of cocoa beans is a complex process that involves several autochthonous microorganisms, such as yeasts, LAB, acetic acid bacteria (AAB), and sporulated bacilli [26,163,164]. This fermentation process is essential to developing the flavor and reaching the final acidity in the cocoa beans to produce quality chocolate [164]. Four different methods are used for the fermentation of cocoa beans: platform, box, heap, and basket. The type of fermentation method selected is related to the region where the cocoa is produced [164,165]. During this fermentation process, the microbial groups mentioned above degrade the pulp. This pulp is composed of water (80–90%), 0–15% of sugars (glucose, sucrose, and fructose), citric acid (1–3%), and pectin (1–1.5%). It also contains proteins (0.5–0.7%), amino acids, some vitamins (mainly vitamin C), and minerals, such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, K^{+}, and Na^{+}. This pulp composition can support microbial growth [164,166]. Cocoa pulp is exposed to a variety of microorganisms derived from the environment (for example, pod surfaces, soil, and banana leaves), tools used to open the cocoa fruits (knives or machetes), workers' hands, and containers where the fermentation process takes place, all of which contribute significantly to microbial development and fermentation of the seeds [26]

During cocoa bean fermentation, microbial growth occurs in three main stages. The main stages of cocoa fermentation are shown in Figure 4. In the first stage, yeast growth occurs mainly in genera *Hanseniaspora*, *Saccharomyces*, *Kluyveromyces*, and *Pichia*. In this stage, yeast produces ethanol. Yeast growth at this stage is favored by the high availability of sugars and the low availability of oxygen. Additionally, these yeasts are responsible for the hydrolysis of the pectin present in the cocoa pulp, as well as for producing a considerable quantity of aromatic compound precursors, such as esters and higher alcohols, which significantly contribute to the development of the chocolate's aromatic profile. Additionally, these yeasts are responsible for the hydrolysis of the pectin present in the cocoa pulp, as well as for producing a considerable quantity of aromatic compound precursors, such as esters and higher alcohols, which significantly contribute to the development of the chocolate's aromatic profile [26,165]. In the second stage, the growth of the LAB occurs, and the yeast populations begin to decrease. At this stage, the main LAB genera are *Limosilactobacillus* and *Lactiplantibacillus*. *Leuconostoc*, *Levilactobacillus*, and *Pediococcus* are other genera that can be found in less significant numbers. The role of LAB in this fermentation process is still controversial because, according to some authors like Ho et al. 2014 [167], they mentioned that LAB is not necessary to obtain good-quality fermented cocoa beans. However, other authors [168–171] highlighted that the presence of some LAB in this fermentation process

may be necessary to produce volatile compounds, such as 2,3-butanediol, diacetyl, and acetoin—compounds required to support bacterial growth and allow a slight increase in pH. In this sense, *Lim fermentum* is a species of LAB that can benefit from this process as it produces the mentioned compounds. Finally, in the last stage, the population of AAB is increasing while that of LAB is decreasing. The principal genera of AAB are *Acetobacter* and *Gluconobacter*. Specifically, *Acetobacter pasteurianus* is the most common AAB species involved in the spontaneous fermentation of cocoa beans, mainly due to its ability to oxidize ethanol, mannitol, and lactic acid, as well as its tolerance to acid and heat [165]. AAB is responsible for the simultaneous oxidation of the ethanol produced by the yeasts and for converting the lactic acid produced by the LAB into acetic acid and acetoin [172,173]. The increase in temperature caused by the oxidation of ethanol and the decrease in pH from 6.5 to 4.8 caused by the penetration of acetic acid and ethanol into the cocoa bean is responsible for the death of the cocoa embryo. Notwithstanding, other bacteria, such as *Bacillus* sp., can be found in concentrations close to 10^7 CFU/g in the final stages of the fermentation of cocoa beans (after 120 h) [166]. Microbial diversity during the cocoa fermentation process has been studied in many studies using both culture-dependent and culture-independent methods [168,174–176]. These studies have shown that the most found microbial species are the yeasts *Pichia kudriavzevii*, *Hanseniaspora opuntiae* and *Saccharomyces cerevisiae*, *Limosilactobacillus fermentum*, and *Lactiplantibacillus plantarum* in the case of LAB, and *Acetobacter pasterianus* in the case of AAB.

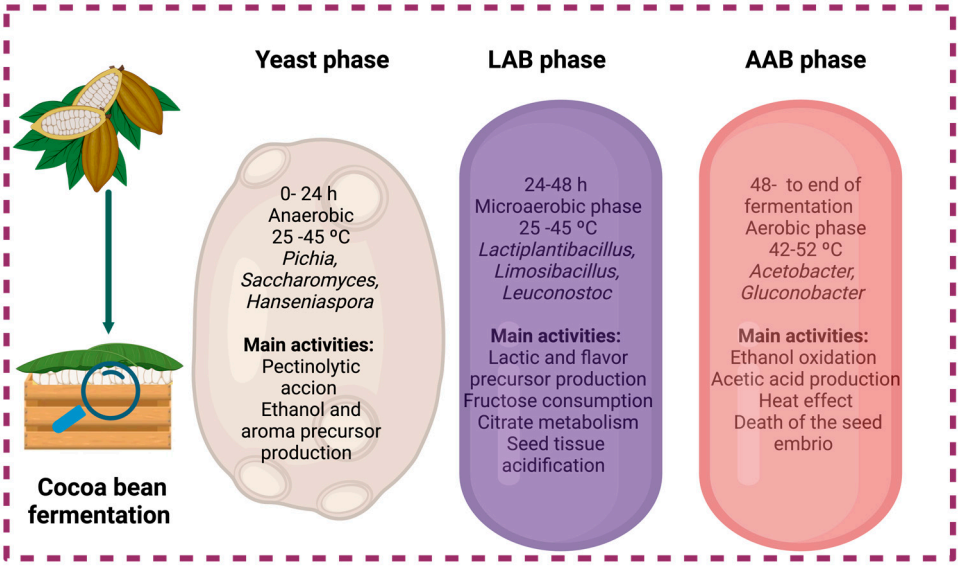


Figure 4. Main stages of cocoa bean fermentation. Adapted from [26,166]. Created by Biorender.

7.2. Probiotic Potential of Autochthonous Cocoa Fermentation Microorganisms

7.2.1. Probiotic Potential of Cocoa Autochthonous LAB Strains

Several studies have highlighted the probiotic potential of yeast strains and LAB autochthonous to cocoa [161,177–183]. Through in vitro and in vivo tests, it has been possible to demonstrate characteristics like those exerted by strains with well-documented probiotic effects. It has even been shown that some strains isolated from cocoa can decrease intestinal inflammation in a murine model with induced colitis [184]. The main potential probiotic characteristics of LAB strains isolated from cocoa are shown in Table 3.

Table 3. Main potential probiotic characteristics of LAB strains isolated from cocoa.

Bacteria Strain(s)	In Vitro Properties	In Vivo Properties	Other Functional Properties	References
Lim. fermentum 5.2 *Lpb. plantarum 6.2* *Lpb. plantarum 7.1*	Surface properties (auto-aggregation and hydrophobicity), Adhesion to vaginal epithelial cells (HMVII), Antimicrobial properties against *G. vaginalis*			[181]
Lpb. plantarum (CH3 and CH41) and *Lim. fermentum* (CH58)	Tolerance to pH 2.0 and bile salt, hydrophobicity, and auto-aggregation capacity			[185]
Lpb. plantarum CH3 and CH41	Adhesion properties to Caco-2 cells			
Lim. fermentum CH58	Antimicrobial activity against antagonistic activity against *L. monocytogenes* and *S. aureus*			
Lim. fermentum fermentum TCUESC01	Resistance to GIT simulated conditions, auto-aggregation, and susceptibility to most antibiotics tested.		Survival after 28-day storage at 4 °C in the milk matrix	[178]
Lpb. plantarum TCUESC02 and *Lpb. plantarum* TCUESC01 strains	Antagonistic activity against pathogenic bacteria			
Lim. fermentum TCUESC01and *Lpb. plantarum* TCUESC02	Antagonism against six pathogenic bacteria (*S. enterica var* Typhimurium, *E. coli*, *E. faecalis*, *L. monocytogenes*, *S. aureus*, *S. flexneri*)	Antagonistic activity against enteropathogen and a lower anti-inflammatory pattern of immune response to infection		[180]
Lpb. plantarum 286	High resistance to the GIT simulation process	High antimicrobial activity against pathogenic strains (*S. enterica* var Typhimurium, *E. coli*, *E. faecalis*, *L. monocytogenes*, *S. flexi*		[182]
L. lactis subsp. lactis CR2 and Lim. fermentum CYF3	Tolerance to acidic conditions, bile salts No hemolytic activity No DNAse activity		Production of bioactive compounds (antioxidant activity and anti-glycemic) Exopolysaccharide (EPS) production	[28]
Lpb. plantarum Lp03, *Lpb. plantarum Lp289*, *Lpb. plantarum Lp291*	Tolerance pH < 4.5, hydrophobicity, auto-aggregation, co-aggregation, biofilm formation, and antimicrobial activity against pathogens (*G. vaginalis* and *N. gonorrhoeae*)			[142]

Table 3. *Cont.*

Bacteria Strain(s)	In Vitro Properties	In Vivo Properties	Other Functional Properties	References
Lpb. plantarum 2.1, *Lpb. plantarum A2*	Tolerance to acid pH, hydrophobic surface, good auto-aggregation and co-aggregation properties Inhibition of growth of pathogens (*Salmonella enteritidis* and *E. coli* EHC)			[141]
Lim. fermentum and *Lpb. plantarum*		Low number of leukocytes, reduced histological damage, anti-inflammatory activity in a rat model		[186]
Lpb. plantarum TCUESC02 and *Lim. fermentum* TCUESC01	Tolerance to acid pH, cell viability, and resistance to GIT digestion during soy yogurt storage			[187]
Lim. fermentum TCUESC01	Anti-adherence and bactericidal activity against planktonic cells of *S. mutans*			[188]
Lim. fermentum C4848.7 and *Leu. pseudomesenteroides C4820.3*	No hemolysis, DNase, or gelatinase activity. No biogenic amine production, Co-aggregation, auto-aggregation and adhesion to CaCo-2 cells			[189]

A study by Ramos et al. [185] evaluated the probiotic potential of 234 lactic acid bacteria isolated from various foods originating in Brazil. A total of 99 of these came from cocoa fermentation. Of all LAB strains, it was found that only 51 LAB strains were able to survive in media with low acidity (pH 2.0). Two strains of *Lactiplantibacillus plantarum* (CH3 and CH41) and one strain of *Limosilactobacillus fermentum* (CH58) from cocoa fermentation were shown to have a high tolerance to bile salts, hydrophobicity, and auto-aggregation capacity. They also found that *Lpb. plantarum strains* CH3 and CH41 were shown to have adhesion properties to Caco-2 cells, like those found for the commercial probiotic strain *L. rhamnosus* GG. The *Lim. fermentum* strain CH58 was shown to have antagonistic activity against *L. monocytogenes* and *S. aureus*.

On the other hand, Melo et al. [178] evaluated the probiotic potential of an autochthonous *Lim. fermentum* strain TCUESC01 from cocoa fermentation. This *Lim. fermentum* TCUESC01 strain showed resistance to gastrointestinal simulated conditions. In addition, *Lim. fermentum* TCUESC01 showed survival after 28-day storage at 4 °C in the milk matrix. Finally, *Lim. fermentum* TCUESC01 had a high auto-aggregation capacity (70.19 ± 1.78%).

In 2018, [180] evaluated the probiotic potential of *Lpb plantarum* and *Lim. fermentum* strains isolated from the cocoa fermentation process by in vitro and in vivo tests. The authors found that *Lpb. plantarum* TCUESC02 and *Lpb. plantarum* TCUESC01 strains exhibited in vitro antagonistic activity against six pathogenic bacteria (*S. typhimurium* ATCC 14028, *E. coli* ATCC 25922, *E. faecalis* ATCC 19433, *L. monocytogenes* ATCC 15313, *Staphylococcus aureus*

ATCC 29213, and *Shigella flexneri* ATCC 12022). In a murine model, these LAB strains were shown to protect against *S. typhimurium* infection. In the in vivo tests, the *Lim. fermentum* and *Lpb. plantarum* strains caused a lower degree of hepatic and splenic translocation of the enteropathogen, higher ileal IgA production, fewer histopathological lesions in the liver, and a lower anti-inflammatory pattern of immune response to infection.

Teles Santos and coworkers [182] evaluated the probiotic potential of various strains of LAB (*Lpb. plantarum* and *Lim. fermentum*) indigenous to cocoa through in vitro and in vivo tests. The *Lpb. plantarum* 286 presented the highest resistance to the gastrointestinal simulation process and the highest antimicrobial activity against pathogenic strains (*S. enterica* var. *typhimurium* ATTCC 14028, *S. flexneri* ATTCC11060, and *E. coli* ATCC 25922).

In a study conducted by Pessoa et al. [181] in 2017, the functional and antagonistic properties of *Lim. fermentum* 5.2, *Lpb. plantarum* 7.1, and *Lpb. plantarum* 6.2, isolated from cocoa fermentation, were studied against infections caused by *Gardnerella vaginalis*. The three strains, as mentioned earlier, were found to have potential use as probiotics by presenting high hydrophobicity (*Lim. fermentum* 53%, *Lpb. plantarum* 71%, and *Lpb. plantarum* 55%) and capacity for auto-aggregation (31; 29; 22%, respectively). In addition, it was observed that the metabolites secreted in the culture medium and the cells of the strains studied can interfere with the growth of *G. vaginalis* to different degrees.

Nandha and Shukla [28], in 2023, studied the probiotic potential of LAB isolated from the fermentation process of Indian Forastero cocoa. They isolated eleven LAB and selected five to evaluate as potential probiotics. In this study, they used tests of resistance to acid and bile salt conditions, tolerance to the gastrointestinal digestion simulation process, auto-aggregation, co-aggregation, hydrophobicity, as well as the production of exopolysaccharides and other bioactive compounds (antioxidant and anti-glycemic activity), and finally, their hemolytic capacity. They found two strains that presented probiotic potential identified as *L. lactis* subsp. *lactis* CR2 and *Lim. fermentum* CYF3, since they presented good viability in acidic conditions and the presence of bile salts, no hemolytic activity, and bioactive compounds were produced.

7.2.2. Probiotic Potential of Cocoa Autochthonous Yeast Strains

Some studies evaluate the probiotic potential of indigenous yeasts from cocoa fermentation. The main potential probiotic characteristics of yeast isolated from cocoa are shown in Table 4.

Table 4. Main potential probiotic characteristics of yeast strains isolated from cocoa.

Yeast Strain(s)	In Vitro Properties	In Vivo Properties	Other Functional Properties	References
K. marxianus, *C. orthopsilosis*, *C. quercitrusa*, *H. uvarum*, *H. opuntiae* and *P. kluyveri*, and *H. uvarum*	Tolerance to pH 2.0, 37 °C, bile salts, auto-aggregation, hydrophobicity, no hemolytic activity		Only strains *P. kluyveri* CCMA0615 and *C. quercitrusa* CCMA0560 showed antioxidant activity (DPPH method) and phytate hydrolysis	[161]
P. kluyveri CCMA 0615	co-aggregation capacity with *E. coli* EPEC, *L. monocytogenes*, and *S. Enteridis*, adhesion properties to Caco-2 cells, 50% inhibition of bacterial infection			[179]
P. kudriavzevii 2P10	Tolerance to acidic pH, bile salts, auto-aggregation, and co-aggregation		Antioxidant activity and H_2O_2 oxidative stress	[183]

One such study was conducted by Menezes et al. [179] in 2020. This study evaluates the probiotic potential of 116 strains isolated from caxiri, kefir, and cocoa, using tests of tolerance to acidic conditions (pH 2.0), tolerance to bile salts, antioxidant activity, hydrophobicity, auto-aggregation and co-aggregation capacity, and hemolytic activity. *Saccharomyces boulardii* (Floratil®) commercial strain was used as a control. The researchers found that only one strain of *P. kluyveri* CCMA 0615 indigenous to cocoa was shown to have relevant probiotic characteristics.

Again, Menezes et al. [161] 2020 investigated the antimicrobial capacity of yeasts, *S. cerevisiae* and *P. kluyveri*, which were previously isolated from fermented foods (autochthonous beverage, kefir, and cocoa), against the adhesion of foodborne pathogens to Caco-2 cells. Quantitative analysis, scanning electron, and confocal laser microscopy evaluated the co-aggregation of yeasts with pathogens. It was found that all yeast strains were able to coaggregate with the pathogens tested; however, this activity was strain-dependent. Inhibition tests showed that the adhesion of *E. coli* EPEC, *L. monocytogenes*, and *S. enteritidis* to Caco-2 cells was reduced by the action of the tested yeasts, with inhibition rates equal to or higher than those of the commercial probiotic *S. boulardii*. The yeasts were able to reduce bacterial infection by up to 50%. The antimicrobial effect was mediated by competition for nutrients or receptors in the intestinal mucosa since the production of antimicrobial compounds was not found. The *P. kluyveri* strain CCMA 0615 shows high probiotic potential.

Wulan and coworkers [183] evaluated the probiotic potential of *P. kudriavzevii* isolated from cocoa fermentation in 2021. They assessed the probiotic potential of 23 indigenous cocoa yeast strains using hemolytic activity, tolerance to bile salts, low pH, auto-aggregation, and co-aggregation tests. They also carried out antioxidant activity and H_2O_2 oxidative stress tests. During this study, no comparison with any commercial strain was performed; however, the probiotic potential of *P. kudriavzevii* 2P10 was demonstrated.

8. Coffee Fermentation as a Source of Potentially Probiotic Microorganisms

8.1. Coffee Fermentation

To obtain the roasted coffee beans needed to prepare the world-famous drink, it is necessary to obtain green coffee beans. These are obtained from different primary processing methods of the coffee cherry, known as the dry, wet, and semi-dry methods [29]. This primary processing involves the fermentation of the coffee cherries by different microorganisms, which causes changes in the aroma and flavor that affect the quality of the cup [29,30].

During the dry process, whole coffee cherries are dried directly in sunlight for 14–30 days, with spontaneous fermentation. This method of processing coffee is the oldest and easiest. It is generally used for *C. robusta* or in places with long sunshine and little rain, such as Ethiopia, Brazil, and Paraguay, to obtain unwashed or natural coffee [29,30]. Wet processing involves pulping the cherries and fermenting them in a tank under water for 24–48 h, after which they are dried until they reach a water content of between 10 and 12% [190]. This method is widely used in Colombia, Mexico, Central America, and Hawaii, where water is more available. This method improves the quality of the coffee by generating aldehydes, organic acids, esters, ketones, alcohols, etc., [31]. The semi-dry method is a hybrid of the dry and wet methods, first used in Brazil. In this process, fermentation takes place directly in the sun. It has been observed that the precursors of coffee flavor depend on the processing method used [29].

Coffee fermentation is a metabolic process of key significance to eliminating mucilage and decreasing its moisture content through the enzymatic action of enzymes found

naturally in the coffee cherry and those acquired exogenously from the environmental microbiota [31]. Furthermore, the microbial metabolites produced during fermentation can migrate to the coffee, affecting physicochemical characteristics such as moisture, simple sugar concentration, aromatic profile, and flavor precursors [29].

Microbial diversity during coffee fermentation depends on the processing method applied and environmental factors. For example, microorganisms are known to be more abundant during the wet process, followed by the dry and semi-dry processes. It has been mentioned that yeast *Pichia caribbica*, LAB *Lev. brevis*, and *Aspergillus ochraceus* (filamentous fungus) are species commonly found in wet and dry coffee processing. Furthermore, *Bacillus megaterium* (bacteria) and *Cladosporium cladosporioides* (filamentous fungus) are commonly found in dry and semi-dry processing [29]. Additionally, the occurrence of other LAB strains, such as *Leuconostoc mesenteroides*, *Weisella cibaria*, and *Lpb. plantarum* has been reported during wet processing. The presence of *H. uvarum*, *K. marxianicus*, *P. guillermondii*, *P. kluyveri*, *C. quercitrosa*, and *H. opuntiae* has also been documented in the wet processing of coffee [29,31].

The role of yeasts during coffee fermentation is key to producing aroma precursors and pectinolytic activity. For example, it has been seen that the presence of yeasts during coffee fermentation causes the drink to have higher sensory scores regarding aromatic profile, flavor, acidity, body, and overall score. Yeasts can produce isoamyl alcohol, ethanol, acetaldehyde, and other flavor compounds in coffee [29]. In the case of LAB, their metabolic activities are essential for eliminating mucilage and metabolizing sugars to produce metabolites of coffee quality. For example, the fermentation of coffee with *Lpb. plantarum*, a microorganism, generates decanol, 2-undecanone; phenol, 2-methyl-; and high quality in the cup with an acidic flavor and a dominant aroma of honey and caramel [191].

8.2. Probiotic Potential of Autochthonous Coffee Fermentation Microorganisms

In the case of the probiotic potential of autochthonous microorganisms from coffee fermentation, no scientific evidence was found for either LAB or yeasts. However, it is well documented that many yeast genera (*Saccharomyces*, *Candida*, *Pichia*, *Hanseniaspora*, and *Kluyveromyces*) and LAB (*Leuconostoc mesenteroides*, *Lev. brevis*, *Lpb. plantarum*, and *Bacillus megaterium*) are involved in this fermentative process, and can exhibit probiotic characteristics [29–31,192]. On the other hand, as there are three methods of coffee processing, this leads to a greater diversity of microorganisms in the coffee fermentation process, with yeasts and LAB, as in the fermentation of cocoa beans, but also many mesophilic bacteria, AAB, sporulated bacteria, and filamentous fungi, which could make it challenging to study the probiotic potential of yeasts and LAB strains of coffee.

9. Potential Application and Challenges of Potential Probiotic Microorganisms Isolated from Cocoa and Coffee Fermentation

Probiotic microorganisms (LAB and yeast) can be added to different food matrices (yogurt, fermented beverages, fermented milk, bread) to functionalize them. Probiotics (validated or potential strains) may produce various metabolites, such as bacteriocins, fatty acids, organic acids, bioactive peptides, hydrogen peroxide, carbon dioxide and aromatic compounds, EPS, and GABA, which improve the techno-functionality and conservation of the final food products [193,194]. In this sense, the production of functionalized foods with improved sensory attributes, with probiotic microorganisms or enriched with postbiotics or bioactive compounds are some of the most relevant applications of probiotic microorganisms in the food industry [194]. Another application that is becoming increasingly popular is using probiotic microorganisms as starter cultures for fermentation dairy products, but it can also be applied to other fermented products. This is due to the ability of these mi-

croorganisms to produce metabolites with antimicrobial, anticarcinogenic, antioxidant, and cholesterol-lowering properties [7]. It is important to mention that a yeast strain (*P. kluyveri* CCMA 0615) with probiotic potential, isolated from the cocoa fermentation process, was co-inoculated with a commercial strain of *Lcb. paracasei* LBC-81 to ferment a maize-based drink. Unfortunately, this strain lost viability during fermentation and refrigerated storage [195].

A critical barrier that must be overcome for probiotic microorganisms or their metabolites to be available in functionalized foods is their viability and stability in food matrices. Probiotic microorganisms are sensitive to environmental factors such as pH, temperature, and exposure to oxygen, reducing their viability and shelf life. In addition, the processing methods used in the food industry can affect the viability and stability of probiotic microorganisms. To overcome these challenges, microencapsulation and stabilization techniques, such as spray drying, freeze-drying, and freezing have been used [196].

10. Conclusions

A growing demand for microorganisms with probiotic characteristics leads to a constant search for them. Food fermentation is an excellent source of probiotic microorganisms. The fermentation of coffee and cocoa involves various strains of LAB and yeasts that may have probiotic capacity. It has been found that some strains of lactic acid bacteria, such as *Lactococcus*, *Lactiplantibacillus*, and *Limosilactobacillus*, and yeasts, such as *Saccharomyces* and *Pichia*, isolated from cocoa, have shown probiotic potential in vitro. The most relevant probiotic characteristics of these native cocoa strains are high hydrophobicity, co-aggregation, auto-aggregation, non-hemolytic activity, antimicrobial activity, and viability during the gastrointestinal tract simulation process.

Furthermore, some of the strains isolated from cocoa, such as *Lim. fermentum* TCUESC01 and *P. kluyveri* CCMA 0615, have been used to functionalize foods. Additionally, it is necessary to continue researching the probiotic potential of the microorganisms isolated from the cocoa fermentation process, using a methodological strategy that includes conducting more in vitro, in vivo, and ex vivo tests, as well as using different omics tools, to guarantee the safety, functionality, and technological properties of the strains evaluated.

On the other hand, no rigorous scientific studies have demonstrated the probiotic potential of the microorganisms native to coffee fermentation. This is because this fermentation process has greater microbial diversity, making it difficult to study probiotic characteristics. However, it represents an area that should be investigated, demonstrating that food fermentation is still a good source of microorganisms with probiotic potential.

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