International Food Research Journal 30(5): 1105 - 1124 (October 2023)
Journal homepage: *http://www.ifrj.upm.edu.my*

Review

Comparative study on biochemical and molecular identification approaches of *Lactobacillus* species

[1]Senjaliya, D. P. and [1,2]*Georrge, J. J.

[1]*Department of Bioinformatics, Christ College, Rajkot, Gujarat 360005, India*
[2]*Department of Bioinformatics, University of North Bengal, Darjeeling District, West Bengal 734013, India*

Article history

Received:
9 June 2022
Received in revised form:
12 November 2022
Accepted:
8 May 2023

Keywords

probiotic,
lactobacilli,
lactic acid bacteria

Abstract

Manufacturers' desire to sell "healthy" food in response to the consumers' desire to lead a healthy lifestyle has increased the use of probiotics during the past few decades. Probiotics are used in dairy products, as well as non-dairy items as a starter culture, encompassing a wide range of goods. Numerous phenotyping, physical characterisation, and genotyping techniques have been developed to identify probiotic lactobacilli to ensure quality management. These techniques are frequently precise enough to categorise probiotic strains by genus and species. Traditional microbiological methods were initially employed for genus and species identification. However, due to their numerous shortcomings as the probiotic ability is often strain-dependent, and that there is no way to differentiate between strains using simple microbiological techniques, new methods that are mostly based on the examination of nucleic acids have been developed. Therefore, the objective of the present review was to provide critical assessment on existing methods for identifying members of the genus *Lactobacillus*, together with newly discovered approaches. The present review aimed to give the most recent information on the scientific techniques used to measure and describe the possible probiotic properties of microorganisms. It will also emphasise molecular and non-molecular tools. Most of these tools are based on 16S ribosomal DNA sequencing, and employ PCR techniques.

DOI
https://doi.org/10.47836/ifrj.30.5.03

Introduction

Probiotics are live microorganisms that can maintain the host's health when consumed in adequate quantities by balancing the microbial population in the gastrointestinal tract, and belong to the lactic acid bacteria (LAB) group. The LAB group includes the genera *Oenococcus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, *Lactobacillus*, *Pediococcus*, *Bifidobacterium*, *Escherichia*, and *Bacillus*, which are the most frequently employed in probiotic formulations. Some *Saccharomyces*-related fungal strains have also been employed (Haghshenas *et al*., 2014; Ozen and Dinleyici, 2015). The human gut is a complex ecosystem that supports a number of crucial biological functions. More than 400 anaerobic and aerobic microbial species, both beneficial and pathogenic, are present in this ecosystem, and they are all directly impacted by the various physiological situations. The large intestine is thought to be the microbiota's final destination. Pathogens and healthy microbiota compete for resources, colonise the gut epithelium, and secrete the by-products of their metabolism. By actively excluding harmful bacteria, probiotics successfully maintain the gut microbiota equilibrium. The gut becomes open to pathogenic colonisation with gut microbial alterations; however, if there is a shift in the microbial makeup, this will result in a severe imbalance between the helpful and potentially pathogenic microorganisms (Youssef *et al*., 2021).

LAB are consumed by people nowadays in fermented foods like dairy products. Due to their vital role in the majority of fermented foods, and their capacity to produce anti-microbial chemicals that promote probiotic qualities such as anti-tumour activity, lactose intolerance relief, a drop in serum cholesterol, stabilisation of gut flora, and immune system stimulation, LAB is currently the focus of extensive international research. The LAB strains developed for exopolysaccharides increase the viscosity and smoothness of fermented milk. Some LAB strains are known to produce mannitol-related

*Corresponding author.
Email: johnjgeorrge@gmail.com

properties that are thought beneficial to health. Probiotic meals have received much attention due to the increased interest in health consciousness. The genera *Lactobacillus* (*L*) and *Bifidobacterium* (*BiI*) contain several probiotic species and strains. Probiotic microorganisms are believed to have a variety of positive health effects. They have an antibacterial effect on enteric pathogenic bacteria, activate mucosal and systemic immune responses in the host, have actions that are anti-inflammatory, anti-cholesterol, and anti-colon cancer, and they also help to enhance nutritional status. They also lessen the effects of allergic responses, lactose intolerance, and diarrhoea (Taye *et al*., 2021). *Lactobacillus* is one of the most important bacteria in dairy and non-dairy-based food products. They are Gram-positive, microaerophilic to strictly anaerobic cocci or bacilli, and ferment carbohydrates. Lactobacilli are generally recognised as safe (GRAS) microorganisms, and used as probiotics granting health benefits to the host (Mathialagan *et al*., 2018).

Mostly, lactobacilli are found in raw milk, dairy products, beverages, meat products, and vegetables. Since ancient days, lactobacilli culture has produced cheese, yoghurts, fermented milk, beverages, sausages, pickles, boza, and others (Wassie and Wassie, 2016). During the fermentation of whey and goat's milk, *Lactobacillus* spp. secrete bioactive compounds that reduce the growth of indicator strains (Biadała *et al*., 2020). Two species of *Lactobacillus,* namely *L. sakei* and *L. curvatus* appeared to be promising candidates to be used in a starter culture, as they enhanced and regulated the fermentation process (Belfiore *et al*., 2013). Recently, a comparative analysis of the volatile profile between spontaneous fermentation and *L. pentosus*-inoculated fermentation was done. The conventional detection method could not identify a volatile profile of spontaneous fermentation; however, increased production of volatile compounds such as phenols, esters, and 4-ethyl phenol was seen when *L. pentosus* was used as a starter culture (de Castro *et al*., 2019). A similar effect was observed in the Sataw-Dong fermentation, in which *L. plantarum* KJ03 was used as a starter culture (Jampaphaeng *et al*., 2018).

The development of host-microorganism associations is essential for the host's health, and disruptions of these interactions in the gastrointestinal tract (GIT) may result in various clinical diseases. Probiotics must both competitively block pathogen adhesion to the GI tract epithelium, and synthesise or create new unique antimicrobial compounds to preserve such microbial equilibrium within the GI tract. Probiotics have been shown to produce chemicals, such as bacteriocins and acids, that have direct inhibitory effects on pathogens. Probiotic-derived antimicrobial compounds can work alone or in concert with other molecules to inhibit the growth of infections (Mazhar *et al*., 2020). They produce lactic acid (sole or end product of fermentation of carbohydrate) and acetic acid, reduce the adherence of pathogens to the gut mucosal surface, boost immunity, produce allergic and anti-cancer properties, reduce fat content in the body, improve irritable bowel syndrome symptoms, and produce various anti-microbial proteins called bacteriocins and organic acids, which give rise to their antagonistic nature against several foodborne pathogens (Karami *et al*., 2017; Fijan *et al*., 2018). The LAB species *L. fermentum*, *L. rhamnosus*, and *Lactococcus lactis* were recently isolated from meat and utilised as bio preservatives (Lengkey *et al*., 2017). The selection of suitable probiotics is the most critical task for improving the bio-therapeutic value of nutraceutical/pharmaceutical products. Several features of bacteria used as probiotics are the capacity to withstand gastric conditions, and adhere to the host intestinal cells (Nami *et al*., 2015). Besides great prevalence, few pathogens, such as *Yersinia enterocolitica* and *Klebsiella pneumoniae*, demonstrate resistance to a group of antibiotics. Therefore, LAB strains isolated from different dairy products can neutralise antibiotic-resistant Gram-negative pathogens. Overconsumption of antibiotics has led to the extensive occurrence of antibiotic resistance genes in probiotic bacteria, which can transmit these genes to other gut microbiota, and cause severe difficulties (Kiani *et al*., 2021).

Recently, isolates were screened for probiotic characterisation, which included the characterisation of their co-aggregation capacity, phenol tolerance, anti-microbial activity, and safety aspects. LAB are only considered safe to be used in the food industry if they are safe (Haghshenas *et al*., 2021). Furthermore, their adhesion properties towards Caco-2 (from cancer coli, colon cancer) and HT-29 (human tumour cell line) cells were documented. All strains showed high (≥ 80.0%) viability rates in gastric and intestinal juices. *L. brevis* CCMA 1284, *L. plantarum* CCMA 0743, and *L. plantarum* CCMA 0359 showed excellent hydrophobicity (95.33, 96.06, and 80.02%, respectively), whereas *L. paracasei* CCMA 0504 and

L. paracasei CCMA 0505 showed the highest auto-aggregation values (45.36 and 52.66%, respectively) (Fonseca *et al*., 2021). Similarly, the probiotic characterisation of *L. plantarum* isolated from fermented raw milk was documented. *L. plantarum* showed excellent viability (93.48 - 96.97%) in simulated gastric juice environment (pH 3). Furthermore, auto-aggregation and hydrophobicity showed that *L. plantarum* might expand the adherence properties of probiotic strains (Nath *et al*., 2020).

Several *Lactobacillus* species produce large molecular weight carbohydrates called exopolysaccharides (EPSs). The lactobacilli EPSs strengthen bacterial cell surface adherence and biofilm development while shielding it from heavy metals. High viscosity, great biocompatibility, and the ability to stabilise emulsions are all characteristics of lactobacilli EPSs. Due to their emulsification ability, they are utilised as bio-thickeners in the food sector. Additionally, they lower cholesterol, hinder pathogenic adhesion, and have anti-tumour, anti-HIV, and immunomodulatory effects (Riaz Rajoka *et al*., 2020b). EPSs from lactobacilli is essential to their probiotic effect. In one study, *L. kefiri* MSR101's EPS (MSR101 EPS) and its structural properties and capacity to inhibit the growth of colon cancer (HT-29) cells were extracted and examined. The newly obtained MSR101 EPS (400 g/mL) appeared to have a good anticancer impact on HT-29 malignant cells (44.1%) and to upregulate the expression of Cyto-c, BAX, BAD, caspase3, caspase8, and caspase9. Overall, the findings indicated that since *Lactobacillus kefiri* MSR101's EPS could prevent colon cancer, it should be used in functional food products, and considered as a topical treatment (Riaz Rajoka *et al*., 2019). The probiotic evaluation *in vitro* was documented by evaluating bile salt, acid tolerance, anti-microbial activity, and adhesion to Caco-2 cells. Strain safety was also assessed through the evaluation of antibiotic resistance and mucin degradation, and haemolytic activity were also determined. *In vivo* analyses were conducted to investigate the anti-inflammatory effects of the strains on a mouse model of 5-FU- induced (5-fluorouracil) mucositis. Results suggested that the used *L. plantarum* strains had good tolerance to bile salts and low pH, and could inhibit gastrointestinal pathogens. Lp2 and Lpl1 strains also exhibited high adhesion rates to Caco-2 cells (13.64 and 9.05%, respectively). Phenotypical resistance to aminoglycosides, vancomycin, and tetracycline was observed for most strains. No strain showed haemolytic or mucolytic activity. Seven strains had a protective effect against histopathological and inflammatory damage induced by 5-FU. Gene expression analysis of inflammatory markers showed that five strains upregulated interleukin 10 (*Il10)*, while four downregulated both interleukin 6 (*Il6*) and interleukin 1b (*Il1b*). Additionally, all strains reduced eosinophilic and neutrophilic infiltration; however, they could not prevent weight loss or reduced liquid/food intake (Coelho-Rocha *et al*., 2022).

The use of silver nanoparticles (AgNPs) in numerous industries has recently attracted much attention. In one study, the EPS of probiotic *Lactobacillus brevis* MSR104 isolated from Chinese *koumiss* was used to characterise the physical properties and biotechnological uses of biosynthesised AgNPs. The anti-microbial assay findings showed that the AgNPs displayed exceptional anti-bacterial activity in a dose-dependent manner against Gram-positive and Gram-negative bacteria. Based on the antioxidant data, the AgNPs demonstrated an excellent rate of free radical scavenging. Additionally, the AgNPs drastically decreased the proportion of viable HT29 cells at greater concentrations. That study concluded that the newly biosynthesised AgNPs would have uses in the food and agriculture industries for anti-microbial, anti-oxidative, and anti-cancer properties (Riaz Rajoka *et al*., 2020a).

What is a probiotic?

The word "probiotic" originates from its Latin root "pro", meaning "for", and the Greek adjective of the noun βίος meaning "life"(Mazhar *et al*., 2020). Food and Agriculture Organization (FAO)/World Health Organization (WHO) define probiotics as "live microorganisms that, whenever administered in adequate amounts, confer a health benefit on the host" (Kaźmierczak-Siedlecka *et al*., 2020). Around 1,000 bacterial species are present in the human intestine. The gut microbial composition depends on factors such as food routine, anxiety and physical activity levels, consumption of prebiotics and probiotics, ingestion of antibiotics, and surgery. *Lactobacillus* spp. are the major microbiota in the human gut (Kaźmierczak-Siedlecka *et al*., 2020). *Lactobacillus* spp. are used either as food/feed preparation by humans/animals or are massively used as non-food components. Currently, many strain-specific health

benefits of lactobacilli have been evaluated (El Manouni El Hassani *et al*., 2019). Therefore, probiotic strain should be characterised by functional, technological, and safety criteria (Sarkar, 2013).

Lactobacilli as probiotics

Due to the probiotic effect of *Lactobacillus* spp. inside the GIT in humans, they have received immense attention. The isolates must be screened for their probiotic potential and must be accessed for their functional characterisation, which includes (1) survival in the gastrointestinal tract, (2) adherence to and colonisation of gut epithelium, (3) colonisation concerning cell surface characters, (4) competition with other gut microbiota including production of antimicrobial metabolites, and (5) production of beneficial enzymes such as bile salt hydrolase and β-galactosidase.

Probiotic work best by adhering to the epithelium of the small intestine (Kinoshita *et al*., 2007). *L. casei* Shirota (LS) was observed to be responsible for building a strong redox mechanism, and protecting the intestinal cell from 2,2''azobis(2-amidinopropane) dihydrochloride-induced inflammatory tension (Finamore *et al*., 2018). The stomach of an average individual is typically acidic (lower than pH 3.0), which chiefly acts as a barrier to most pathogenic microorganisms. Still, since *Lactobacillus* are acid-tolerant, they can colonise inside the stomach, and positively affect the host (Bazukyan *et al*., 2018). Increased production of three unique protein markers, namely 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase 2, elongation factor G, and 50s ribosomal protein L10 by *L. pentosus* AP2-15 and AP2-18 strains have been observed under acidic conditions (Pérez Montoro *et al*., 2018). Published data show the antagonistic nature of *L. plantarum* having a positive consequence against diarrheal bacteria (Toualbia *et al*., 2018).

Antibiotic-resistant microorganisms can colonise the intestinal mucosa, posing a global health threat. *L. rhamnosus* strain GG is effective against pathogenic enterococci (E) as it alters the adsorption capacity of the pathogen to the intestinal mucosa (Lebeer *et al*., 2018). Lactobacilli contain bile salt hydrolase (BSH) enzyme, which efficiently removes the toxic effect of bile salts, thus showing bile salt tolerance property (du Toit *et al*., 1998). *L. johnsonii* La1 (producing BSH) showed a strong effect against protozoan infection (Allain *et al*., 2018). Lactobacilli have shown antagonistic interaction against several foodborne pathogenic bacteria. They show competitive exclusion properties, decrease redox potential, inter-bacterial aggregation, or produce antimicrobial substances such as organic acids, and other inhibitory primary metabolites such as hydrogen peroxide and antifungal peptide, and produce an antimicrobial protein called bacteriocins (Roselli *et al*., 2019).

A study evaluated the efficiency of *Lactobacillus* spp. isolated from the gut of prawns as an antagonist against *Vibrio* (*V*). A reduction in the growth of *V. harveyi* was observed after eight weeks (Ahmmed *et al*., 2020). Several strains of lactobacilli showed their high survivability rate in adverse GI conditions once the S-layer protein (Slps) was expressed. It has been observed that encapsulating them with alginate enhanced the viability of these bacteria further (Banić *et al*., 2018). Recent studies have revealed that *L. plantarum* SLC 13 produces EPS with antimicrobial potential, and can tolerate stimulated intestinal conditions (Huang *et al*., 2018b). Probiotic lactobacilli have been used in the accessory treatment to sustain equipoise in the human gut microorganisms, and as antibiotic treatment for dysbiosis. Lactobacilli have shown antibiotic resistance against antibiotic groups like lactam, macrolide, aminoglycoside, chloramphenicol, and tetracycline (Sharma *et al*., 2018). Similarly, *L. plantarum* has also shown resistance against methicillin, oxacillin, cefoxitin, cefmetazole, teicoplanin, vancomycin, ciprofloxacin, ofloxacin, streptomycin, tobramycin, clindamycin, and fusidic acid (Thakur *et al*., 2018). However, further studies have reported the transfer of this antibiotic-resistance gene from lactobacilli to other members, thus creating a huge concern (Das *et al*., 2020). Recently, isolates that showed tolerance toward harsh GIT conditions were continued for functional characterisation *viz*. adhesion capability, aggregation and co-aggregation ability, antimicrobial activity, and antibiotic resistance.

The broad-spectrum anti-microbial activity, haemolytic activity, auto-aggregation, and cell-surface hydrophobicity were shown by *L. casei* isolated from infant faeces (Wang *et al*., 2020). *L. plantarum* strongly disliked this parameter (Gupta and Bajaj, 2017). Similarly, cell-surface hydrophobicity, anti-oxidative activity, BSH (bile salt hydrolase) activity, phenol tolerance, anti-microbial, and antibiotic resistance activity were

assessed for isolates that grew in the presence of bile salt and low pH. *L. rhamnosus* showed viability in such conditions (Riaz Rajoka *et al*., 2017).

Approaches in identifying lactobacilli at genus and species levels

The intuitive selection and identification of probiotics can be understood once the mode of action of lactobacilli is clear. Furthermore, it will lead to substantiating health claims. Due to the various developed multifarious biochemical and molecular techniques, identifying probiotics has been easy. Biochemical and morphological tests are done as the primary indicator for the identification of specific strains of lactobacilli. However, these simple, cost-effective techniques do not give complete information. Different identification approaches have helped reorganise probiotic strains in the same source. Each isolate's probiotic potential and viability status should be identified, as the viability of lactobacilli is a major concern in developing an efficient probiotic. Therefore, each probiotic product's name and viability status should be mentioned on the label (Saad *et al*., 2013). A rapid, reproducible, inexpensive, and sensitive method should be used. Therefore, more potent molecular and analytical techniques are used in combination, such as MALDI-TOF MS (matrix-assisted laser desorption/ionisation time-of-flight mass-spectrometer), PFGE (pulse-field gel electrophoresis), RAPD (random amplified polymorphic DNA), MLST (multi-locus sequence typing), qPCR (quantitative polymerase chain reaction), SSR (simple sequence repeat), and 16S rRNA (16S ribosomal ribonucleic acid) sequencing for the identification of probiotic lactobacilli (Table 1).

Table 1. Approximate duration and level of detection of each method.

Method	Required time (hour)	Detection level	
		Genus	Species
Morphological profile	~ 48 h	-*	-
API 50 CHL	~ 96 h	-*	+
MALDI-TOF MS	~ 96 h	+	+
PFGE	~ 72 h	+	+
MILST	~ 110 h	+	+
16S rRNA sequencing	~ 144 h	+	+
qPCR	~ 62 h	+	+
RAPD	~ 62 h	+	+
SSR	~ 62 h	+	+
WGS	~ 120 h	+	+

*detection at genus/species level is not possible.

Morphological profile

In this approach, typical colony morphology (Table 2), motility testing, Gram-staining, and catalase and oxidase reactions are useful in identifying lactobacilli (Mathialagan *et al*., 2018). Although colony morphology does not provide absolute strain or species recognition, it is mainly used to detect isolates at the genus level. However, this technique has limitations, such as lack of reproducibility, poor differentiation power, and labour-intensive (Van Belkum *et al*., 2012). Hence, a more efficient approach is needed to get specific results.

Table 2. Typical colony morphology of *Lactobacillus* species.

Colony parameter	*Lactobacillus* species
Size	~ 2 to 5 µm
Type	Small/Large
Colour	Pale yellow/white
Margin	Entire
Elevation	Raised/Convex
Opacity	Translucent/Opaque
Pigmentation	No pigmentation
Consistency	Mucoid/butyrous

Analytical profile index carbohydrate identification kit (API 50 CHL system)

Probiotic lactobacilli have been identified using this phenotypic approach. Lactobacilli can be identified effectively by API 50 CHL system from BioMérieux (fermentative and phenotypic profiling) since they differ in their fermentation potential (Alvarez-Olmos *et al*., 2004). Various physiological tests can be preferred, including substrates covering carbohydrates, heterosides, polyalcohols, and uronic acids. Following the guidelines mentioned on the test kit, isolates should be recognised within 48 h of incubation. Furthermore, the API web database of BioMérieux is extensively utilised to detect lactobacilli (Herbel *et al*., 2013). *L. acidophilus* (7.14%), *L. fermentum* (7.14%), *L. paracasei* (35.71%), *L. plantarum* (14.29%), *L. delbrueckii* (21.43%), and *L. rhamnosus* (14.29%) were isolated successfully from raw cow milk (Karakas-Sen and Karakas, 2018); and *L. pentosus, L. acidophilus, L. collinoides, L. fermentum, L. salivarius,* and *L. brevis* were isolated from the vaginal swab, infant stool, and sheep and cow milk (Maikhan and Amin, 2017). The API 50 scheme was used to test the variability of the fermentation profile of different strains of lactobacilli LRV 1, LRV 2, SP, and S1. LRV 2 was able to ferment raffinose as well as hydrolyse hippuric acid, whereas the S2 strain showed negative results (Pelinescu *et al*., 2009). However, several reports suggest that this method is not preferable to the molecular method because of their similar nutritional requirement (Soofy and Ahmed, 2019). Hence, once the results are obtained from the API 50 CHL approach, a more assertive approach should be considered for lactobacilli identification (Domingos-Lopes *et al*., 2017).

In conclusion, the API 50 CHL system appears acceptable for supporting findings from genomic approaches. However, considering the large degree of phenotypic heterogeneity among lactobacilli, this labour-intensive method should not be the only one employed. Results from API 50 CHL stripes may also reflect acidification activities rather than growth or fermentation processes. The output may be impacted by oxygen or a variation in the density of the bacterial culture. Misidentification and unintelligible results are two glaring drawbacks of this approach (Herbel *et al*., 2013). Although this method is impeccable for identification at the genus level; however, lactobacilli show phenotypic dissimilarity; hence, this method should be combined with a more robust and reliable method.

MALDI-TOF MS

Another method used to identify various microorganisms is MALDI-TOF MS (Table 3).

Table 3. Several Isolates identified by MALDI-TOF.

Sr. no.	Source	Score value	Identified strain	Reference
1	Vagina	≥ 1.70	*L. murinus*, *L. crispatus*	Fosch *et al*. (2018)
2	Faecal and vagina	≥ 1.9	*L. crispatus*, *L. helveticus*	Foschi *et al*. (2017)
3	Spoiled food, juices, and beers	2 - 3	*L. curvatus*, *L. brevis*, *L. rhamnosus*	Bucka-Kolendo *et al*. (2020)
4	Cheese	≥ 2	*L. curvatus*, *L. paracasei*, *L. plantarum*, *L. rhamnosus*	Sánchez-Juanes *et al*. (2020)
5	Cheese	≥ 2	*L. paracasei*, *L. curvatus*, *L. fructivorans*, *L. parabuchneri*	Nacef *et al*. (2017)

In this method, giant non-volatile proteins are simultaneously ionised and vaporised, and unique fingerprints are created for particular genus/species/subspecies (Febbraro *et al*., 2016). This approach has been used for the last few years to rapidly identify microorganisms based on their protein-profiling (Laroche *et al*., 2018). The modified MALDI-TOF MS with Bruker Biotyper system is more efficient and cost-effective than the time-consuming 16S ribosomal sequencing approach to identify anaerobic microorganisms. *L. casei* strains were identified efficiently with a mean score of 2.45 ± 0.1. It showed unity with the data collected from the sequencing of the mutL gene (Shannon *et al*., 2018). Using this technique, *L. gasseri* and *L. crispatus* were identified from vaginal samples before and after taking the contraceptive dose (Fosch *et al*., 2018). A comparative study of this method with genotypic identification resulted in only 1 out of 40 conflicts (97.5%). An average score value of ≥ 1.9 was shown by each strain with ten replicates (Table 3) (Foschi *et al*., 2017).

Phenotypic characteristics and/or physiological and biochemical standards are used to identify lactobacilli from various sources. These are intricate and time-consuming techniques, and they have the potential to overestimate or underestimate the microbial diversity of a food environment. The MALDI-TOF MS profiling-based bacterial identification depends on several features, including high throughput, robustness, recognition-based methodology, rapid tendency test, usability, and low cost per test. In the initial stage of characterising the culturable microbial community in dairy products, MALDI-TOF MS profiling complemented the phenotypic approaches for identification. It is simple to apply as a routine analysis. This quick, inexpensive, consistent, and resilient method of identifying bacteria offers a compelling alternative to biochemical and even molecular biology-based identification procedures because it does not require nucleic acid extraction, PCR (polymerase chain reaction), or sequencing stages (Nacef *et al*., 2017). The identification of *Lactobacillus* spp. showed that the MALDI-TOF MS technique had a greater success rate for species-level assignment (93%) than PCR (77%) (Dušková *et al*., 2012). Therefore, the MALDI-TOF-MS method—presented as an inexpensive, reliable, and sustainable method—could support improved microbial identification management. This approach is preferable to other methods because only a tiny quantity of samples is required, and it is much more rapid and reproducible. Furthermore, slow-growing and fastidious microorganisms have been identified using this approach (Veloo *et al*., 2017). However, this method is not preferable for microbiotic populations as it is not present in the MALDI Biotyper reference database (Kraková *et al*., 2018).

PFGE

This powerful approach identifies lactobacilli based on data revealed from the separation of either large DNA molecules or small RE (restriction endonuclease)-digested fragments of the genome and the entire genome (Hicks *et al*., 2018). It can differentiate between valuable probiotic species such as *L. casei*, *L. rhamnosus*, *L. helveticus*, and *L. johnsonii* (Ben Amor *et al*., 2007). PFGE profiling was used to study and evaluate microbial oscillation from raw milk to cheese production. The microbial population in raw milk detected by PFGE profiling showed massive diversity, including *La. lactis*, *L. plantarum*, *L. curvatus*, *S. gallinarum*, *mycobacterium*, and *E. faecalis*. However, only *La. lactis*, *L. plantarum*, and *E. faecalis* were viable at the end of cheese production (Yunita and Dodd, 2018). Genotyping of the genus *Lactobacillus* (*L. fermentum*, *L. gasseri*, *L. plantarum*, and *L. rhamnosus*) was also documented using this technique (Gosiewski and Brzychczy-Wloch, 2015).

The tools for PFGE and the techniques for analysis of electrophoretic data have noticeably been enhanced; however, the sample groundwork for PFGE remains monotonous. Agarose plugs are handled and separated case-by-case, which is time-consuming and difficult for scale-up. Different isolates can get messed up and merged, thus leading to the misidentification of isolates (Hicks *et al*., 2018).

MLST

In this method, distant evolutionary processes are used to differentiate *Lactobacillus* strains, either at the intraspecies or subspecies level, using variation in the housekeeping gene sequence (Buhnik-Rosenblau *et al*., 2012). Various MLST databases such as PubMLST (public database for molecular typing) (http://pubmlst.org/) or MLST (WWW.mlst.net) are used. MLST and multiplex-

polymerase chain reaction (PCR) was used to identify *L. sanfranciscensis* isolated from sourdoughs. MLST system was decreased from six gene lysate (gdh, gyrA, mapA, nox, pgmA, and pta) to five (gdh, gyrA, mapA, nox, and pta) as the pgmA lysate showed only single allelic pattern (Yang *et al*., 2017). Next, 13 sequence types from 13 different isolates were epitomised as one strain. Furthermore, 83 polymorphic locations were recognised from seven locations; the numbers of these sites ranged between four for rpoB and 24 for recA. Furthermore, the number of alleles was observed between 2 of dnaK and 13 of recA. Splits Tree recombination was used to separate three chief categories from every 13 isolates (Sharma *et al*., 2017). Eleven different unique loci (clpx, dnaA, dnaK, groEL, murc, murE, pepX, pyrG, recA, rpoB, and uvrc) present in the genome of *L. fermentum* were revealed by genomic characterisation and identification of *L. fermentum* from ready-to-eat foodstuffs (Dan *et al*., 2015). Characterisation has been done on a variety of *L. rhamnosus* and *L. fermentum* strains isolated from faeces, saliva, and vaginal cavities of healthy women. The outcomes of the investigation of genetic and genomic polymorphism, and multi-locus sequence typing were equivalents. Sequence types (ST) of *L. rhamnosus* and *L. fermentum* were isolated from diverse locations on the same person's body in different and identical forms. Additionally, identical ST were isolated from other women, thus indicating that these strains may be part of a larger group of strains that are circulating around the population (Poluektova *et al*., 2017).

Various species of LAB present in food can be differentiated using the MLST method. However, in a daily diagnosis, it is a more tedious and time-consuming method when dealing with a large number of strains (Yang *et al*., 2017).

16S ribosomal RNA analysis

Probiotic microorganisms can be easily identified using molecular-based technologies, which are frequently used as a substitution for less effective conventional techniques. Probiotic bacteria have strain-specific health-promoting properties; hence it is necessary to distinguish and identify them at the strain level using reliable and effective strain-identification methods like 16S rRNA sequencing. The process of amplifying the 16S rDNA gene, which is highly conserved in some areas, has been repeatedly documented in the literature to identify new probiotic bacterial strains. However, it contains highly variable areas that can offer a signature unique to a certain strain. Consequently, the 16S rDNA gene should be amplified using PCR, and the PCR results should then be sequenced to confirm the presence of *Lactobacillus* (Nami *et al*., 2015; 2018). Using 16S rRNA permits the enumeration of microorganisms inaccessible to cultivate using the methods now in use or that have died during transport and storage. These techniques have effectively identified the gut microbiota, the "pool" of probiotic origin. All bacteria share highly conserved sections of the 16S rDNA gene, while some germs have highly variable sequences unique to them (Stefanis *et al*., 2016). Because phylogenetically related LAB species frequently exhibit similar physiological profiles, it is insufficient to rely solely on biochemical approaches for identification. Based on research findings, 16S rRNA sequencing is precise and accurate for identifying LAB (Reuben *et al*., 2019). Sequencing the 16S ribosomal region has been used to identify lactobacilli correctly at the species level. This genome region can differentiate nearly identical species (Masalam *et al*., 2018). The 16S ribosomal RNA region is the most ordinary spot for recognising lactobacilli at the species level, where species-specific/universal primers (Table 4) are utilised to amplify the precise target of the genome with the help of PCR (Bested *et al*., 2013a). Additionally, Sanger or pyrosequencing method can be used for accurate species identification. BLAST (basic local alignment search tool) or the Megalign® alignment suite (Lasergene DNAStar, USA) using the ClustalW algorithm helps in the inside analysis by sequence data analysis (Bested *et al*., 2013b).

Lactobacilli are categorised using intergenic spacer regions (ITS) of rRNA. Colony PCR is used to rapidly identify lactobacilli, where a crude cell lysate and species-specific primers targeting the 16S rRNA give the outcome within three hours after isolation (duration of cultivation: 48 h) (Luo *et al*., 2012). The 16S rRNA profile of lactobacilli isolated from chickens with different dilutions showed a lower level of lactobacilli, thus indicating that higher dilution can change the *Lactobacillus* profile (Adhikari and Kwon, 2020). This approach was used to identify *L. plantarum*, *L. casei*, and *L. rhamnosus* from the faecal sample (Chandra and Vij, 2018). Furthermore, evaluation of the proteolytic activity of these

Table 4. Several primers used for 16s rRNA gene amplification.

Primers	Sequence (5' to 3')	Identified strains	Reference
fD1 rD1	AGAGTTTGATCCTGGCTCAG TAAGGAGGTGATCCAGGC	*L. fermentum*, *L. gasseri*, *L. brevis*	Dimitonova *et al*. (2008)
16s:f27 16s:r27	AGAGTTTGATCCTGGCTCAG AAGGAGGTGATCCAGCCGCA	*L. casei*, *L. plantarum*	Masalam *et al*. (2018)
27f 1492r	AGAGTTTGATCCTGG CTCAG GGTTACCTTGTTACGACTT	*L. plantarum*, *L. fermentum*, *L. pentosus*	Tajabadi *et al*. (2013)
MLB PLB	GGCTGCTGGCACGTAGTTAG AGAGTTTGATCCTGGCTCAG	*L. gasseri*, *L. crispatus*	Fosch *et al*. (2018)
Joh 16SI Joh 16SII	GAGCTTGCCTAGATGATTTTA ACTACCAGGGTATCTAATCC	*L. johnsonii*	Awd *et al*. (2020)
fD1 R1530	AGA GTT TGA TCC TGG CTC AG AAG GAG GTG ATC CAG CCG CA	*L. salivarius*, *L. johnsonii*, *L. ingluviei*	Dec *et al*. (2016)

organisms suggests that these probiotics can be exploited as a potential starter culture for the production of skim milk and whey fermentation.

Sequencing the 16S ribosomal RNA region resulted in a new strain of *L. metriopterae* Hime 5–1^T. Identical sequence resemblance with *L. furfuricola* (96.5%), *L. tucceti* (96.7%), and *L. versmoldensis* (96.3%) were displayed by this strain (Chiba *et al*., 2018). A recent study has published the effect of *L. plantarum* on pathogenic bacteria, especially against *E. fergusonii*. 16S rDNA sequencing with the universal primers 27F and 1492R was used to identify *L. plantarum* isolated from camel milk. Furthermore, camel milk enriched with these bacteria released several antagonistic compounds to prevent further the effect of diarrhoea in infants (Toualbia *et al*., 2018). Similarly, rD1 and fD1 primers were used to identify *L. paracasei* and *L. brevis* isolated from various dough samples. Each isolate was an acid producer (Tilahun *et al*., 2018).

L. mucosae was isolated from cattle faeces, pen soil, and feed rations. Few identified isolates were hydrophilic, whereas few strains were hydrophilic and auto-aggregative. Variability in biofilm development was observed due to variation in the concentration of Tween 80 in the MRS medium (Maldonado *et al*., 2018). A comparative study conducted in the vaginal community of 236 African and white women showed that women lacking lactobacilli in their vagina were susceptible to HIV infection. They showed an elevated number of activated mucosal $CD4^+$ T cells in their vagina. The presence of diverse communities in these volunteers was shown by sequencing the V4 region; *L. crispatus* dominated 10%, *L. iners* dominated 32%, and lactobacilli dominated 90% in white women (Gosmann *et al*., 2017). Usually, bacterial identification can be accomplished quickly and accurately without the need for phenotypic characterisation by amplifying the 16S rRNA gene. 16S rRNA sequencing, however, is unable to distinguish between closely related species. Therefore, sequencing of alternative genes with more discriminating ability, such as rpoA, should be combined with 16S rRNA sequencing (Farahmand *et al*., 2021). The sequencing approach perfectly makes identification at the genus and species levels possible; however, it is time-consuming daily, as much time is needed for sequencing and analysis of PCR products.

qPCR

It is a molecular technique used to identify and quantify the species/strains of lactobacilli in a sample. Due to genetic variability in nearly identical species, it is critical to identify at the species level (Demkin and Koshechkin, 2017). Varying PCR techniques have been used to detect the amplification process by genus or species-specific primers (Fujimoto *et al*.,

2008). Primers like SYBR® Green, TaqMan® labelled primers, or molecular beacons are universally utilised in qPCR techniques (Pogačić *et al*., 2010). Each primer has a different constitution and properties. SYBR® Green is a fluorescence dye, and anneals with double-stranded DNA. TaqMan®-labelled primer is a DNA-binding primer that binds to the DNA flanking primer's internal sequence. On the other hand, a secondary structure with a target region is formed by molecular beacons, and they become fluorescent in their hybridisation state (Herbel *et al*., 2013). Viable *L. paracasei* isolated from yogurt was detected using the ordinary qPCR with propidium monoazide modified into PMA-qPCR. PMA-qPCR permits the detection of bacteria with a limit of 10^4 copies of the genome (Scariot *et al*., 2018). For the identification and enumeration of *L. plantarum* PM411, a novel approach entitled viability quantitative PCR (v-qPCR) was established. Three different primers were set up with amplicons lengths of 92,188 and 319 bp with one TaqMan probe (Daranas *et al*., 2018). This technique was used to detect lactobacilli along with bacterial vaginosis in the vagina and chorioamnion of pregnant women (Lannon *et al*., 2019). Four closely similar species make up the *Latilactobacillus sakei* group, thus making it challenging to separate them with precision using conventional markers such as the 16S rRNA gene. A comparative pan-genomic analysis was used to identify novel markers for PCR detection, and differentiation of *L. sakei* group species and subspecies. 119,899 coding genes were found in the 63 genome sequences of *L. sakei* group species, resulting in 5,741 pan-genomes, 831 core genomes, 3,347 accessory genomes, and 1,563 unique genomes. The accessory genome was compared to identify unique candidate genes shared only by the same species' genomes. Afterwards, the candidate genes were compared to the genomes of other bacteria to identify flag genes that were common to all genomes of a certain species but not to those of others. In that study, the marker genes for *L. sakei*, *L. sakei* subsp. *sakei*, *L. sakei* subsp. *carnosus*, *L. curvatus*, *L. graminis*, and *L. fuchuensis* were identified. These markers included the arginine/ornithine antiporter, putative cell surface protein precursor, sodium: solute symporter, and primer pairs were created for each marker, and demonstrated 100% specificity for 48 reference strains of LAB. To show that the marker genes offered a competitive alternative to the 16S rRNA gene, 106 bacteria isolated from fermented foods were examined using the PCR technique described in that study (Kim *et al*., 2021).

This method is faster, and can identify minor populations of lactobacilli over major populations of other bacteria. Non-culturable species are also detected using this method and quantified using qPCR and PCR. As both qPCR and RT-qPCR (real time) are cost-effective, they are appropriate for everyday analysis. This method becomes more advantageous after amplification, and there are no chances of contamination as no modifications are needed (Postollec *et al*., 2011). Therefore, this method is suitable for species-specific quantification and identification of lactobacilli (Junick and Blaut, 2012; Daranas *et al*., 2018).

RAPD

RAPD is an easy, rapid, and economical genotyping system based on PCR. In this method, arbitrary primers are used, which anneal with low stringency to partially or entirely complementary sequences of an organism's genome. An RAPD primer anneals at target DNA randomly, and gives the band "fingerprints" (Fujimoto *et al*., 2008). Rapid manifold sample recognition is done by RAPD (Satokari *et al*., 2003). According to several researchers, this method can easily identify lactobacilli effortlessly due to its heterogeneous nature (Andrighetto *et al*., 2004). Currently, the Ready-To-Go-RAPD kit is extensively used to screen bacterial communities faster as it has necessary primers (Plengvidhya *et al*., 2004). Lactobacilli can be identified more accurately using PCR variants. Primers derived from RAPD were used to modify the multiplex-PCR assay. *L. plantarum* 2035 and *L. plantarum* ACA-DC 2640 isolated from feta cheese were identified using this approach (Galanis *et al*., 2015). However, this tool could not recognise a few isolates. A broad range of activity and shielding properties of vaginal lactobacilli has been discovered. RAPD, species-specific, and BOX-PCR were used to elucidate this evidence to identify *L. fermentum*, *L. gasseri*, *L. brevis*, and *L. salivarius* from vaginal isolates (Dimitonova *et al*., 2008). This method can differentiate among different species of either lactobacilli or *Bifidobacterium*, thus proving highly efficient. Any microbial variability occurring during fermentation can be evaluated using this method with the help of RAPD (Stefanis *et al*., 2016). This technique was used to identify lactobacilli isolated from traditional dairy products unambiguously.

Results showed that polymorphic information content (PIC) oscillated between 15.9 and 34.4% using a 1,254 primer. The results obtained from that study also showed that diversity within species (with 94% of all variance) was more significant than diversity between species (with 6% of all variance) (Delshad *et al*., 2018). Although reports suggested that data obtained from RAPD analysis could be highly ambiguous, as each isolate was nearly identical to *L. johnsonii* by 16S rRNA sequencing, RAPD analysis of these isolates showed much sequence variation when compared with reference strain *L. johnsonii* (Doi *et al*., 2013).

RAPD technique is easy, cost-effective, and expedient to execute. No prior sequence is required for bacterial identification. However, the major disadvantage of RAPD is its lack of accuracy, as analogous strains cannot be differentiated (Pingault *et al*., 2007). In a study, the results of 24 isolates of *L. brevis* showed a low diversity by the RAPD-PCR technique, which suggested using a combination of more powerful techniques along with RAPD-PCR (Noohi *et al*., 2021). Another drawback of the RAPD is that it shows low reproducibility. The same sample with minor variations can give dissimilar results. Therefore, a more unambiguous method is required to identify results obtained from RAPD. However, this method can confirm the results of lactobacilli identified by PFGE.

SSR

Lactobacillus spp. can be identified with a high rate of mutation in their genome using the SSR technique. The SSR loci are distributed evenly and abundantly in the genome of *Lactobacillus* species, in both coding and non-coding regions, which can be utilised to characterise bacteria. This technique is much more efficient at species and strain levels, as it has high resolution and differentiation capacity. SSR loci showed variability within the same strain isolated from a different host. *L. johnsonii* isolated from a different host resulted in SSR loci dissimilarity in each strain. This suggested that each LAB strain could be host-specific (Buhnik-Rosenblau *et al*., 2012).

WGS

This approach gives an inside view of the genome, and was used to identify genetic and structural variations of sequenced *Lactobacillus* (Table 5). This approach further helps study comparative and functional genomics (1000 Genomes Project Consortium, 2010; Zhang *et al*., 2018). Amplified source DNA is attached to beads, and sequenced later for high throughput sequencing. Next-generation sequencing (NGS) helps in sequencing and constructing multiple short-sequence reads (Ansorge, 2009). Currently, high-throughput sequencing is used to find genetic trouble in biology and medicine. New methodologies have been developed to analyse genome structure, variation among different strains, and metabolic capacities (Soon *et al*., 2013). Whole genome sequences (WGS) were used to identify the development of streptomycin resistance in *L. plantarum*. After 25 days, the MIC reached 131,072 µg/mL (Zhang *et al*., 2018). Nowadays, thousands of sequencing-by-synthesis operations are done simultaneously by largely parallel sequencing or ultra-high-throughput sequencing (UHTS) (1000 Genomes Project Consortium, 2010). Readouts are evaluated together by light production after adding a new nucleotide in Sanger sequencing and pyrosequencing (Rothberg and Leamon, 2008).

L. curvatus is believed to be involved in the fermentation of food. It has a unique genetic sequence in response to the environment where it resides. To determine further, the 12 strains of *L. curvatus* were evaluated using WGS with the help of Illumina MiSeq and Oxford Nanopore MinION. WGS of this strain revealed that they do possess 1,911,461 bp, and having 42% GC content, 5rRNA operon, 65 tRNA, and 1,946 coding sequences. The SLC13 genome was sequenced, showing a plantaricin gene cluster responsible for the antagonistic activity and 2-gene clusters involved in EPS production (Huang *et al*., 2018b). Pacific Biosciences (PacBio)_20K sequencing data was used for the complete genome sequence of *L. rhamnosus* 4B15 and *L. gasseri* 4M13, and then they were analysed in Chunlab, Inc. (Seoul, Korea). The resultant contigs were further scaffolded using PacBio SMRT Analysis version 2.3.0 (Oh *et al*., 2018). The structure and function of S-layer protein were revealed by WGS of *L. brevis* SF 9B strain isolated from sauerkraut (Banić *et al*., 2018). High EPS production and antagonistic capability were shown by *L. pentosus* SLC 13. The WGS of L. *plantarum* EM was recently studied, and its genetic correlation with its probiotic properties was evaluated. It had 3,560 coding regions, among which genes encoding for acid, bile, temperature, and stress tolerance were present (Kim *et al*., 2020a). The gene

encoding for P II-like protein, present only in five out of 280 species of *Lactobacillus*, was recently recognised first time by WGS in *L. hilgardii*. This gene is responsible for the regulation of cellular metabolism. Unlike the typical P II gene, it is present inside the pot ABCD operon of *L. hilgardii*, with unique genetic content, and hence termed pot N (pot-protein, nucleotide-binding protein). It carries two genes encoding for proteins responsible for similar functions (Zhuravleva *et al*., 2020). *Lactobacillus* spp. with close genetic and phenotypic similarities such as *L. casei*, *L. paracasei*, and *L. rhamnosus* are difficult to differentiate by most commonly used 16S rRNA sequencing. Recently, WGS with genome comparisons along with average nucleotide identity (ANI) and digital DNA-DNA hybridisation (DDDH) were used as gold standard approaches for the discrimination of phylogenetic relationships among closely related species (Huang *et al*., 2018a).

Table 5. Characteristics of several whole genome sequenced isolates.

Sr. no.	Strain used for WGS	Characteristic of isolate	Reference
1	*L. johnsonii* ZLJ010	Genes responsible for stress adaptation, biosynthesis, metabolism, transport of amino acid, secretion, and the defence machinery were identified out of 1,959 protein-coding sequences (CDSs).	Zhang *et al*. (2019)
2	*L. rhamnosus* 4B15, *L. gasseri* 4M13	Both are novel genomic strains. Both displayed the highest probiotic potential and showed an effect on immune health by modulation of pro-inflammatory cytokines.	Oh *et al*. (2018)
3	*Lactobacillus hilgardii* LMG 7934	The presence of a PII-like protein (central regulators of cellular metabolism) encoding gene in *Lactobacillus hilgardii* was documented for the first time. *Lactobacillus* does not possess any genes for PII proteins, because of excessive nutrient surroundings.	Zhuravleva *et al*. (2020)
4	*L. casei*, *L. paracasei*, *L. rhamnosus*, *L. chiayiensis*	Based on comparative genome analysis results, species-specific genes were recognised which helped to design primers to identify closely related species	Kim *et al*. (2020b)
5	*L. plantarum* LP9010	Genome-wide analysis revealed that LP9010 possesses potential carbohydrate transporter, excellent adhesion, antioxidant and antibacterial activities, tolerance to the simulated human gastrointestinal tract, and novel gene clusters of exopolysaccharide biosynthesis were analysed.	Liu *et al*. (2022)
6	*L. pentosus* SLC13	A plantaricin gene cluster, which is responsible for bacteriocin biosynthesis and could be associated with its broad-spectrum antimicrobial activity, was identified based on comparative genomic analysis. Two gene clusters involved in EPS production were also identified.	Huang *et al*. (2018b)

Conclusion

Various culture-specific methods (morphology, API 50 CHL) are used for genus identification; however, they are time-consuming and have low reproducibility. THE MALDI-TOF MS technique is more efficient than other phenotypic methods as it needs only a small sample, and is a rapid method. Furthermore, it is used for the identification of slow-growing and fastidious microorganisms. However, this method cannot provide correct results for microbiota that are not present in the MALDI Biotyper reference database. 16S ribosomal RNA sequencing, MLST, and SSR are accurate methods for identification at the species level. However, several species of LAB having a conserved region do

not respond to the 16S rRNA sequencing approach. For such strains or species of lactobacilli, species-specific variants of PCR might be the accurate method. The qPCR technique is a cost-effective method used to identify microorganisms isolated from food products, and simultaneously, quantification can be done within 7 h. Taxonomic differentiation of closely related species is only possible with WGS, and it should be used as a gold standard for the phylogenetic analysis of such species. WGS is a preferred and advantageous method as it gives an inside view of the entire genome sequence of an organism, which later can be used for primer designing for 16s/23s/5s sequencing; however, it becomes expensive while dealing with a large number of species.

References

1000 Genomes Project Consortium. 2010. A map of human genome variation from population-scale sequencing. Nature 467: 1061-1073.

Adhikari, B. and Kwon, Y. M. 2020. Cell density alters bacterial community structure in culture - enriched 16S rRNA gene microbiota profiling. BMC Research Notes 13: 269.

Ahmmed, F., Ahmmed, M. K., Khushi, S. S., Sumon, M. S., Karamcheti, S. S. and Sarower, M. G. 2020. Host gut-derived probiotic *Lactobacillus* sp. improves resistance of giant freshwater prawn *Macrobrachium rosenbergii* against *Vibrio harveyi*. Aquaculture International 28: 1709-1724.

Allain, T., Chaouch, S., Thomas, M., Vallée, I., Buret, A.G., Langella, P., … and Florent, I. 2018. Bile-salt-hydrolases from the probiotic strain *Lactobacillus johnsonii* La1 mediate anti-giardial activity *in vitro* and *in vivo*. Frontiers in Microbiology 8: 2707.

Alvarez-Olmos, M. I., Barousse, M. M., Rajan, L., Van Der Pol, B. J., Fortenberry, D., Orr, D. and Fidel, P. L. 2004. Vaginal lactobacilli in adolescents: Presence and relationship to local and systemic immunity, and to bacterial vaginosis. Sexually Transmitted Diseases 31: 393-400.

Andrighetto, C., Marcazzan, G. and Lombardi, A. 2004. Use of RAPD-PCR and TTGE for the evaluation of biodiversity of whey cultures for Grana Padano cheese. Letters in Applied Microbiology 38: 400-405.

Ansorge, W. J. 2009. Next-generation DNA sequencing techniques. New Biotechnology 25: 195-203.

Awd, A., Ismail, A., Abdelfatah, K. and Khater, A. 2020. Identification and phylogenetic studies of a new probiotic *Lactobacillus* spp. Egyptian isolate based on 16s rRNA gene. Acta Scientific Nutritional Health 4(3): 104-110.

Banić, M., Uroić, K., Leboš Pavunc, A., Novak, J., Zorić, K., Durgo, K., … and Kos, B. 2018. Characterisation of S-layer proteins of potential probiotic starter culture *Lactobacillus brevis* SF9B isolated from sauerkraut. LWT – Food Science and Technology 93: 257-267.

Bazukyan, I., Matevosyan, L., Toplaghaltsyan, A. and Trchounian, A. 2018. Antifungal activity of lactobacilli isolated from Armenian dairy products: An effective strain and its probable nature. AMB Express 8: 87.

Belfiore, C., Raya, R. R. and Vignolo, G. M. 2013. Identification, technological and safety characterisation of *Lactobacillus sakei* and *Lactobacillus curvatus* isolated from Argentinean anchovies (*Engraulis anchoita*). Springer Plus 2: 257.

Ben Amor, K., Vaughan, E. and de Vos, W. 2007. Advanced molecular tools for the identification of lactic acid bacteria. The Journal of Nutrition 137: 741S-747S.

Bested, A. C., Logan, A. C. and Selhub, E. M. 2013a. Intestinal microbiota, probiotics and mental health: From Metchnikoff to modern advances: Part II - contemporary contextual research. Gut Pathogens 5: 3.

Bested, A. C., Logan, A. C. and Selhub, E. M. 2013b. Intestinal microbiota, probiotics and mental health: From Metchnikoff to modern advances: Part I - autointoxication revisited. Gut Pathogens 5: 5.

Biadała, A., Szablewski, T., Lasik-Kurdyś, M. and Cegielska-Radziejewska, R. 2020. Antimicrobial activity of goat's milk fermented by single strain of kefir grain microflora. European Food Research and Technology 246: 1231-1239.

Bucka-Kolendo, J., Sokołowska, B. and Winiarczyk, S. 2020. Influence of high hydrostatic pressure on the identification of *Lactobacillus* by MALDI-TOF MS - Preliminary study. Microorganisms 8(6): 813.

Buhnik-Rosenblau, K., Matsko-Efimov, V., Jung, M., Shin, H., Danin-Poleg, Y. and Kashi, Y. 2012. Indication for co-evolution of *Lactobacillus johnsonii* with its hosts. BMC Microbiology 12: 1.

Chandra, P. and Vij, S. 2018. Molecular characterisation and identification of bioactive peptides producing *Lactobacillus* sps. based on 16S rRNA gene sequencing. Food Biotechnology 32: 1-14.

Chiba, M., Itabashi, T., Hirai, K., Sakamoto, M., Ohkuma, M., Ishige, T. and Kawasaki, S. 2018. *Lactobacillus metriopterae* sp. nov., a novel lactic acid bacterium isolated from the gut of grasshopper *Metrioptera engelhardti*. International Journal of Systematic and Evolutionary Microbiology 68: 1484-1489.

Coelho-Rocha, N. D., de Jesus, L. C. L., Barroso, F. A. L., da Silva, T. F., Ferreira, E., Gonçalves, J. E., … and Azevedo, V. A. C. 2022. Evaluation of probiotic properties of novel Brazilian *Lactiplantibacillus plantarum* strains. Probiotics and Antimicrobial Proteins 15: 160-174.

Dan, T., Liu, W., Song, Y., Xu, H., Menghe, B., Zhang, H. and Sun, Z. 2015. The evolution and population structure of *Lactobacillus fermentum* from different naturally fermented products as determined by multilocus sequence typing (MLST). BMC Microbiology 15: 107.

Daranas, N., Bonaterra, A., Francés, J., Cabrefiga, J., Montesinos, E. and Badosa, E. 2018. Monitoring viable cells of the biological control agent *Lactobacillus plantarum* PM411 in aerial plant surfaces by means of a strain-specific viability quantitative PCR method. Applied and Environmental Microbiology 84: e00107-18.

Das, D. J., Shankar, A., Johnson, J. B. and Thomas, S. 2020. Critical insights into antibiotic resistance transferability in probiotic *Lactobacillus*. Nutrition 69: 110567.

de Castro, A., Sánchez, A. H., Cortés-Delgado, A., López-López, A. and Montaño, A. 2019. Effect of Spanish-style processing steps and inoculation with *Lactobacillus pentosus* starter culture on the volatile composition of cv. Manzanilla green olives. Food Chemistry 271: 543-549.

Dec, M., Puchalski, A., Urban-chmiel, R. and Wernicki, A. 2016. 16S-ARDRA and MALDI-TOF mass spectrometry as tools for identification of *Lactobacillus* bacteria isolated from poultry. BMC Microbiology 16: 105.

Delshad, A., Seyed Mostafa, H., Batoul, K., Alireza, M. and Mohammad Reza, A. 2018. Identification of *Lactobacillus* species isolated from traditional dairy products using RAPD-PCR. Avicenna Journal of Clinical Microbiology and Infection 5: 7-13.

Demkin, V. V. and Koshechkin, S. I. 2017. Characterisation of vaginal *Lactobacillus* species by *rplK*-based multiplex qPCR in Russian women. Anaerobe 47: 1-7.

Dimitonova, S. P., Bakalov, B. V., Aleksandrova-Georgieva, R. N. and Danova, S.T. 2008. Phenotypic and molecular identification of lactobacilli isolated from vaginal secretions. Journal of Microbiology, Immunology and Infection 41: 469-477.

Doi, K., Phuong, O., Kawatou, F., Nagayoshi, Y., Fujino, Y. and Ohshima, T. 2013. Identification and characterisation of lactic acid bacteria isolated from fermented rice bran product. Advances in Microbiology 3: 265-272.

Domingos-Lopes, M. F. P., Stanton, C., Ross, P. R., Dapkevicius, M. L. E. and Silva, C. C. G. 2017. Genetic diversity, safety and technological characterisation of lactic acid bacteria isolated from artisanal Pico cheese. Food Microbiology 63: 178-190.

du Toit, M., Franz, C. M. A., Dicks, L. M. T., Schillinger, U., Haberer, P., Warlies, B., … and Holzapfel, W. H. 1998. Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. International Journal of Food Microbiology 40: 93-104.

Dušková, M., Šedo, O., Kšicová, K., Zdráhal, Z. and Karpíšková, R. 2012. Identification of lactobacilli isolated from food by genotypic methods and MALDI-TOF MS. International Journal of Food Microbiology 159: 107-114.

El Manouni El Hassani, S., de Boer, N. K. H., Jansen, F. M., Benninga, M. A., Budding, A. E. and de Meij, T. G. J. 2019. Effect of daily intake of *Lactobacillus casei* on microbial diversity and dynamics in a healthy pediatric population. Current Microbiology 76: 1020-1027.

Farahmand, N., Ouoba, L. I. I., Naghizadeh Raeisi, S., Sutherland, J. and Ghoddusi, H. B. 2021. Probiotic lactobacilli in fermented dairy products: Selective detection, enumeration and identification scheme. Microorganisms 9(8): 1600.

Febbraro, F., Rodio, D. M., Puggioni, G., Antonelli, G., Pietropaolo, V. and Trancassini, M. 2016. MALDI-TOF MS Versus VITEK®2: Comparison of systems for the identification of microorganisms responsible for bacteremia. Current Microbiology 73: 843-850.

Fijan, S., Šulc, D. and Steyer, A. 2018. Study of the *in vitro* antagonistic activity of various single-strain and multi-strain probiotics against *Escherichia coli*. International Journal of Environmental Research and Public Health 15: 1539.

Finamore, A., Ambra, R., Nobili, F., Garaguso, I., Raguzzini, A. and Serafini, M. 2018. Redox role of *Lactobacillus casei* Shirota against the cellular damage induced by 2,2'-azobis (2-amidinopropane) dihydrochloride-induced oxidative and inflammatory stress in enterocytes-like epithelial cells. Frontiers in Immunology 9: 1131.

Fonseca, H. C., de Sousa Melo, D., Ramos, C. L., Dias, D. R. and Schwan, R. F. 2021. Probiotic properties of lactobacilli and their ability to inhibit the adhesion of enteropathogenic bacteria to Caco-2 and HT-29 cells. Probiotics and Antimicrobial Proteins 13: 102-112.

Fosch, S. E., Ficoseco, C. A., Marchesi, A., Cocucci, S., Nader-Macias, M. E. F. and Perazzi, B. E. 2018. Contraception: Influence on vaginal microbiota and identification of vaginal lactobacilli using MALDI-TOF MS and 16S rDNA sequencing. The Open Microbiology Journal 12: 218-229.

Foschi, C., Laghi, L., Parolin, C., Giordani, B., Compri, M., Cevenini, R., … and Vitali, B. 2017. Novel approaches for the taxonomic and metabolic characterisation of lactobacilli: Integration of 16S rRNA gene sequencing with MALDI-TOF MS and ^{1}H-NMR. PLoS One 12(2): e0172483

Fujimoto, J., Matsuki, T., Sasamoto, M., Tomii, Y. and Watanabe, K. 2008. Identification and quantification of *Lactobacillus casei* strain Shirota in human feces with strain-specific primers derived from randomly amplified polymorphic DNA. International Journal of Food Microbiology 126: 210-215.

Galanis, A., Kourkoutas, Y., Tassou, C. C. and Chorianopoulos, N. 2015. Detection and identification of probiotic *Lactobacillus plantarum* strains by multiplex PCR using RAPD-derived primers. International Journal of Molecular Sciences 16: 25141-25153.

Gosiewski, T. and Brzychczy-Wloch, M. 2015. The use of PFGE method in genotyping of selected bacteria species of the *Lactobacillus* genus. In Jordan, K. and Dalmasso, M. (eds). Pulse Field Gel Electrophoresis - Methods and Protocols, p. 225-240. United States: Springer.

Gosmann, C., Anahtar, M. N., Handley, S. A., Farcasanu, M., Abu-Ali, G., Bowman, B. A., … and Kwon, D. S. 2017. *Lactobacillus*-deficient cervicovaginal bacterial communities are associated with increased HIV acquisition in young South African women. Immunity 46: 29-37.

Gupta, M. and Bajaj, B. K. 2017. Functional characterisation of potential probiotic lactic acid bacteria isolated from Kalarei and development of probiotic fermented oat flour. Probiotics and Antimicrobial Proteins 10: 654-661.

Haghshenas, B., Kiani, A. and Nami, Y. 2021. Probiotic potential and safety evaluation of lactic acid bacteria isolated from colostrum. Journal of Biosafety 14: 37-60.

Haghshenas, B., Nami, Y., Abdullah, N., Radiah, D., Rosli, R. and Khosroushahi, A. Y. 2014. Anti-proliferative effects of *Enterococcus* strains isolated from fermented dairy products on different cancer cell lines. Journal of Functional Foods 11: 363-374.

Herbel, S. R., Vahjen, W., Wieler, L. H. and Guenther, S. 2013. Timely approaches to identify probiotic species of the genus *Lactobacillus*. Gut Pathogens 5: 27.

Hicks, L., Van der Graaf, C. M., Childress, J., Cook, E., Schmidt, K., Rosenzweig, F. and Kroll, E. 2018. Streamlined preparation of genomic DNA in agarose plugs for pulsed-field gel electrophoresis. Journal of Biological Methods 5: 86.

Huang, C. H., Li, S. W., Huang, L. and Watanabe, K. 2018a. Identification and classification for the *Lactobacillus casei* group. Frontiers in Microbiology 9: 1974.

Huang, M. L., Huang, J. Y., Kao, C. Y. and Fang, T. J. 2018b. Complete genome sequence of *Lactobacillus pentosus* SLC13, isolated from mustard pickles, a potential probiotic strain with antimicrobial activity against foodborne pathogenic microorganisms. Gut Pathogens 10: 1.

Jampaphaeng, K., Ferrocino, I., Giordano, M., Rantsiou, K., Maneerat, S. and Cocolin, L. 2018. Microbiota dynamics and volatilome profile during stink bean fermentation (Sataw-Dong) with *Lactobacillus plantarum* KJ03 as a starter culture. Food Microbiology 76: 91-102.

Junick, J. and Blaut, M. 2012. Quantification of human fecal *Bifidobacterium* species by use of quantitative real-time PCR analysis targeting the *groEL* gene. Applied and Environmental Microbiology 78: 2613-2622.

Karakas-Sen, A. and Karakas, E. 2018. Isolation, identification and technological properties of lactic acid bacteria from raw cow milk. Bioscience Journal 34(2): 385-399.

Karami, S., Roayaei, M., Hamzavi, H., Bahmani, M., Hassanzad-Azar, H., Leila, M. and Rafieian-Kopaei, M. 2017. Isolation and Identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens. International Journal of Pharmaceutical Investigation 7: 137-141.

Kaźmierczak-Siedlecka, K., Ruszkowski, J., Fic, M., Folwarski, M. and Makarewicz, W. 2020. *Saccharomyces boulardii* CNCM I-745 - A non-bacterial microorganism used as probiotic agent in supporting treatment of selected diseases. Current Microbiology 77: 1987-1996.

Kiani, A., Nami, Y., Hedayati, S., Elieh Ali Komi, D., Goudarzi, F. and Haghshenas, B. 2021. Application of Tarkhineh fermented product to produce potato chips with strong probiotic properties, high shelf-life, and desirable sensory characteristics. Frontiers in Microbiology 12: 657579.

Kim, E., Chang, H. C. and Kim, H.-Y. 2020a. Complete genome sequence of *Lactobacillus plantarum* EM, A putative probiotic strain with the cholesterol-lowering effect and antimicrobial activity. Current Microbiology 77: 1871-1882.

Kim, E., Yang, S.-M., Cho, E.-J. and Kim, H.-Y. 2020b. Novel real-time PCR assay for *Lactobacillus casei* group species using comparative genomics. Food Microbiology 90: 103485.

Kim, E., Yang, S.-M., Kim, D. and Kim, H.-Y.,2021. Real-time PCR method for qualitative and quantitative detection of *Lactobacillus sakei* group species targeting novel markers based on bioinformatics analysis. International Journal of Food Microbiology 355: 109335.

Kinoshita, H., Uchida, H., Kawai, Y., Kitazawa, H., Miura, K., Shiiba, K., … and Saito, T. 2007. Quantitative evaluation of adhesion of lactobacilli isolated from human intestinal tissues to human colonic mucin using surface plasmon resonance (BIACORE assay). Journal of Applied Microbiology 102: 116-123.

Kraková, L., Šoltys, K., Otlewska, A., Pietrzak, K., Purkrtová, S., Savická, D., … and Pangallo, D. 2018. Comparison of methods for identification of microbial communities in book collections: Culture-dependent (sequencing and MALDI-TOF MS) and culture-independent (Illumina MiSeq). International Biodeterioration and Biodegradation 131: 51-59.

Lannon, S. M. R., Adams Waldorf, K. M., Fiedler, T., Kapur, R. P., Agnew, K., Rajagopal, L., … and Fredricks, D. N. 2019. Parallel detection of lactobacillus and bacterial vaginosis-associated bacterial DNA in the chorioamnion and vagina of pregnant women at term. Journal of Maternal-Fetal and Neonatal Medicine 32: 2702-2710.

Laroche, M., Bérenger, J. M., Gazelle, G., Blanchet, D., Raoult, D. and Parola, P. 2018. Corrigendum: MALDI-TOF MS protein profiling for the rapid identification of chagas disease triatomine vectors and application to the triatomine fauna of French Guiana. Parasitology 145: 676.

Lebeer, S., Bron, P. A., Marco, M. L., Van Pijkeren, J.-P., O'Connell Motherway, M., Hill, C., … and Klaenhammer, T. 2018. Identification of probiotic effector molecules: Present state and future perspectives. Current Opinion in Biotechnology 49: 217-223.

Lengkey, H. A. W., Balia, R. L., Siwi, J. A., Tasbac, B. A. and Togoe, I. 2017. Isolation and Identification of *Lactobacillus* bacteria from culled hens meat for meat biopreservator. KnE Life Sciences 2(6): 518-528.

Liu, D.-M., Huang, Y.-Y. and Liang, M.-H. 2022. Analysis of the probiotic characteristics and adaptability of *Lactiplantibacillus plantarum* DMDL 9010 to gastrointestinal environment by complete genome sequencing and corresponding phenotypes. LWT – Food Science and Technology 158: 113129.

Luo, Y., Ma, B. C., Zou, L. K., Cheng, J. G., Cai, Y. H., Kang, J. P., … and Xiao, J. J. .2012. Identification and characterisation of lactic acid bacteria from forest musk deer feces. African Journal of Microbiology Research 6: 5871-5881.

Maikhan, H. K. and Amin, S. M. M. 2017. Isolation, biochemical identification and antioxidant activity of locally isolated *Lactobacillus* spp. in Garmian area. Eurasian Journal of Science and Engineering 3: 128-136.

Maldonado, N. C., Ficoseco, C. A., Mansilla, F. I., Melián, C., Hébert, E. M., Vignolo, G. M. and Nader-Macías, M. E. F. 2018. Identification, characterisation and selection of autochthonous lactic acid bacteria as probiotic for feedlot cattle. Livestock Science 212: 99-110.

Masalam, M. S., Bahieldin, A., Alharbi, M. G., Al-Masaudi, S., Al-Jaouni, S. K., Harakeh, S. M. and Al-Hindi, R. R. 2018. Isolation, molecular characterization and probiotic potential of lactic acid bacteria in Saudi raw and fermented milk. Evidence-Based Complementary and Alternative Medicine 2018: 7970463.

Mathialagan, M., Johnson Thangaraj Edward, Y. S., David, P. M. M., Senthilkumar, M., Srinivasan, M. R. and Mohankumar, S. 2018. Isolation, characterisation and identification of probiotic lactic acid bacteria (LAB) from honey bees. International Journal of Current Microbiology and Applied Sciences 7: 894-906.

Mazhar, S. F., Afzal, M., Almatroudi, A., Munir, S., Ashfaq, U. A., Rasool, M., … and Khurshid, M. 2020. The prospects for the therapeutic implications of genetically engineered probiotics. Journal of Food Quality 2020: 9676452.

Nacef, M., Chevalier, M., Chollet, S., Drider, D. and Flahaut, C. 2017. MALDI-TOF mass spectrometry for the identification of lactic acid bacteria isolated from a French cheese: The Maroilles. International Journal of Food Microbiology 247: 2-8.

Nami, Y., Haghshenas, B. and Khosroushahi, A. Y. 2018. Molecular identification and probiotic potential characterisation of lactic acid bacteria isolated from human vaginal microbiota. Advanced Pharmaceutical Bulletin 8: 683-695.

Nami, Y., Haghshenas, B., Haghshenas, M. and Khosroushahi, A. Y. 2015. Antimicrobial activity and the presence of virulence factors and bacteriocin structural genes in *Enterococcus faecium* CM33 isolated from ewe colostrum. Frontiers in Microbiology 6: 782.

Nath, S., Sikidar, J., Roy, M. and Deb, B. 2020. *In vitro* screening of probiotic properties of *Lactobacillus plantarum* isolated from fermented milk product. Food Quality and Safety 4: 213-223.

Noohi, N., Papizadeh, M., Rohani, M., Talebi, M. and Pourshafie, M. R. 2021. Screening for probiotic characters in lactobacilli isolated from chickens revealed the intra-species diversity of *Lactobacillus brevis*. Animal Nutrition 7: 119-126.

Oh, N. S., Joung, J. Y., Lee, J. Y. and Kim, Y. 2018. Probiotic and anti-inflammatory potential of *Lactobacillus rhamnosus* 4B15 and *Lactobacillus gasseri* 4M13 isolated from infant feces. PLoS One 13(2): e0192021.

Ozen, M. and Dinleyici, E. 2015. The history of probiotics - The untold story. Beneficial Microbes 6(2): 159-165.

Pelinescu, D. R., Sasarman, E., Chifiriuc, M. C., Stoica, I., Nohit, A. M., Avram, I., … and Dimov, T. V. 2009. Isolation and identification of some *Lactobacillus* and *Enterococcus* strains by a polyphasic taxonomical approach. Romanian Biotechnological Letters 14: 4225-4233.

Pérez Montoro, B., Benomar, N., Caballero Gómez, N., Ennahar, S., Horvatovich, P., Knapp, C. W., … and Abriouel, H. 2018. Proteomic analysis of *Lactobacillus pentosus* for the identification of potential markers involved in acid resistance and their influence on other probiotic features. Food Microbiology 72: 31-38.

Pingault, N. M., Lehmann, D., Bowman, J. and Riley, T. V. 2007. A comparison of molecular typing methods for *Moraxella catarrhalis*. Journal of Applied Microbiology 103: 2489-2495.

Plengvidhya, V., Breidt, F. and Fleming, H. P. 2004. Use of RAPD-PCR as a method to follow the progress of starter cultures in sauerkraut fermentation. International Journal of Food Microbiology 93: 287-296.

Pogačić, T., Kelava, N., Zamberlin, Š., Dolenčić-Špehar, I. and Samaržija, D. 2010. Methods for culture-independent identification of lactic acid bacteria in dairy products. Food Technology and Biotechnology 48: 3-10.

Poluektova, E. U., Yunes, R. A., Epiphanova, M. V., Orlova, V. S. and Danilenko, V. N. 2017. The *Lactobacillus rhamnosus* and *Lactobacillus fermentum* strains from human biotopes characterised with MLST and toxin-antitoxin gene polymorphism. Archives of Microbiology 199: 683-690.

Postollec, F., Falentin, H., Pavan, S., Combrisson, J. and Sohier, D. 2011. Recent advances in quantitative PCR (qPCR) applications in food microbiology. Food Microbiology 28: 848-861.

Reuben, R. C., Roy, P. C., Sarkar, S. L., Alam, R. U. and Jahid, I. K. 2019. Isolation, characterisation, and assessment of lactic acid bacteria toward their selection as poultry probiotics. BMC Microbiology 19: 253.

Riaz Rajoka, M. S., Mehwish, H. M., Fang, H., Padhiar, A. A., Zeng, X., Khurshid, M., … and Zhao, L. 2019. Characterisation and antitumor activity of exopolysaccharide produced by *Lactobacillus kefiri* isolated from Chinese kefir grains. Journal of Functional Foods 63: 103588.

Riaz Rajoka, M. S., Mehwish, H. M., Siddiq, M., Haobin, Z., Zhu, J., Yan, L., … and Shi, J. 2017. Identification, characterisation, and probiotic potential of *Lactobacillus rhamnosus* isolated from human milk. LWT – Food Science and Technology 84: 271-280.

Riaz Rajoka, M. S., Mehwish, H. M., Zhang, H., Ashraf, M., Fang, H., Zeng, X., … and He, Z. 2020a. Antibacterial and antioxidant activity of exopolysaccharide mediated silver nanoparticle synthesised by *Lactobacillus brevis* isolated from Chinese *koumiss*. Colloids and Surfaces B - Biointerfaces 186: 110734.

Riaz Rajoka, M. S., Wu, Y., Mehwish, H. M., Bansal, M. and Zhao, L. 2020b. *Lactobacillus exopolysaccharides*: New perspectives on engineering strategies, physiochemical functions, and immunomodulatory effects on host health. Trends in Food Science and Technology 103: 36-48.

Roselli, M., Schifano, E., Guantario, B., Zinno, P., Uccelletti, D. and Devirgiliis, C. 2019. *Caenorhabditis elegans* and probiotics interactions from a prolongevity perspective. International Journal of Molecular Sciences 20: 5020.

Rothberg, J. M. and Leamon, J. H. 2008. The development and impact of 454 sequencing. Nature Biotechnology 26: 1117-1124.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J. M. and Bressollier, P. 2013. An overview of the last advances in probiotic and prebiotic field. LWT – Food Science and Technology 50: 1-16.

Sánchez-Juanes, F., Teixeira-Martín, V., González-Buitrago, J. M., Velázquez, E. and Flores-Félix, J. D. 2020. Identification of species and subspecies of lactic acid bacteria present in Spanish cheeses type "Torta" by MALDI-TOF MS and *pheS* gene analyses. Microorganisms 8(2): 301.

Sarkar, S. 2013. Microbiological considerations for probiotic supplemented foods. International Journal of Microbiology and Advanced Immunology 1: 1-5.

Satokari, R. M., Vaughan, E. E., Smidt, H., Saarela, M., Mättö, J. and de Vos, W. M. 2003. Molecular approaches for the detection and identification of bifidobacteria and lactobacilli in the human gastrointestinal tract. Systematic and Applied Microbiology 26: 572-584.

Scariot, M. C., Venturelli, G. L., Prudêncio, E. S. and Arisi, A. C. M. 2018. Quantification of *Lactobacillus paracasei* viable cells in probiotic yoghurt by propidium monoazide combined with quantitative PCR. International Journal of Food Microbiology 264: 1-7.

Shannon, S., Kronemann, D., Patel, R. and Schuetz, A. N. 2018. Routine use of MALDI-TOF MS for anaerobic bacterial identification in clinical microbiology. Anaerobe 54: 191-196.

Sharma, A., Kaur, J., Lee, S. and Park, Y. S. 2017. Molecular discrimination of *Lactobacillus brevis* strains isolated from food products in South Korea using multilocus sequence typing. LWT – Food Science and Technology 86: 337-343.

Sharma, P., Anand, S., Tomar, S. K. and Goswami, P. 2018. Antibiotic susceptibility of *Lactobacillus* sp. isolated from commercial probiotic products by E-test strip method. International Journal of Current Microbiology and Applied Sciences 7: 3499-3517.

Soofy, B. Y. and Ahmed, K. D. 2019. Isolation and identification of *Lactobacillus* using biochemical and molecular methods. Iraqi Journal of Biotechnology 18: 116-125.

Soon, W. W., Hariharan, M. and Snyder, M. P. 2013. High-throughput sequencing for biology and medicine. Molecular Systems Biology 9: 640.

Stefanis, C., Mantzourani, I., Plessas, S., Alexopoulos, A., Galanis, A., Bezirtzoglou, E., … Varzakas, T. 2016. Reviewing classical and molecular techniques regarding profiling of probiotic character of microorganisms. Current Research in Nutrition and Food Science Journal 4: 27-47.

Tajabadi, N., Mardan, M., Saari, N., Mustafa, S., Bahreini, R. and Manap, M. Y. A. 2013. Identification of *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus fermentum* from honey stomach of honeybee. Brazilian Journal of Microbiology: 44: 717-722.

Taye, Y., Degu, T., Fesseha, H. and Mathewos, M. 2021. Isolation and identification of lactic acid bacteria from cow milk and milk products. The Scientific World Journal 2021: 4697445.

Thakur, N., Sharma, C., Rokana, N., Pal Singh, B., Devidas Gulhane, R., Kumar Mishra, S., … and Panwar, H. 2018. *In vitro* assessment of antibiotic resistance pattern among *Lactobacillus* strains isolated from goat milk. International Journal of Current Microbiology and Applied Sciences 7: 2108-2116.

Tilahun, B., Tesfaye, A., Muleta, D., Bahiru, A., Terefework, Z. and Wessel, G. 2018. Isolation and molecular identification of lactic acid bacteria using 16s rRNA genes from fermented *Teff* (*Eragrostis tef* (Zucc.)) dough. International Journal of Food Science 2018: 8510620.

Toualbia, M., Bouras, A. E. K. D., Koiche, M. and Kerkoud, M. 2018. Isolation, identification and characterisation of *Lactobacillus plantarum* from camel milk and its antagonist effect against diarrheal bacteria. Emirates Journal of Food and Agriculture 30: 283-287.

Van Belkum, A., Welker, M., Erhard, M. and Chatellier, S. 2012. Biomedical mass spectrometry in today's and tomorrow's clinical microbiology laboratories. Journal of Clinical Microbiology 50: 1513-1517.

Veloo, A. C. M., Friedrich, A. W., Jean-Pierre, H., Jean-Pierre, H., Justesen, U. S., Morris, T., … and Friedrich, A. W. 2017. A multi-center ring trial for the identification of anaerobic bacteria using MALDI-TOF MS. Anaerobe 48: 94-97.

Wang, X., Wang, W., Lv, H., Zhang, H., Liu, Y., Zhang, M., … and Tan, Z. 2020. Probiotic potential and wide-spectrum antimicrobial activity of lactic acid bacteria isolated from infant feces. Probiotics and Antimicrobial Proteins 13: 90-101.

Wassie, M. and Wassie, T. 2016. Isolation and identification of lactic acid bacteria from raw cow milk. International Journal of Advanced Research in Biological Sciences 3: 44-49.

Yang, H., Liu, T., Zhang, G., Chen, J., Gu, J., Yuan, L. and He, G. 2017. Genotyping of *Lactobacillus sanfranciscensis* isolates from Chinese traditional sourdoughs by multilocus sequence typing and multiplex RAPD-PCR. International Journal of Food Microbiology 258: 50-57.

Youssef, M., Ahmed, H. Y., Zongo, A., Korin, A., Zhan, F., Hady, E., … and Li, B. 2021. Probiotic supplements: Their strategies in the therapeutic and prophylactic of human life-threatening diseases. International Journal of Molecular Sciences 22(20): 11290.

Yunita, D. and Dodd, C. E. R. 2018. Microbial community dynamics of a blue-veined raw milk cheese from the United Kingdom. Journal of Dairy Science 101: 4923-4935.

Zhang, F., Gao, J., Wang, B., Huo, D., Wang, Z., Zhang, J. and Shao, Y. 2018. Whole-genome sequencing reveals the mechanisms for evolution of streptomycin resistance in *Lactobacillus plantarum*. Journal of Dairy Science 101: 2867-2874.

Zhang, W., Wang, J., Zhang, D., Liu, H., Wang, S., Wang, Y. and Ji, H. 2019. Complete genome sequencing and comparative genome characterization of *Lactobacillus johnsonii* ZLJ010, a potential probiotic with health-promoting properties. Frontiers in Genetics 10: 812.

Zhuravleva, D. E., Iskhakova, Z. I., Ozhegov, G. D., Gogoleva, N. E., Khusnutdinova, D. R., Shagimardanova, E. I., … and Kayumov, A. R. 2020. Complete genome sequence of *Lactobacillus hilgardii* LMG 7934, carrying the gene encoding for the novel PII-like protein PotN. Current Microbiology 77: 3538-3545.