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# Lactic acid bacteria: isolation–characterization approaches and industrial applications

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## ABSTRACT

The current state-of-art research pertaining to lactic acid bacteria (LAB) calls for the screening and isolation of robust LAB strains to achieve holistic exploitation of LAB and their metabolites of marketable importance. Hence it is imperative to comprehend LAB sources, growth requisites, isolation and characterization strategies necessary for featured cataloging and appropriate culturing. This review comprehensively describes various growth media and biomasses used for supporting LAB sustenance, assay procedures needed for the isolation and characterization of LAB strains, and their application in diverse sectors. The various industrial patents and their summarized claims about novel LAB strains isolated and identified, methods and media (used for detection/screening, isolation, adaptation, culturing, preservation, growth improvement), the techniques and/or methodologies supporting LAB fermentation, and applications of produced industrial metabolites in various market scenarios are detailed.

## KEYWORDS

Application; isolation; culture; industrial; lactic acid bacteria; media; sources

## Introduction

Lactic acid bacteria (LAB) are gram-positive, non-sporulating, acid-tolerant, microaerophilic, bacilli/cocci-shaped microorganisms of the *Lactobacillales* bacterial order (Motta and Gomes 2015; Florou-Paneri, Christaki, and Bonos 2012; Bintsis 2018), including genera like *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* (fundamental LAB group), *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (peripheral LAB group) (Petrova and Petrov 2020). LAB possess a unique metabolism of transforming macromolecular carbohydrates/polysaccharides in foods/feedstocks into value-added chemicals, commodities, and health products (Wang et al. 2021; Papagianni 2012; Hatti-Kaul et al. 2018). LAB chiefly find applications in food industries as starters and fermentation agents (Rama et al. 2019). Food-related LAB species generally belong to genera of the fundamental LAB group, *Enterococcus*, and *Weissella* (Zheng et al. 2020). Based upon technological properties, LAB are classified as homofermentative (lactic acid is the dominant product formed) and heterofermentative (acetate, carbon dioxide, and ethanol are formed as products); the former function as starter cultures promoting rapid acidification of foods, while the latter serve as non-starter cultures for promoting sensorial food qualities owing to their glycolytic/lipolytic/proteolytic activities (Motta and Gomes 2015; Gatti et al. 2014; Guarrasi et al. 2017). Fermented foods produced using LAB include table olives,

carrot puree, fermented fruit juice, fermented shallot roots, leek, fermented small round aubergines, pickled cabbage, fermented banana leaves, mashed tomatoes, sourdough, meat sausages, raw fermented fish/pork (nem Trey/chua), cheese, yogurt, etc. (Bintsis 2018; Hurtado et al. 2012; Juvonen et al. 2015; Yien Ong et al. 2012; Wouters et al. 2013; Alan and Yildiz 2021; Gobetti et al. 2016; Nediani et al. 2017; Lorn et al. 2021; Domingos-Lopes et al. 2017). Due to the production of various metabolites, LAB cultures find applications as industrial probiotics, food quality/flavor enhancers, bio-preservatives, and antimicrobials, that ensure food safety by thwarting the growth of food-borne pathogens (Bintsis 2018; Hatti-Kaul et al. 2018; Geria and Caridi 2014).

Fermentation of LAB strains results in the production of several products and metabolites: Lactic acid (LA), LA derivatives like poly lactic acid, organic acids (acetic acid, phenyl lactic acid, butyric acid, formic acid, propionic acid, succinic acid), amines, bacteriocins, antifungal and antibacterial peptides, short-chain fatty acids, vitamins, extracellular polysaccharides,  $\gamma$ -aminobutyric acids, flavor precursors, antioxidant substances, diacetyls, acetoin, polyols, hydrogen peroxides, etc. (Wang et al. 2021; Rajanikar et al. 2021; Zheng et al. 2011; Egan et al. 2016; Pandey et al. 2021). LA is the chief commercial and most demanded product of LAB fermentation, and can be produced cost-effectively using renewable biomass substrates procured from agro-food industries like milk/cheese whey, pear processing residues, potato/tomato pomace (Costa et al. 2020). The production

of LA globally has been estimated to be about 270,000 tonnes/year owing to its various applications (Alexandri et al. 2019), its global market size was valued as USD 2.7 billion in 2020 and will reach USD 5.02 billion by 2028, with expansion possibility at 8.0% CAGR (compound annual growth rate) from 2021–2028 (Lactic Acid Market 2021; Global Lactic Acid Market 2021). The cost price for LA in 2011 ranged from USD 1.30–2.30/kg, however, it has increased recently to USD 3.0–4.0/kg (González et al. 2007; Ahmad, Banat, and Taher 2020). Other marketable products of LAB fermentation are poly lactic acid and polyhydroxy-alkanoates used as biodegradable plastics replacing synthetic plastics (Naser, Deiab, and Darras 2021), bacteriocins (nisin, pediocin, pediocin, lactococin, acidocin, helveticin) used as food preservatives (De Vuyst and Leroy 2007), lipoteichoic acids used for preventive treatment of oral infectious diseases (due to anti-biofilm potential) and treatment of colitis and immunomodulation (due to anti-inflammatory properties) (Kim et al. 2019; Lebeer, Claes, and Vanderleyden 2012; Ryu et al. 2009), phenyl lactic and hydroxy-phenyl lactic acids used as antimicrobial agents, food flavor/quality enhancers, and bio-preservatives (Valerio et al. 2004; Chaudhari 2016), miscellaneous compounds like flavors, antimicrobials, pharmaceuticals, texturizing compounds, vitamins, sweeteners, and nutraceuticals (Mays and Nair 2018).

Hereby, as LAB and their fermentation products are of marketable importance and are apposite for numerous industrial purposes, it is imperative to comprehend LAB sources, growth requisites, isolation and characterization methodologies necessary for featured cataloging and appropriate culturing. This review comprehensively describes various growth media and biomasses used for supporting LAB sustenance, assay procedures needed for isolation, identification and characterization of LAB strains, and their application in diverse sectors.

### Isolation of lactic acid bacteria (LAB)

LAB are versatile entities that are strewn all over the planet across a plethora of diverse and manifold ecosystems. They have dynamically interacted and coevolved along with both plant and animal kingdoms, by exhibiting multifaceted ecological and/or functional properties established through several modes of nutrition viz., mutualism, symbiosis, commensalism, and parasitism (George et al. 2018). They serve as beneficial microbial cell factories to produce probiotics chiefly by acting as starter cultures, hence it's imperative to explore novel and/or unknown niches for isolating robust and adaptive LAB strains (Ruiz Rodríguez et al. 2019). LAB can be isolated from myriad sources viz., traditional or homemade pickles, kimchi, dry-cured meats; silage of plants; milk, milk products, cheese; fermented beverages, jams, fishes; fruits like cupuaçu, strawberry, cherry tomato, blueberry, blackberry, cherry, apple, and flowers; intestinal tracts of honey bee, Mediterranean trout, wild boar; seed meal; ferments of cocoa beans, cricket powder, cassava, cereal foods, and sourdoughs, etc. Table 1 enlists a complete compendium of versatile sources for isolating LAB as evidenced by recent research. Both novel and traditional plant/animal

materials can serve as promising sources for the isolation of LAB.

The de Mann Rogosa Sharpe (MRS) agar medium supplemented with 1% CaCO<sub>3</sub> is commonly and widely used as defined growth medium developed for culturing of LAB, as it can provide sustenance and support the growth of almost all LAB genera viz., *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, *Weissella*, when it's made selective through reduction of the pH to 5.7 and addition of sorbic acid (0.14%). It is chemically comprised of ammonium citrate, beef extract, di-potassium hydrogen orthophosphate, glucose, magnesium sulfate, manganese(II) sulfate, sodium acetate, sorbitan monooleate, casein tryptic digest, yeast extract, and agar added with distilled/deionized water. MRS agar can give a perfect colony count and determine the characteristic size and morphologies of LAB strain colonies, whilst the other microbes can be excluded through specific colonial appearance and confirmation tests (Monika et al. 2017; Corry, Curtis, and Baird 2003). Samples from procured sources (like traditional pickles or cured meats) are diluted in saline, plated upon the MRS agar plates, and incubated at 30–37°C for 2–3 days in anaerobic conditions to induce growth of LAB, such grown colonies are detected by a characteristic clear zone of hydrolysis around (Monika et al. 2017; Zeng et al. 2020; Yi et al. 2020; Won et al. 2020). Plants materials viz., fruits (Li et al. 2021b; das Neves Selis et al. 2021; Fevria and Hartanto 2019), flowers (Ruiz Rodríguez et al. 2019; Xia et al. 2021), silage (*Lolium perenne*, *Sorghum dochna*, *Zea mays* and *Medicago sativa* L) (Peng et al. 2021), *Zanthoxylum bungeanum* seed meal (Li et al. 2021a), rye flour sourdough (Bartkiene et al. 2019; Revuelta, Ledesma-Amaro, and Jiménez 2016), African fan palm (*Borassus aethiopicum* Mart. Sap) (Oumarou et al. 2021), and animal sources viz., fishes (*Channa striata*, *Puntius filamentosus*, *Oreochromis mossambicus*, *Cirrhinus mrigala*, *Rasbora daniconius*) (Govindaraj et al. 2021), intestinal tracts of honey bees (Elzeini et al. 2021) and wild boars (Li et al. 2020) have reportedly been used as sources for isolating LAB strains using MRS agar/broth medium. Unwanted growth of yeasts and fungi can be suppressed by supplementing MRS agar plates with cycloheximide (0.01% v/v) (Elzeini et al. 2021) or Nystatin (100 mg/L) (Oumarou et al. 2021), to promote LAB growth selectively. MRS agar plates can be supplemented with bromocresol purple which can promote the distinct visibility of pure LAB colonies by forming visible discoloration rings around (Li et al. 2021b).

Usage of other media for selective LAB isolation has also been suggested based upon the specific biochemical requirements of the particular LAB species. *Lactobacillus*, *Lactococcus*, and *Streptococcus* genera isolated from milk/milk products (raw milk, cheese, and yogurt), could be grown upon selective media like MRS agar, M17 agar, and Rogosa SL agar respectively (Kostelac et al. 2021; Taye et al. 2021). Replacement of glucose of MRS medium with starch effectively promoted the growth of *Enterococcus* and *Lactobacillus* strains from fermented food samples. *Lactiplantibacillus plantarum* subsp. *plantarum* 445 was isolated from fermented cereals using MRS broth wherein the

**Table 1.** Compendium showing isolation of lactic acid bacteria from various sources from diverse environments, selective growth medium supporting isolation, and applications.

S. No.	Source	Lactic acid bacteria	Growth medium	Application	References
1	Indian pickles	<i>Enterococcus faecalis</i> , <i>Lactobacillus plantarum</i> , <i>Lactococcus lactis</i> , <i>Leuconostoc mesenteroides</i> , <i>Pediococcus pentosaceus</i> <i>Enterococcus</i> sp.,	MRS agar	Probiotics	(Monika et al. 2017)
2	Chinese homemade pickles	<i>Lactobacillus plantarum</i> , <i>Lactobacillus paraplantarum</i> , <i>Lactobacillus farsiminus</i> , <i>Lactobacillus futsaii</i> , <i>Lactobacillus formosensis</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus buchneri</i> , <i>Lactobacillus parabuchneri</i> , <i>Lactobacillus fermentum</i> , <i>Lactococcus taiwanensis</i> , <i>Leuconostoc mesenteroides</i> , <i>Weissella</i> sp., <i>Enterococcus</i> sp., <i>Pediococcus acidilactici</i>	MRS agar	Probiotics, Food preservatives	(Zeng et al. 2020)
3	Chinese pickles	<i>Lactobacillus buchneri</i> GBS3	MRS broth	Antimicrobial	(Guan et al. 2019)
4	Chinese traditional pickles	<i>Lactobacillus</i> spp. ZX1	MRS broth	Antimicrobial	(Xu, Zhang, and Ni 2016)
5	Chinese fermented vegetable- Stinky xiancaigeng	<i>Lactiplantibacillus plantarum</i> CXG9	MRS broth	Antibacterial	(Zhang et al. 2022)
6	Pickled vegetables: sour bamboo shoots, salted tomatoes, pickled onions, salted figs	<i>Lactobacillus</i> sp. MX3.2	MRS broth	Antibacterial, food preservative	(Dung et al. 2021)
7	Dry cured meats and pickles	<i>Lactobacillus plantarum</i> , <i>Lactobacillus pentosus</i> , <i>Lactobacillus casei</i> , <i>Staphylococcus</i> sp., <i>Staphylococcus hominis</i> , <i>Pediococcus ethanolidurans</i> , <i>Weissella confuse</i> , <i>Weissella paramesenteroides</i>	MRS agar	Production of bacteriocins with broad antibacterial activity	(Yi et al. 2020)
8	Fermented beverage and finfish	<i>Leuconostoc</i> spp., <i>Enterococcus</i> spp., <i>Weissella</i> spp., <i>Pediococcus</i> spp.	MRS agar	natural food preservative and antimicrobial agent	(Dejene, Regasa Dadi, and Tadesse 2021)
9	Fermented food	<i>Lactobacillus plantarum</i> KP3, <i>Lactobacillus plantarum</i> KP4, <i>Leuconostoc mesenteroides</i> K8, <i>Leuconostocparacasei paracasei</i> DP2	MRS broth, Porphyra residues	Biopreservative, improving <i>Porphyra</i> -based bioeconomy	(Huang et al. 2021)
10	Fermented shallot roots, fish, pork, banana leaves	<i>Lactiplantibacillus plantarum</i> C022-2B, <i>Lactiplantibacillus plantarum</i> C022-3B, <i>Lactiplantibacillus plantarum</i> V0023-4B2, <i>Limosilactobacillus (L.) fermentum</i> V013-1A	MRS agar/broth	Aromatic starters in fermented vegetables	(Lorn et al. 2021)
11	Kimchi	<i>Lactobacillus sakei</i> ADM14	MRS agar	Therapeutic supplement with anti-adipogenic effect and as probiotic	(Won et al. 2020)
12	Sourdough of rye flour	<i>Enterococcus pseudoavium</i> 242, <i>Lactobacillus brevis</i> 173, <i>Lactobacillus casei</i> 210, <i>Lactobacillus coryniformis</i> 71, <i>Lactobacillus curvatus</i> 51, <i>Lactobacillus farraginis</i> 206, <i>Lactobacillus paracasei</i> 244, <i>Lactobacillus plantarum</i> 122, <i>Lactobacillus uvarum</i> 245, <i>Leuconostoc mesenteroides</i> 225, <i>Pediococcus acidilactici</i> 29, <i>Pediococcus pentosaceus</i> 183	MRS agar	agri-food industries	(Bartkiene et al. 2019)

(Continued)

Table 1. (Continued)

S. No.	Source	Lactic acid bacteria	Growth medium	Application	References
13	Milk	<i>Lactiplantibacillus plantarum</i> UM55	MRS broth	Antifungal effect activity	(Guimarães, Venancio, and Abrunhosa 2018)
14	Equid milk	<i>Lactobacillus plantarum</i> M2, KO9	MRS agar	Probiotic and anti-inflammatory properties	(Kostelac et al. 2021)
15	Fermented rose jam	<i>Pediococcus pentosaceus</i> MP3, MP11, MP13, MP16, MY8	MRS agar	Probiotic, starter culture for traditional fermentation and functional foods.	(Xia et al. 2021)
16	Cricket Powder's ferments	<i>Lactiplantibacillus plantarum</i> CR L1, <i>Weissella confusa</i> CR L2, <i>Latilactobacillus curvatus</i> CR L13, <i>Lactococcus garvieae</i> CR L14, <i>Lactococcus garvieae</i> CR L31, <i>Latilactobacillus sakei</i> CR L15, <i>Enterococcus durans</i> CR L36	MRS agar	Potential starters for cricket-wheat bread production	(Galli et al. 2020)
17	Intestinal tracts of honey bees	<i>Enterococcus faecalis</i> MG890204, <i>Enterococcus faecalis</i> KX073783, <i>Enterococcus faecalis</i> EU594564, <i>Lactobacillus brevis</i> MH191230, <i>Lactobacillus casei</i> KT273339	MRS agar	Probiotic in honey bees, natural food preservatives	(Elzeini et al. 2021)
18	Human intestine	<i>Limosilactobacillus reuteri</i> R29	MRS broth + Phenyl-alanine/glycerol	Antifungal activity	(Schmidt et al. 2018)
19	Cherry, tomato, Apple, blueberry, blackberry	<i>Lactobacillus plantarum</i> LSJ-TY-HYB-T9, <i>Lactobacillus plantarum</i> LSJ-TY-HYB-T7, <i>Lactobacillus fermentum</i> LSJ-TY-HYB-C22, <i>Lactobacillus fermentum</i> LSJ-TY-HYB-L16	MRS agar	Production of fermented fruit juices	(Li et al. 2021b)
20	Tomato carrot pineapple cheese	<i>Lactobacillus plantarum</i> POM1, <i>Lactobacillus plantarum</i> C1, <i>Lactobacillus plantarum</i> 1LE1, <i>Lactobacillus plantarum</i> 285; <i>Lactobacillus rhamnosus</i> 2178, 2140, 2360, 1473,1019; <i>Lactobacillus casei</i> 2246, 2306, 2057, 2107; <i>Lactobacillus paracasei</i> 4186	MRS agar/broth	Starters for cherry juice fermentation	(Ricci et al. 2019)
21	ZBM seed meal	<i>Lactobacillus paracasei</i> KQ, <i>Lactobacillus acidipiscis</i> LCM	MRS agar	Processing ZBM as feed additive.	(Li et al. 2021a)
22	Silage	<i>Lactobacillus rhamnosus</i> BDy3-10, <i>Lactobacillus buchneri</i> TSy1-3	MRS agar	Improve fermentation quality of alfalfa	(Peng et al. 2021)
23	Silage	<i>Pediococcus acidilactici</i> CRL1753	MRS broth/ Dough	Bio-preservative, starter in bread making	(Bustos, Font de Valdez, and Gerez 2018)
24	African fan palm	<i>Enterococcus gilvus</i> BL30; <i>Leuconostoc mesenteroides</i> BL50, BL58; <i>Enterococcus</i> sp. BL64	MRS agar	Sap fermentation, biotechnological applications.	(Oumarou et al. 2021)
25	Grapes, must, grape wine	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus alimentarius</i> , <i>Lactobacillus amylolyticus</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus coryniformis</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>delbrueckii</i> , <i>Lactobacillus fermentum</i> , <i>Lactobacillus fructivorans</i> , <i>Lactobacillus hilgardii</i> , <i>Lactobacillus nageli</i> , <i>Lactobacillus oris</i> , <i>Lactobacillus parabuchneri</i> , <i>Lactobacillus paracasei</i> subsp. <i>paracasei</i> , <i>Lactobacillus paracasei</i> subsp. <i>tolerans</i> , <i>Lactobacillus pentosus</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus saerimneri</i> , <i>Lactobacillus sakei</i> , <i>Lactococcus lactis</i> , <i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> , <i>Pediococcus acidilactici</i> , <i>Pediococcus pentosaceus</i> , <i>Weissella uvarum</i>	MRS agar	Brewing and improvement of wine quality	(Kačániová et al. 2020)

(Continued)

Table 1. (Continued)

S. No.	Source	Lactic acid bacteria	Growth medium	Application	References
26	Brazilian cupuaçu (fruit)	<i>Lactobacillus casei</i> Lc24; <i>Lactobacillus fermentum</i> Lf38, Lf47; <i>Lactobacillus plantarum</i> Lp81, Lp90	MRS agar or broth	Probiotic properties against genital pathogens	(das Neves Selis et al. 2021)
27	Gastrointestinal tract of wild boar	<i>Lactobacillus mucosae</i> M6-29, <i>Lactobacillus salivarius</i> M2-71, <i>Enterococcus hirae</i> M5-8, <i>Enterococcus durans</i> M2-3, <i>Enterococcus faecium</i> M6-29	MRS broth	Development of animal feed additives	(Li et al. 2020)
29	Food matrix	<i>Lactiplantibacillus plantarum</i> UFG121	MRS broth	Antimicrobial	(Russo et al. 2017)
30	Food matrix	<i>Lactobacillus brevis</i> ŁOCK 0944; <i>Lactobacillus casei</i> ŁOCK 0906, ŁOCK 1020; <i>Lactobacillus delbrueckii</i> ŁOCK 0987	MRS agar/broth	Anticandidal activity, food preservatives	(Lipinska-Zubrycka et al. 2020)
31	Cow milk and milk products	<i>Lactobacillus</i> spp., <i>Lactococcus</i> spp., <i>Streptococcus</i> spp., <i>Leuconostoc</i> spp., <i>Pediococcus</i> spp., <i>Bifidobacteria</i> spp.	MRS agar, M17 agar, Rogasa SL agar	Probiotics	(Taye et al. 2021)
32	Home-made curd	<i>Limosilactobacillus fermentum</i> C14	MRS broth	Long-term storage of post-packaged bread, Cell suspension as a bio-preservative	(Barman et al. 2017)
33	strawberry	<i>Lactobacillus bulgaricus</i>	Nutrient agar, Oxoid MRS medium	Digestive aid, regulating immune system, lipid metabolism, Antibiotic	(Fevria and Hartanto 2019)
34	Fermented cereal foods	<i>Lactobacillus plantarum</i> 445	MRS broth (glucose replaced by soluble starch)	Cereal-based probiotic products	(Xu et al. 2020)
35	Andean fermented vegetal foods (chicha, tocosh)	<i>Lactobacillus fermentum</i> T3M3, <i>Lactobacillus plantarum</i> M5MA1, <i>Lactobacillus plantarum</i> M9MM1, <i>Leuconostoc mesenteroides</i> T1M3	MRS broth	Antimicrobial, Food preservatives	(Yépez et al. 2017)
36	Sourdough	<i>Lactiplantibacillus plantarum</i> 21B, 19A; <i>Lactobacillus fermentum</i> 18B; <i>Lactobacillus brevis</i> 18F	Defined medium, phenyl-pyruvic acid	Antimicrobial, food preservative	(Valerio et al. 2004; Valerio et al. 2016)
37	Fermented chia sourdough	<i>Lactococcus lactis</i> CH179, <i>Lactobacillus rhamnosus</i> CH34, <i>Weissella cibaria</i> CH28	MRS agar (glucose replaced by starch)	Novel and functional-starter cultures for production of gluten-free fermented baked foods	(Dentice Maidana et al. 2020)
38	Fruits and flowers	<i>Lactococcus lactis</i> subsp. <i>lactis</i> FN3-317, F-Cq1-484-2; <i>Weissella cibaria</i> FMy1-3, FMy2-19; <i>Weissella minor</i> G1-E-15, G1-E-16, G1-E-19, G1-E-21, G1-E-27; <i>Leuconostoc mesenteroides</i> FMy1-2, FMy1-4, FYPMr2-345, FYP-G1-1, FYP-G1-7, P3-60, F-Mr2-348; <i>Lactobacillus brevis</i> FCh-38, F-G2-29, F-G2-31; <i>Lactobacillus plantarum</i> G2-E-39; <i>Lactobacillus rhamnosus</i> H3-213; <i>Enterococcus casseliflavus</i> FMy2-26, P1-1; <i>Enterococcus mundtii</i> F30-Ch2-119; <i>Enterococcus faecium</i> F30-P1-154, F-Mr2-358; <i>Enterococcus durans</i> F-H2-428; <i>Fructobacillus fructosus</i> F-H2-401; <i>Fructobacillus tropaeola</i> F-H3-450	MRS agar, MRSf agar (MRS agar with 2% fructose replacing glucose)	Manufacture of fermented fruit-based products, design of novel functional foods	(Ruiz Rodríguez et al. 2019)
39	Goatling isolate	<i>Limosilactobacillus reuteri</i> K05	MRS supplemented with 1.0% D-glucose monohydrate, 0.1% L-phenylalanine, 1.0% glycerol	Antimicrobial, Immunomodulatory	(Greifová et al. 2017)

(Continued)

Table 1. (Continued)

S. No.	Source	Lactic acid bacteria	Growth medium	Application	References
40	Coffee beans	<i>Lacticasibacillus zeae</i> Y44	MRS broth supplemented with PPA and phenylalanine	Antimicrobial	(Yoo, Lim, and Yoon 2016)
41	Ezine Cheese	<i>Enterococcus lactis</i> PMD7	kanamycin aesculin azide agar	Probiotic, bacteriocinogenic	(Uymaz Tezel 2019)
42	Parmigiano Reggiano cheese	<i>Lactobacillus rhamnosus</i> 2360, <i>Lactobacillus plantarum</i> 285	MRS agar	Starters for Cherry Juice Fermentation	(Ricci et al. 2019)
43	Pecorino cheese	<i>Lactobacillus paracasei</i> 4186	MRS agar	Starters for Cherry Juice Fermentation	(Ricci et al. 2019)
44	Freshwater fishes	<i>Limosilactobacillus fermentum</i> URLP18; <i>Lactococcus lactis</i> URLA2; <i>Weissella cibaria</i> URLP4; <i>Enterococcus</i> sp. URLM3, URLC1, URLD9; <i>Enterococcus faecalis</i> URLB1	Nutrient agar, MRS agar	Probiotic, dietary supplement for freshwater aquaculture, antimicrobial	(Govindaraj et al. 2021)
45	Cocoa bean ferments	<i>Lactobacillus plantarum</i> LPBF30, LPBF35; <i>Pediococcus acidilactici</i> LPBF 66; <i>Pediococcus pentosaceus</i> ; <i>Bacillus subtilis</i> ; <i>Leuconostoc pseudomesenteroides</i>	Fructose-yeast extract-peptone agar/broth	Starter cultures inducing cocoa microbial activity, and improved fructose consumption	(Viesser et al. 2020)
46	Gastrointestinal tract of trout fish	<i>Carnobacterium maltaromaticum</i> L1, L2, L3, L5, L9, L11, L13, L14, L18, L20, B5, B7, M21C, M21CR, 2CR, 2ACR, 11V, 12V, 18V, 25V, 42V, 43V, 45V, 46V 1T, 2T, 3T, 4T; <i>Lactiplantibacillus plantarum</i> B2, 23V, 33V, 36V, 37V, 38V, 63V, 64V, 65V, 66V, 67V, 68V, 6T, 6V; <i>Lactobacillus acidophilus</i> 3V; <i>Lactiplantibacillus pentosus</i> L8; <i>Lactococcus lactis</i> L10, L12, L14B, L19, B6, B34; <i>Enterococcus faecalis</i> L4, 2V, 4V, 7V, 31V, 32V, 34V; <i>Vagococcus fluvialis</i> 15V; <i>Weissella paramesenteroides</i> 39V, 57V, 61V	MRS agar, M17 medium	Improving fish microbiome for disease control and boosting health.	(Iorizzo et al. 2021)

glucose component (1%) was replaced with soluble starch (Xu et al. 2020), several other strains of *Enterococcus*, *Lactococcus*, *Weissella*, and *Lactobacillus* were also isolated by replacing glucose component (0.5%) with starch in MRS agar (Dentice Maidana et al. 2020). 21 LAB strains belonging to the genera *Enterococcus*, *Fructobacillus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Weissella* were isolated from ripe fruits (custard apple, fig, khaki, guava, papaya, passion fruit, medlar, mulberry), and flowers (custard apple, medlar, papaya, passion fruit) using MRS agar and MRSf agar (MRS agar altered by replacing 2% glucose with fructose) (Ruiz Rodríguez et al. 2019).

*Enterococcus lactis* PMD74 was isolated from ezine cheese samples (diluted in Ringer's solution) where the supporting growth medium used was kanamycin aesculin azide (KAA) agar incubated at 37 °C for 2 days aerobically (Uymaz Tezel 2019). Strawberries (*Fragaria vesca*) serve as a source of LAB and also as a natural growth medium/substrate for nurturing LAB. In a research study undertaken using strawberries, colonies of *Lactobacillus delbrueckii* subsp. *bulgaricus* were isolated by (i) plating the strawberry tissue directly into nutrient agar, (ii) fermenting the strawberry tissue and

plating upon oxoid MRSA medium (Fevria and Hartanto 2019). *Pediococcus acidilactici* LPBF 66; *P. pentosaceus*; *Lactiplantibacillus plantarum* subsp. *plantarum* LPBF30, LPBF35; *Bacillus subtilis*; *Leuconostoc pseudomesenteroides*, were isolated from cocoa bean ferments using FYP (fructose-yeast extract-peptone) agar medium supplemented with 0.5% (w/v) CaCO<sub>3</sub> with incubation for a day at 30 °C (Viesser et al. 2020). Isolated and identified LAB strains can be preserved and sub-cultured for long term usage by storing in typical MRS agar/broth (containing 100 g skim milk, 5 g yeast extract, 10 g glucose, 10% v/v glycerol) (Ruiz Rodríguez et al. 2019) and Tryptone soya agar (90%) supplemented with MRS (10%) (Kačaniová et al. 2020), and maintained as stocks by freezing at -80 °C in cryoprotective media containing glycerol (30%) (Hwanhlem et al. 2017).

### LAB Identification and characterization methodologies

Identification of isolated LAB can be conducted through polyphasic approaches, taking into consideration dependant factors like the sample source, quantity of isolated strains,

genotypic, phenotypic, and biochemical features of strains. Regular protocols followed for clinical isolate identification use phenotypic tests, while food isolates and vegetable/fruit isolates demand molecular approaches and molecular typing methods respectively. Universal and well-established LAB identification by genotypic methods is 16S rRNA gene sequencing where conserved genes represent adequate variability serving as phylogenetic markers for genus and species level of identification. The sequenced nucleotides are analyzed for similarities and aligned for the construction of phylogenetic trees using programs like BLAST of NCBI, ClustalW, etc. (Di Cagno et al. 2013; Emerenini et al. 2013; Moraes et al. 2013).

Biochemical and physiological characterization of LAB isolates can be done by testing for cell/colony morphology and characteristics, gram staining affinity, catalase and oxidase production (Menconi et al. 2014), carbohydrate fermentation, tolerance to NaCl contents (1.5–10%), their growth at variable ranges of pH (3.0–8.5) and temperature (15–45°C), arginine hydrolysis, utilization of citrate (Monika et al. 2017), and lactic acid production (Ahire et al. 2021). The influence of pH, NaCl, and bile salts on the LAB strains can be assessed using microplates: 2 mL ( $10^8$  CFU/mL) of LAB strain cultured anaerobically in MRS broth (30°C, 24 hours) is centrifuged at 8000 rpm for 5 minutes, washed with 2 mL phosphate buffer twice, and obtained bacterial suspensions are tested for NaCl, acid, and bile salt tolerances. The appropriate pH values of MRS medium are maintained with pH 2 and pH 3 using HCl with a control set at pH 6.5, tolerance to NaCl is inspected with 2, 4, 6, 8% levels of sodium chloride, and tolerance to the levels of bile salts set at 1%, 0.5%, 0.25%. For these three assays, the microplates were incubated for 24 hours at 30°C, the spectrometric readings were taken at 600 nm wavelength (Szutowska and Gwiazdowska 2021). Bile tolerance can also be tested by inoculating the chosen LAB suspension (0.5%) in MRS broth and MRSO (MRS supplemented with bile oxgall) for obtaining final concentrations of 0.05%, 0.1%, 0.15%, 0.3%, and incubating at 37°C for 24 hours, while the absorbance read at hourly 560 nm. The cholesterol-lowering capacity can also be studied by adding water-soluble cholesterol (100 µg/mL) to MRSO (containing 0.3% oxgall), inoculating with LAB strain and incubating at 37°C for 20 hours, and harvesting the cells to determine remainder cholesterol content (Zergui 2014; Pereira and Gibson 2002). LAB cultures grown overnight are inoculated (1% v/v) in MRS broth, incubated anaerobically (37°C, 24 hours), centrifuged ( $11,000 \times g$ , 4°C, 10 minutes) to obtain supernatants, which are measured for D- and L-lactic acid contents using the D/L-lactic acid kit (Ahire et al. 2021).

Antimicrobial activity is a key metabolic feature of the LAB group and can be determined using agar well diffusion protocol: MRS agar medium (1% w/v agar) is seeded with food-spoilage/foodborne pathogenic bacteria mixed, poured upon petri plates, and allowed to set for making 5 mm diametric wells in the agar; diluted cell-free supernatants of chosen LAB are poured into the wells, plates incubated and observed for inhibition zones. *Lactococcus lactis* subsp. *lactis*

KT2W2L was found to produce nisin Z which could inhibit the growth of food-spoilage pathogens like *Brochothrix thermosphacta* and *Staphylococcus aureus* as determined by using agar spot test and agar well diffusion (Hwanhlem et al. 2017). Diluted cell-free supernatants (CFS) of LAB to be used in well diffusion assays are prepared by taking 1 mL of chosen LAB isolate ( $10^8$  CFU/mL), inoculating in 50 mL MRS broth for incubation at 37°C for 48 hours, centrifuging at  $11,200 \times g$  for 10 minutes at 4°C (separating supernatant and bacterial cells) and filtering using 0.45 µm membranes. The CFS of *Lactobacillus* strains were tested for antimicrobial activity against pathogenic strains like *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Salmonella Paratyphi*, and *Yersinia enterocolitica*. Here 0.1 mL ( $1.5 \times 10^8$  CFU/mL) of the pathogenic strain culture was added to 20 mL of Mueller-Hilton agar (0.75%) and poured into petri plates, 5 mm-diameter wells were made in the set agar and filled with 50 µL of (LAB) CFS and MRS broth (negative control) followed by incubation at 37°C for 24 hours. The CFS obtained from *Lactiplantibacillus plantarum* subsp. *plantarum* showed antimicrobial effect against *S. paratyphi* A with 22.5 mm-diameter inhibitory halo zone and from *Lactobacillus raffinolactis* showed inhibitory effect against *Listeria monocytogenes* with 23.5 mm-diameter zones (Yazgan et al. 2021).

Probiotic potential is the chief attribute of LAB and can be assessed by testing cell survival under simulated gastric/pancreatic digestion conditions using standard protocols. *Lactobacillus* strain is inoculated in 100 mL MRS broth and incubated at 37°C for 12–14 hours for obtaining ( $10^9$  CFU/mL) culture. Obtained culture is harvested, washed, resuspended in 100 mL sterile gastric/pancreatic base solution, and incubated at 37°C with rotary shaking (200 rpm) for 4/6 hours. The incubated samples (5 mL) collected hourly are centrifuged ( $5000 \times g$ , 10 minutes, 4°C), washed with saline (0.85% w/v NaCl), diluted serially, plated upon set MRS agar petri plates, and incubated aerobically at 37°C for 48 hours. Survival of cells is calculated analyzing experimental values using the equation: % Survival =  $\log N_t / \log N_0 \times 100$ , ( $N_0$  = cell number at time zero,  $N_t$  = cell number at time when sample is taken) (Bhushan et al. 2021).

As acidification process is a vital trait responsible for preservation of food products by LAB, it is quintessential to analyze acidifying properties of LAB by determining pH levels (Stojanovski et al. 2010). Colorimetric methodology using spectrophotometry measures bacteria-caused pH changes with the aid of bromocresol purple (pH indicator dye). Here the standard curve was calibrated using various buffer solutions (pH increments ranging 4.4–7.0), followed by adding 5 µL solution of bromocresol purple (0.5 mg/mL) to 200 µL of each buffer in a microplate, and measuring absorbance at 430 nm using microtitre reader. Suspensions of LAB ferments were checked for absorbance with and without bromocresol purple dye and comparatively analyzed using the standard curve of the calibrated assay, this method was found advantageous as it allowed simultaneous and fast screening of LAB acidifying property. Acidifying activities in LAB could also be measured by monitoring pH changes



in skimmed milk and whey at various incubation periods by means of pH meters (Ribeiro, Coelho, and Silva 2021).

Biogenic amines (BA) are microbial toxins of LAB fermented food products causing food-borne intoxications, hence LAB strains must be tested for BA production to ensure food safety (Perin and Nero 2017). BA production was reported from *Lactobacillus* strain adopting a characterization protocol: 10 mL tyrosine decarboxylase broth (TDB) was mixed with 0.5 mL of LAB culture ( $\sim 10^8$  CFU/mL) and incubated at 37°C for 3 days to extract 4 mL of LAB supernatant. Post extraction, verification of BA formation was done using rapid HPLC method with a reversed-phase column employing a gradient elution program. Calibration curves for the amines were prepared (0–50 µg/mL range) and correlation coefficients of peak areas against amine standard concentrations were calculated (Yazgan et al. 2021). Quantitative and qualitative determination of BA content produced by *Limosilactobacillus reuteri* strains (sheep origin) were determined using RP-HPLC with UV detector, amongst the produced biogenic amines the most potent and dominant was tyramine (Body et al. 2021).

Antibiotic susceptibility of LAB strains must be studied to comprehend their application as potential probiotics. Disks containing antibiotics like penicillin (10 units), erythromycin (15 µg), vancomycin (30 µg), teicoplanin (30 µg), clindamycin (2 µg), ofloxacin (5 µg), azithromycin (15 µg), and tetracycline (30 µg), can be placed upon MRS agar plates (spread with 100 µL LAB culture) and incubated for 24 hours at 30°C to observe the zone of inhibition (Monika et al. 2017). Another recent research has interpreted the use of MRS agar disc-diffusion method for assaying antibiotic susceptibility. Saline suspensions ( $1.5 \times 10^7$  CFU/mL) were prepared from LAB strains (grown in MRS broth at 30°C for 24 hours), and 1 mL was poured along with 20 mL MRS agar into petri plates for setting. Eleven antibiotics viz., ampicillin, gentamicin, erythromycin, chloramphenicol, streptomycin, penicillin G, clindamycin, vancomycin, tetracycline, kanamycin, neomycin, were used in preparing disks that are placed upon the set agar and incubated at 30°C for 48 hours, and observed for the zones of inhibition. The experiment was done in triplicate and results were analyzed based on the sensitivity patterns/zones of tested LAB against standard antibiotics as resistant ( $\leq 14$  mm), intermediate (15–19 mm), and susceptibility ( $\geq 20$  mm) (Szutowska and Gwiazdowska 2021). Results based upon antibiotic susceptibility assays have generally stated that majority of LAB strains were resistant to vancomycin and kanamycin, around 93% to neomycin, and 96% to gentamicin (Szutowska and Gwiazdowska 2021; Colombo, Nero, and Todorov 2020; Michalak et al. 2018; Argyri et al. 2013).

Other miscellaneous tests usually done for LAB strain characterization include: observing for exopolysaccharide (EPS) production, hemolytic activity,  $\beta$ -Glucosidase activity, phytase activity, amylase activity, and gelatin activity. EPS production in LAB is performed by spot-inoculation of 5 µL of chosen strain (grown overnight) upon ruthenium red milk agar (5 g yeast extract, 100 g skimmed milk powder, 10 g sucrose, 15 g agar, 0.08 g ruthenium red, per 1 L of distilled water), incubating at 37°C anaerobically for

48 hours, and observing white and red colony patterns. White ropy colonies are EPS producers, while the non-ropy red colonies do not produce EPS and can pick up the red-stain in their cell walls (Ahire et al. 2021; Stinglee, Neeser, and Mollet 1996). Ropiness of *Lactiplantibacillus plantarum* subsp. *plantarum* colonies could be confirmed through the formation of sticky filaments when touched using loops (Bhushan et al. 2021). Hemolytic activity in LAB strains is confirmed by streaking grown cultures upon sheep blood agar plates, incubating for 24–48 hours at 30–37°C, and observing for hydrolysis zones viz.,  $\alpha$ -hemolysis (partial hydrolysis),  $\beta$ -hemolysis (clear hydrolysis),  $\gamma$ -hemolysis (no hydrolysis) (Monika et al. 2017; Bhushan et al. 2021).  $\beta$ -Glucosidase activity can be determined using p-nitrophenyl- $\beta$ -D-glucopyranoside as a substrate (Matsuda et al. 1994), and confirmed by the appearance of blue colonies on incubated MRS agar plates (containing 60 µL of 1 mg/mL 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside, and 40 µL of 1 mg/mL isopropyl  $\beta$ -D-thiogalactopyranoside solution as inducer). Quantitative screening can be done using 4 mg/mL o-nitrophenol- $\beta$ -D-galactopyranoside in 0.1 M sodium phosphate buffer at pH 6.8, the released o-nitrophenol content measured spectrophotometrically at 410 nm after 10 minutes of reaction, where a unit denotes the enzyme amount that catalyzes/liberates 1 µmol o-nitrophenol- $\beta$ -D-galactopyranoside per minute (Monika et al. 2017). The phytase activity of LAB isolates can be confirmed by streaking the isolate cultures upon petri plates and flooding with aqueous cobalt chloride (ACC) (2% w/v solution), incubating for 5 minutes at room temperature and draining, adding equal volumes of aqueous ammonium molybdate and ammonium vanadate solutions, incubating for another 5 minutes and examining for zones of hydrolysis after draining (Monika et al. 2017). Amylase activity is determined using 3,5-dinitrosalicylic acid as per standard protocol (Bernfeld and Colowick 1955), and gelatinase activity analyzed by spot inoculating grown LAB cultures upon nutrient gelatin agar plates, incubating at 37°C for 24–48 hours anaerobically, flooding with saturated ammonium sulfate solution for precipitating un-hydrolyzed gelatin, and observing for clear zones of hydrolysis around LAB colonies (Ahire et al. 2021).

## LAB Patents and industrial applications

This section describes the various industrial patents and their summarized claims viz., novel LAB isolated and identified, methods and media used for “their detection/screening, isolation, adaptation, culturing, preservation, improvement of robustness,” and the techniques and/or methodologies supporting LAB fermentation for production of industrial metabolites like LA, poly lactic acid, organic acids (acetic acid, phenyl lactic acid, butyric acid, formic acid, propionic acid, succinic acid), amines, bacteriocins, antifungal/antibacterial peptides, short-chain fatty acids, vitamins, extracellular polysaccharides,  $\gamma$ -aminobutyric acids, flavor precursors, antioxidant substances, diacetyls, acetoin, polyols, hydrogen peroxides, etc. (Table 2).

**Table 2.** Compendium listing patents claimed on LAB strains and their applications in various sectors

	Patent no.	LAB	Summary/ Claim of patent: application	Ref.
1	JP2016059328A	<i>Lactococcus lactis lactis</i> BF3	Isolation of novel lactic acid bacterium from intestinal contents of salmon fish	(Eto 2016)
2	KR20020050048A	<i>Lactobacillus</i> sp. DW-1	Isolation of <i>Lactobacillus</i> sp. DW-1 and its identification system	(Park Jong 2002)
3	JP2017209021A	<i>Lactobacillus plantrum</i> HOKU-1	Isolation of novel plant LAB and uses for fermenting yoghurt	(Sato 2017)
4	JP2010215665A	-	Selective breeding method for isolation of LA-producing antimicrobial strains with therapeutic applications	(Farmer 2010)
5	WO2008/003782A1	<i>Lactobacillus casei</i> V, <i>Lactobacillus casei</i> W, <i>Lactobacillus casei</i> T, <i>Lactococcus lactis</i> S, <i>Bifidobacterium infantis</i> S, <i>Streptococcus salivarius thermophilus</i>	Isolation of LAB and adaptive growth upon 100% vegetable isolation and adaptation media: application as vegetable food product/ ingredient.	(De Schinkel and De Buyser 2008b)
6	CN109337836 A	-	Isolation medium for LAB: method for preparing isolation medium and LAB screening medium	(Yang et al. 2019)
7	JP 2020022393 A	-	LAB medium composed of food materials: used for culturing appropriate number of bacteria	(Nakao et al. 2020)
8	CN 112080457 A	-	LAB enrichment culture medium designed to suit industrial production, its production process	(Han et al. 2020)
9	KR 20070006960 A	-	LAB culturing using Mung Bean medium, its preparation method and cosmetic composition	(Park Chang et al. 2007)
10	KR20150004088 A	-	Method for culturing LAB using the soybean meal extract	(Lee Jong and Koo Bon 2015)
11	WO 2021/005813 A1	<i>Lactobacillus acidophilus</i> NBRC 13951, <i>L. brevis</i> NBRC 3960, <i>L. delbrueckii</i> subsp. <i>lactis</i> NBRC 3376, <i>L. helveticus</i> NBRC 15019, <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> NBRC 13953, <i>L. lactis</i> subsp. <i>cremoris</i> NBRC 100676, <i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> NBRC 100496, <i>Streptococcus thermophilus</i> NBRC 111149	culture medium for selectively separating LAB strains	(Teramura, Ogura, and Fujiwara 2021)
12	CN 103031361 A	-	Improved yoghurt lactic acid bacteria counting culture medium	(Lv 2013)
13	US2016/0024459A1	<i>Lactobacillus brevis</i> , <i>Lactobacillus hilgardii</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus fructivorans</i>	Isolation of novel <i>Lactobacillus</i> strains: used for preservation of foods, animal feedstuff, pharmaceuticals, and/or cosmetic compositions.	(Goelling, Heilmann, and Lang 2016)
14	US2006/0270020A1	<i>Lactobacillus salivarius</i> ss <i>salicinius</i> NCIMB 41287 (AHF 122 A), <i>Lactobacillus animalis</i> NCIMB 41288 (AHF 223 C), <i>Lactobacillus reuteri</i> NCIMB 41289 (AHF 5119)	<i>Lactobacillus</i> strains isolated from resected and washed feline gastrointestinal tract: animal probiotic uses.	(Boileau Thomas et al. 2006b)
15	US2005/0158294A1	<i>Bifidobacteria pseudolongum</i> NCIMB 41199	LAB isolated from resected & washed canine gastrointestinal tract: animal probiotic uses.	(Boileau Thomas et al. 2005)
16	US2006/0269534A1	<i>Bifidobacterium longum</i> NCIMB 41290 (AHF5340), <i>Bifidobacterium longum</i> NCIMB 41291 (AHF231)	<i>Bifidobacteria</i> strains isolated from resected and washed feline gastrointestinal tract: animal probiotic uses.	(Boileau Thomas et al. 2006a)
17	CN210856093U	-	Simultaneously culturing various LAB strains using a convenient fermentation culture device	(Chen et al. 2020)
18	US7887794B2	<i>Lactobacillus acidophilus</i> I-1492, <i>Lactobacillus casei</i>	Isolation of novel properties of LAB strains: prevention and/ or treatment of cancer	(Luquet, Baldwin, and Lacroix 2011)
19	US2020/0360448A1	<i>Bacillus coagulans</i> FF-7	Isolation and characterization of novel fructophilic LA- producing strains: therapeutic applications.	(Majeed et al. 2020)
20	WO2014/140123A1	<i>Lactobacillus brevis</i> , <i>Lactobacillus hilgardii</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus fructivorans</i> .	Novel <i>Lactobacillus</i> strains: preservation of food, animal feedstuffs, pharmaceuticals and cosmetic compositions.	(Goelling, Heilmann, and Lang 2014)
21	WO2008/003781A1	<i>Lactobacillus casei</i> V, <i>Lactobacillus casei</i> W, <i>Lactobacillus casei</i> T, <i>Lactococcus lactis</i> S, <i>Bifidobacterium infantis</i> S, <i>Streptococcus salivarius thermophilus</i>	Method and medium for preserving LAB in viable condition	(De Schinkel and De Buyser 2008a)

(Continued)

Table 2. (Continued)

	Patent no.	LAB	Summary/ Claim of patent: application	Ref.
22	CN110373351A	–	Freeze-drying protective agent: application in preparation of LAB freeze-dried powder	(Li et al. 2019)
23	EP 1038951 A1	<i>Lactobacillus</i> genera, <i>Bifidobacteria</i> genera	Novel defined medium for identification and/or isolation of bioactive molecules or functional metabolites.	(Elli et al. 2000)
24	UA 84665 U	–	Method for pH stabilization of LAB cultivating nutrient media: preparation of nutrient media with mineral substances and beet molasses to support LAB growth.	(Vasyleva Natalia et al. 2013)
25	WO 2011/041402 A1; US 9169499 B2	<i>Lactobacillus plantarum</i> DWS2269, DWS2279; <i>Lactobacillus plantarum</i> PN0512	Engineering method for genetic modification and isolation of LAB cells: used to produce isobutanol contents.	(Paul Brian and Suh 2011; Paul Brian and Suh 2015)
26	US 10563271 B2	<i>Lactococcus lactis</i> CS4616m	Genetically engineered LAB for high-level production of diacetyl	(Solem, Jensen Peter, and Liu 2020)
27	US 11111474 B2	<i>Lactococcus lactis</i> 43103	<i>Lactococcus lactis</i> obtained by protoplast fusion: production of acetoin and/or 2,3-butanediol (2,3-BDO)	(Roncal Martínez et al. 2021)
28	EP2206505A1	<i>Lactobacillus brevis</i> FERM BP-4693	Nano-Level LAB cells ( $\leq 1.0 \mu\text{m}$ ): production of INF $\pm$	(Hasegawa and Kan 2010)
28	US2012/0312743A1	–	LAB strains used for detoxification of soluble sulfates present in environmental effluents	(Ray Chaudhuri and Thakur Ashoke 2012)
29	WO 2010/087551 A1	<i>Lactobacillus plantarum</i> , <i>Bifidobacterium longum</i> , <i>Lactobacillus rhamnosus</i> , <i>Streptococcus lactis</i>	Method for producing freeze-dried LAB powder using membrane bioreactor	(Cho Young et al. 2010)
30	EP 3301173 A1	<i>Lactobacillus johnsonii</i> , <i>Lactobacillus curvatus</i> , <i>Lactobacillus bulgaricus</i> , <i>Lactobacillus acidophilus</i>	Growth medium (yeast extract, carbohydrate, lecithin) and process for producing LAB	(Ananta et al. 2018)
31	US 2010/0047396A1	<i>Lactococcus lactis</i> subsp. <i>cremoris</i> I-3557	Method for obtaining variants of LAB: Vitamin K2 production, preparing food products	(Garault et al. 2010)
32	CN 111471613 A	–	Edible LAB culture medium: used in food fermentation	(Guo et al. 2020)
32	EP 1177794 A2	<i>Lactobacillus bulgaricus</i> , <i>Streptococcus thermophilus</i>	LAB composition: treatment of gastrointestinal disorders, obesity, hyperlipidemia, autoimmune diseases	(Bojrab Gregory 2002)
33	US 2017/0020936 A1	<i>Lactobacillus reuteri</i> 6475	Selection method for specific probiotic LAB producing histamine: treatment/prophylaxis of inflammatory conditions in mammals	(Versalovic, Thomas Carissa, and Connolly 2017)
34	KR 20140018718 A	–	LAB culture medium comprised of fermented solution of washed rice water, and its method of preparation	(Kim Keun, Kim Min, and Yan 2014)
35	EP 3027035 B1	<i>Streptococcus thermophilus</i> 10.44, <i>Lactobacillus delbrueckii</i> spp. <i>bulgaricus</i> 9 A	A large-scale screening method for identifying glucose-secreting LAB	(Klinkenberg, Holo, and Øyaas 2018)
36	WO 2010/100047 A1	<i>Lactobacillus acidophilus</i> LA-5, <i>Lactobacillus gasseri</i> SBT-2055.	An improved method for preparing LAB cultures: manufacturing of food, feed, and pharmaceutical products	(Kringelum Boerge and Soerensen Niels 2010)
37	WO 2011/098843 A2	<i>Lactobacillus amylovorus</i> DSM 20531 <sup>T</sup>	Procedure for producing lactic acid or its salts through simultaneous starch-saccharification and sugar-fermentation	(Slavica et al. 2011)
38	CN 105112465 A	–	LAB culture medium for production of conjugated linoleic acid. using extensive sources and at reduced production costs.	(Chen et al. 2015)
39	US 9615594 B2	<i>Lactobacillus plantarum</i> OH22, <i>Pediococcus pentosaceus</i> OH19, <i>Lactobacillus casei</i> OH12, <i>Lactobacillus paracasei</i> OH14, <i>Lactobacillus sakei</i> OK1, <i>Leuconostoc citreum</i> OK2	A mixed LAB culture fluid to inhibit LAB proliferation in fermented kimchi to delay kimchi ripening.	(Koo et al. 2017)
40	US 2021/0307347 A1	<i>Lactobacillus delbrueckii</i> , <i>Streptococcus thermophilus</i>	Method for obtaining polylactic acid from cheese whey using LAB	(Cuervo Garces Laura 2021)
41	CN 112842951 A	–	Secondary fermented birch juice (using LAB and <i>Saccharomycetes</i> ): used in compositions for skin anti-eczema.	(Wang, Duan, and Hong 2021)

(Continued)

Table 2. (Continued)

	Patent no.	LAB	Summary/ Claim of patent: application	Ref.
42	WO 2021/080537 A1	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> PNGR_B6 MN792802, PNGR_B8 MN811207; <i>Streptococcus thermophilus</i> PNGR_K1 MN812674, PNGR_K2 MN812707, PNGRJC5 MN814043, PNGR_K13 MN814030, PNGR_K31 MN814032, PNGRJC55MN812789	Production of fermented milk products using natural LAB isolate starter cultures	(Şahiner 2021)
43	CA 3112751 A1	–	LAB cocultures expressing a bacteriocin system	(Steele James et al. 2021)
44	US 2020/0054695 A1	<i>Lactobacillus plantarum</i> Gkm3	Composition of active substances of <i>Lactobacillus Plantarum</i> Gkm3, and application for promoting Longevity	(Chen et al. 2020)
45	US 2020/0199633 A2	<i>Bacillus acidiproducens</i> , <i>Bacillus coagulans</i>	Process for producing LA or its salts by fermenting thermotolerant <i>Bacillus</i> bacteria	(Thongchul, Tolieng, and Prasitchoke 2020)
46	US 2021/0207077 A1	<i>Lactobacillus casei</i> CBT LC5, <i>Lactobacillus plantarum</i> CBT LP3, <i>Lactobacillus rhamnosus</i> CBT LR5, <i>Bifidobacterium longum</i> CBT BG7, <i>Bifidobacterium lactis</i> CBT BL3, <i>Bifidobacterium bifidum</i> CBT BF3, <i>Streptococcus thermophilus</i> CBT ST3, <i>Pediococcus pentosaceus</i> CBT SL4	Medium composition (comprising growth factors) for promoting LAB growth	(Chung Myung 2021)
47	EP 3708649 A1	<i>Pediococcus pentosacus</i> AB160011	Isolating novel LAB, defining culture medium composition: used as antibacterial, cosmetic, and functional food	(Lee Jong 2020)
48	EP 2945496 B2	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Improved Nisin Production Process using LAB strains	(Dierdorp-Andraea Brenda et al. 2020)
49	US 10898529 B2	<i>Lactobacillus reuteri</i> 6475	Production and use of bacterial histamine from probiotic LAB	(Versalovic, Thomas Carissa, and Connolly 2021)
50	US 10570366 B2	<i>Lactobacillus kunkeei</i> BPS402, BPS104	LAB producing IgA promoting activity and application thereof	(Matsuura et al. 2020)
51	EP0415470B1	<i>Lactococcus lactis</i> subsp. <i>cremoris</i> Wg2	Aminopeptidase isolated and characterized from LAB for preparing polyclonal / monoclonal antibodies: preparing food products -cheese, dairy products, meat products, protein hydrolysates	(Tan Paris Som et al. 1995)
52	EP 2988602 A1	–	LAB composition for manufacturing fermented dairy products of improved texture properties.	(Maljaars Cornelia Elizabeth et al. 2016)
53	JP 2021107446 A	<i>Enterococcus faecalis</i>	Combined culturing of LAB cell and acetic acid bacterium: used for immunostimulatory purposes	(Hiramatsu and Soma 2021)
54	CN 112899117 A	–	Co-fermenting 'red date and coix seed vinegar' with high $\gamma$ -aminobutyric acid content by culturing ' <i>Monascus</i> , LAB, and spore bacteria' for producing table vinegar	(Xu et al. 2021)
55	US 2021/0284560 A1	LAB chosen from genera: <i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Enterococcus</i> , <i>Lactococcus</i> , <i>Pediococcus</i> , <i>Leuconostoc</i>	Animal and poultry solid waste material are treated using preferred lactobacillus organism, for significantly reducing odor	(Kaneshika 2021)
56	EP 3442348 B1	<i>Lactobacillus rhamnosus</i> I-4993	<i>Lactobacillus rhamnosus</i> used for preparing of fermented products	(Garault, Daval, and Marchal 2020)
57	US 10653161 B2	<i>Lactobacillus rhamnosus</i> CHCC12697	LAB used for flavor enhancement and preparing dairy products	(Jimenez et al. 2020)

Selective detection and separation of LAB from a mixed bacterial culture could be accomplished by supplementing MRS agar (culture medium) with an inorganic metal salt of an organic acid and glycine (Teramura, Ogura, and Fujiwara 2021). Isolation of LAB from natural environments or culture collections, adapting them upon suitable animal-free isolation medium (composed of vegetable peptone, yeast component, buffering agent, fermentable carbohydrate) and culturing LAB for preparing a fermented dairy analogue using LAB culture was adopted for strains *Lacticaseibacillus casei* V (LMG P-23504), *Lacticaseibacillus*

*casei* W (LMG P-23505), *Lacticaseibacillus casei* T (LMG P-23506), *Lactococcus lactis* S (LMG P-23669), *Bifidobacterium infantis* S (LMG P-24096), and/or *Streptococcus salivarius* subsp. *thermophilus* (LMG P-24095) (De Schinkel and De Buyser 2008b). Another medium comprised of soy peptide and yeast extract was used for LAB cultivation to trigger growth and achieve sufficient number count of LAB (Nakao et al. 2020).

Several novel LAB strains isolated from plant sources have been patented reportedly: novel *Lactiplantibacillus plantarum* subsp. *plantarum* HOKU-1 strain isolated from

*Cucumis melo* (Yubari King), used to prepare flavored yoghurt storable for more than a year (Sato 2017); fructophilic-probiotic *Weizmannia coagulans* FF7 isolated from honey mixed with saline (in 1:10 w/v ratio), cultured in fructose-containing medium (MRS medium, glucose yeast extract agar, tryptone soya broth, sporulation media, mueller hinton agar) for 48 hours at 35–37 °C, found with biological applications and/or therapeutic uses for managing eating disorders related to high fructose intake (Majeed et al. 2020); LAB from fermented grains isolated and screened using 25–100 mL/L of a Daqu-fermented grain extraction solution (Yang et al. 2019); *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* isolated from Anatolian village yoghurts, used as commercial starter cultures for production of creamy yoghurt, homogenized yoghurt, and fermented milk products like yogurt and ayran with different fat/dry matter contents (Şahiner 2021). Many novel LAB strains have been isolated from animal sources: *Lactobacillus delbrueckii* subsp. *lactis* BF3 isolated from intestinal contents of salmon fish, bearing tolerance to 55–70 times bile of 0.25–2.5% w/v content,  $\geq 7$  times acid of pH 4–5,  $\geq 1.3$  times salt of 4–5% mass content,  $\geq 2$  times of protective action to reactive oxygen species toxicity, and with ability to ferment soybean milk (Eto 2016); *Bifidobacterium longum* strains (NCIMB 41290 and NCIMB 41291) isolated from resected-washed feline gastrointestinal tract (GIT), exhibiting probiotic activity in animals and odor-reduction potential in litter boxes (Boileau Thomas et al. 2006a); probiotic *Bifidobacterium pseudolongum* NCIMB 41199 isolated from canine GIT (Boileau Thomas et al. 2005); probiotic *Lactobacillus* strains (*Lactobacillus salivarius* ss *salicinius* NCIMB 41287, *Lactobacillus animalis* NCIMB 41288, *Lactobacillus reuteri* NCIMB 41289) isolated from resected-washed feline GIT (Boileau Thomas et al. 2006b); *Lactobacillus* sp. DW-1 isolated from the dog's feces, and treated with “simulated gastric juice and bile acid solution (containing oxgall)” for inducing improved therapeutic activities for treating intestinal disorders and inhibiting pathogenic bacteria like *E. coli* and *Salmonella* sp. with acid resistance and bile acid resistance (Park Jong 2002).

Various kinds of growth media compositions are adopted for suiting nutrient requirements and metabolic needs of particular groups of LAB. A defined synthetic medium suited for cultivating *Lactobacillus* and/or *Bifidobacteria* strains could be used for the isolation of bioactive molecules or functional metabolites, and its nutrients components were defined as: a carbon source (glucose/fructose/lactose/saccharose/mixture), buffer ( $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4/\text{diammonium hydrogen citrate}/\text{NaHCO}_3/\text{Na}_2\text{CO}_3/\text{mixture thereof}$ ), a nitrogen source (one or more amino acids/diammonium hydrogen citrate/mixture thereof), trace elements (Cu-, Zn-, Mn-, Mg-, Co-compounds), antioxidants (ascorbic acid, cysteine, thiol compounds) and vitamins (nicotinic acid, pantothenate, cobalamine, p-aminobenzoic acid, pyridoxal-HCl, riboflavin, biotin, folic acid) (Elli et al. 2000). Glucose-secreting LAB strains like *Lactococcus* spp., *Streptococcus* spp. (*Streptococcus thermophilus*), *Lactobacillus* spp. (*Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus*

*casei*), *Leuconostoc* spp., *Pseudoleuconostoc* spp., *Brevibacterium* spp., *Enterococcus* spp., *Propionibacterium* spp., and *Bifidobacterium* spp., could be screened by subjecting to mutagenizing conditions (X-radiation, UV-radiation, chemical mutagens), selecting the mutated colonies and culturing them upon lactose-rich growth media (Klinkenberg, Holo, and Øyaas 2018). For culturing LAB viz., *Lacticaseibacillus casei* CBT LC5, *Lactiplantibacillus plantarum* subsp. *plantarum* CBT LP3, and *Lacticaseibacillus rhamnosus* CBT LR5, *Bifidobacterium longum* CBT BG7, *Bifidobacterium lactis* CBT BL3, and *Bifidobacterium bifidum* CBT BF3, *Streptococcus thermophilus* CBT ST3, *Pediococcus pentosaceus* CBT SL4, a growth medium composition was developed comprising of a carbon source (glucose and fructooligosaccharide), and nitrogen source (yeast extract, isolated soy protein) (Chung Myung 2021). LAB like *Lactococcus lactis*, *Leuconostoc lactis*, *Leuconostoc pseudomesenteroides*, *Leuconostoc mesenteroides*, *Leuconostoc dextranicum*, *Enterococcus faecium*, and *Propionibacterium* sp., were cultivated upon “bacitracin and peroxide” containing culture media resulting in production of higher quantities of vitamin K2 under standard fermentation conditions, lactic ferments of such variants found application in preparing food products (fermented, dairy products) enriched in vitamin K2 for strengthening bone health (Garault et al. 2010). Thermotolerant LAB like *Weizmannia acidiproducens* and *Weizmannia coagulans* could be used for production of lactic acid (100–200 g/L) or its salts from media containing fermentable sugars like glucose, fructose, galactose, sucrose, lactose, maltose, cellobiose, raffinose, isomaltotriose, maltotriose, nigerotriose, kestose, or mixtures (Thongchul, Tolieng, and Prasitchoke 2020). *Weizmannia coagulans* adapted for tolerant growth in delactosed whey permeate was found to convert delactosed whey permeate (substrate) into lactic acid with 90% (w/w) yields (O'Connor et al. 2018). Amyolytic LAB *Lactobacillus amylovorus* DSM 20531<sup>T</sup> could be used for simultaneous saccharification and fermentation of semi-solid substrate (corn grits and MRS-medium ingredients) using bioreactors for the production of lactic acid and/or its salts (Slavica et al. 2011).

The compositions/nutrients of LAB growth/culture media could also be adjusted to trigger the production of particular metabolites and/or industrial products considering cost-cutting strategies. An LAB culture medium comprised of 6–14% jerusalem artichoke extractive juice, 0.5–4% glucose, 1–6% yeast extracts, 2–6% sodium acetate, 2% dipotassium phosphate, 0.05% magnesium sulfate, 0.05% manganese sulfate, 1 ml/L Tween-80 is used, water as solvent, pH 6.8, was used for production of conjugated linoleic acid ensuring practicality and reducing production costs (Chen et al. 2015). Edible medium comprised of raw liquids like 1–5% soy sauce precipitate, 1–4% sugar mash liquid, water, with pH value of 5.4–5.6, could be used for cultivating/culturing LAB for fermenting foods with added flavors at low costs (Guo et al. 2020). Fermented rice water (used for washing rice) could be used as inexpensive substrate for cultivating LAB contributing to economic aspects and preventing environmental pollution (Revuelta, Ledesma-Amaro,

and Jiménez 2016; Kim Keun, Kim Min, and Yan 2014). Beet molasses and mineral substances like sodium acetate, ammonium citrate, magnesium sulfate can also be used as components of cultivation nutrient media whilst stabilizing pH conditions added in order to trigger LAB growth (Vasyleva Natalia et al. 2013). Polyoxyethylene-sorbitan mono-oleate may be substituted with diacetyl tartaric acid esters of mono- and di-glycerides as a yield enhancing agent in LAB fermentation media, and such cultures could be used for manufacturing of food, feed and pharmaceutical products (Kringelum Boerge and Soerensen Niels 2010). LAB inoculation and culturing in medium comprised of “mung bean extract and monosodium glutamate (MSG)” lead to production of gamma-aminobutyric acid with uses featuring skin anti-aging/improving effects, collagen synthesis, anti-inflammatory, and skin-irritation alleviating effects (Park Chang et al. 2007). An enrichment medium for industrial production of LAB cultures was prepared from components viz., 400–430 parts of glucose, 400–430 parts of soybean meal zymolyte, 35–45 parts of growth factor, 5–15 parts of vitamins, 75–93 parts of buffer agent, 3.5–4.8 parts of magnesium sulfate, and 0.5–1.5 parts of manganese sulfate, so that the culture cost of LAB in production is reduced (Han et al. 2020). A culture medium (comprised of 4–7 g peptone, 4–7 g beef extract powder, 4–7 g yeast powder, 15–22 g glucose, 15–22 g lactose, 7.5–13 g calcium carbonate, 0.03–0.07 g neutral red, 10–16 g agar powder, was made up with distilled water, per 1000 ml medium) was devised for accurately counting yogurt LAB, and had advantages that the proportioning step was simple, enough nutrients were provided, the solidifying effect was good, and the quality control of the yogurt was facilitated (Lv 2013). LAB cultivated in soybean meal extract at high concentration in relatively short time using fermentation was used for manufacturing applied lactic acid products at low costs for industrial uses including agriculture, fishery, lives stock, etc. (Lee Jong and Koo Bon 2015).

LAB strains and their ferments also find applications in the storage and preservation of food stuff. An invention outlines methodology using vegetable-based adaptation-culture media comprised of 0.1–10% papaic digest of soybean protein, 0.1–10% yeast component, 0.1–10% phosphate buffer, 0.1–10% vegetable derived fermentable carbohydrate could be adopted for isolation, adaptation, culturing, preservation, and long-term storage of LAB genera like *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Weissella*, *Oenococcus*, *Enterococcus*, *Propionibacterium*, and *Bifidobacterium*. Such strains find applications in preparing dairy analogues, food ingredients, functional foods, and dietary supplements suitable for vegetarian/vegan consumer groups of people (De Schinkel and De Buyser 2008a). Novel heterolactic LAB ferments of strains viz., *Levilactobacillus brevis*, *Lentilactobacillus hilgardii*, *Lactiplantibacillus plantarum* subsp. *plantarum*, *Fructilactobacillus fructivorans*, find applications in preserving foods, animal feedstuffs, pharmaceutical compositions and/or cosmetic compositions as they could inhibit pathogenic fungal growth in foods like yogurt, milk, cheese, cream, curd cheese (Goelling, Heilmann, and

Lang 2016; Goelling, Heilmann, and Lang 2014). Carboxymethyl pachyman was discovered as a novel and efficient LAB freeze-drying protective/preservative agent, its application in the preparation of LAB freeze-dried powder was far superior compared to conventional freeze-drying protective agents as it could increase the survival rate of LAB (Li et al. 2019). High yields of LAB (from *Lactobacillus* genera) were achieved using a growth medium containing yeast extract and lecithin (replacement for polyoxyethylene-sorbitan-mono-oleate) ensuring consumption by most sensitive consumers, because the use of lecithin improved LAB survival during drying, storage, and/or reconstitution with a liquid. LAB products find applications as nutritional compositions for infant formula, infant cereals, follow-up formula, growing-up milk, functional milk and milk products for pregnant and lactating women (Ananta et al. 2018). High concentrations of LAB production could also be achieved using membrane reactors adopting certain cultivation steps: membrane-separating product from medium supply apparatus, supplying culture medium through the medium supply apparatus, continuously discharging culture filtrate for product-separation, recycling LAB continuously to bioreactor, producing LAB powder using freeze drying preservatives; the obtained LAB also exhibited superior physical and chemical stability compared to simple freeze-dried LAB powder (Cho Young et al. 2010). A mixed LAB culture fluid containing six types of LAB viz., *Lactiplantibacillus plantarum* subsp. *plantarum* OH22, *Pediococcus pentosaceus* OH19, *Lactobacillus casei* OH12, *Lactobacillus paracasei* OH14, *Lactobacillus sakei* OK1, *Leuconostoc citreum* OK2, could be used for delayed-ripening of kimchi specifically by inhibiting LAB proliferation in fermented kimchi (Koo et al. 2017). A utility model for fermentation culture was devised to feature simultaneous cultivation of multiple groups of LAB strains together (Chen 2020).

Food and pharmaceutical applications of LAB and their ferments include usage as probiotics, therapeutics, dietary supplements, and as medicinal drugs. *Lactiplantibacillus plantarum* subsp. *plantarum* was found to produce active metabolites that can promote longevity, reduce mitochondrial damage, and delay aging conditions like nerve degeneration and sarcopenia (Chen et al. 2020). Novel properties discovered in *Lactobacillus acidophilus* I-1492 and *Lactocaseibacillus casei* promote their application as anti-cancer agents in the treatment/prevention of colon cancer (Luquet, Baldwin, and Lacroix 2011). LAB strains resistant to anti-microbial agents were selectively bred and isolated through a novel methodology with therapeutic applications as pharmacologically acceptable carriers for administration to the gastrointestinal tract of vertebrates to prevent pathogenic colonization (Farmer 2010). A novel *Pediococcus pentosaceus* AB160011 strain and its cell-free culture was found to have antimicrobial properties with applications in cosmetic compositions and as health functional foods for treating inflammatory bowel disease (Lee Jong 2020). *Limosilactobacillus reuteri* 6475 was cultured in glucose media under anaerobic conditions at 37 °C, and screened

for histamine production by detecting the presence of *hdcA/hdcB* genes, and/or the histidine/histamine antiporter genes; its probiotic composition producing  $\geq 250$  pg/ml histamine could be used in treatment or prophylaxis of inflammatory conditions in mammals like colitis, inflammatory bowel disease, irritable bowel syndrome, diverticulosis, gingivitis, vaginitis (Versalovic, Thomas Carissa, and Connolly 2017). Aminopeptidases isolated from *Lactococcus lactis* subsp. *cremoris* Wg2 ferments could be used in preparing polyclonal/monoclonal antibodies with affinity/specificity for the aminopeptidase, and in preparing food products like cheese, other dairy products, meat products, protein hydrolysates that could be characterized using aminopeptidases (Tan Paris Som et al. 1995). LAB cells cultured by controlling medium pH (from 5–8) were found to have a mode value of 1.0  $\mu\text{m}$  particle size distribution, and such nano-sized LAB cells were excellent in INF- $\pm$  producing capacity along with superb water dispersibility. The cell compositions of *Levilactobacillus brevis* FERM BP-4693 were found to have immune-potentiating activities, and such dispersions could also be used in freeze drying or spray drying (Hasegawa and Kan 2010). *Apilactobacillus kunkeei* strains (BPS402, BPS104) produced high IgA levels with immunostimulant properties with applications in food, pharmaceutical, and cosmetic compositions for preventing respiratory/oesophageal infections (Matsuura et al. 2020). A probiotic composition comprised of a fermented culture having *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and a carbohydrate enriched media, could be used in treating patients with gastrointestinal disorders, hyperlipidemia, autoimmune diseases or obesity (Bojrab Gregory 2002). “LAB and *Saccharomycetes*” strain combinations were used for fermenting “birch juice and optional cereal powder” for usage as an anti-eczema cosmetic composition treating external skin treatments (Wang, Duan, and Hong 2021). LAB and their ferments/metabolites also have various other miscellaneous industrial applications apart from food, therapeutic, and pharmaceutical uses. Bioremediation of soluble sulfates from environmental effluents was achieved using a combination of LAB and sulfate-reducing bacterial cultures, wherein the LAB were provided a suitable fermentation medium for producing LA which is directed to culture sulfate-reducing bacteria as substrate/electron donor (Ray Chaudhuri and Thakur Ashoke 2012). Animal and poultry solid waste materials were treated using preferred *Lactobacillus* strain (5%) and non-chlorinated water, for significantly reducing odor (Kaneshika 2021). A combined culture of a recombinant *Lacticaseibacillus paracasei* cell expressing a bacteriocin, another recombinant LAB (immune to bacteriocin) partly capable of bioconversion, and a yeast cell could be used for bioconversion of corn-biomass into a fermentation product like ethanol (Steele James et al. 2021). A utility model featuring a fermentation culture device was found suitable for the simultaneous cultivation of multiple groups of LAB strains together (Chen 2020). Polylactic acid production from cheese whey was reported using homofermentative LAB like *Lactobacillus delbrueckii* and *Streptococcus thermophilus* (Cuervo Garcés Laura 2021). Combined fermentation of

*Monascus*, LAB, and spore bacteria was conducted with mixed substrate “germinated coix seeds, coix seed bran, enzymatically decomposed red date juice, L-sodium glutamate,” resulting in the production of high yields of “gamma-aminobutyric acid, nonvolatile acid, flavone and Monacolin K” with applications in resisting oxidation, lowering cholesterol and blood pressure (Xu et al. 2021). *Lacticaseibacillus rhamnosus* I-4993 could ferment milk to produce fermented dairy products (Garault, Daval, and Marchal 2020) like low-fat yogurts or cheeses with high diacetyl contents and enhanced creamy flavors, without affecting rheologies/fermentation time/post-acidification of dairy products (Jimenez et al. 2020). Diacetyl production was also reported from genetically modified LAB cultivated under aerobic conditions in lactose-rich media (supplemented with iron-containing porphyrin sources, and metal ions like  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ) (Solem, Jensen Peter, and Liu 2020). Genetically engineered LAB strains like *Lactiplantibacillus plantarum* subsp. *plantarum* DWS2269, DWS2279, and PN0512, lacking lactate dehydrogenase and acetolactate decarboxylase activities showed production of improved isobutanol (Paul Brian and Suh 2011; Paul Brian and Suh 2015). *Lactococcus lactis* 43103 modified through protoplast fusion of two wild strains showed an increased ability to produce acetoin and/or 2,3-butanediol (Roncal Martínez et al. 2021).

## Conclusion

LAB are a diverse cluster of industrially important microorganisms that are primarily probiotic starter cultures and act as biocatalysts for biotechnological processes for transforming renewable feedstocks into value-added chemicals, health products, and other marketable commodities. The objective of this review is to familiarize the reader with sources, substrates, growth media and requisite components, methods, ideologies and research efforts on culturing of LAB strains. There is also an added briefing upon the various patents claimed upon LAB culturing/fermentation and allied applications in variable industrial domains. Owing to their excellent metabolic abilities, LAB can thrive efficiently upon a plethora of media/substrates thereby finding applications chiefly in food and dairy industries as starters, probiotics, therapeutics, dietary supplements, and quality enhancers. They also find uses as antimicrobials, medicinal drugs, pharmaceuticals, and cosmetic compositions. Genetic and metabolic engineering technologies, optimized fermentation, and co-cultivation with other microbes could be used to improve LAB metabolic abilities and phenotypic traits to aid the biosynthesis of value-added/industrial bioproducts. However, the myriad applications of LAB, still face technological hurdles in lesser developed countries, and other social setbacks like reluctance to accept new-fangled functional foods by consumers influenced by culture/ethics/religion. Moreover, more research needs to be done to have a profound comprehension of LAB metabolic pathways in order to commercially exploit their genotypes for the production of industrial bioproducts.

## Note

Throughout the text of the manuscript, new genus names have been provided owing to the reclassification of *Lactobacillus* (Zheng et al. 2020) (Available from: <http://lactobacillus.uantwerpen.be/>), and *Bacillus* (Available from:  [genera.](https://criver.widen.net/s/c2kbp9gjws/ms-2021-bacillus-reclassification-technical-sheet.)

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## Author’s contributions

Haritha Meruvu: Conceptualization, Data curation, Investigation, Resources, Writing - original draft, review, editing. Sebnem Tellioglu Harsa: Validation, Resources.

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