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EXPLORING LACTOCOCCUS SPP. IN DAIRY FERMENTATION AS A LACTIC ACID BACTERIA FROM RAW COW MILK

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ARTICLE INFO	ABSTRACT
Keywords: Lactococcus spp, Gram-positive bacteria, Facultatively anaerobic, Dairy fermentation, Cheese production. **Corresponding Author:** **Bakhtawar Khan,** Institute of Microbiology, University of Veterinary and Animal sciences, Lahore, Pakistan, Email:khanbakhtawar330@gmail.com	This short review presents the Lactococcus spp. are gram-positive, facultatively anaerobic bacteria widely recognized for their essential role in dairy fermentation processes, particularly in cheese and yogurt production. Their ability to produce lactic acid and contribute to flavor and texture makes them indispensable in the dairy industry. This review explores the latest advancements in the isolation and identification techniques of Lactococcus spp. from cow milk, highlighting their fermentation potential and biotechnological applications. Furthermore, the article discusses the challenges and future perspectives in enhancing the efficiency and reliability of Lactococcus strains for commercial applications. Our discussion covers various bioactive compounds such as Gamma Amino Butyric Acid (GAMMA), Exopolysaccharides (EPs), Bacteriocins, Conjugated Linoleic acid (CLA), Bioactive peptides, lactic acid and citrate, vitamins and enzymes detailing their composition and potential application.

INTRODUCTION

One of the most important food products is milk, a biological fluid constituted of nutrimental components and composition. Milk is not only a good diet source for young mammals and adults but it also examined as a substrate in fermentation process due to the presence of lactic acid bacteria (LAB) (Deddefo et al., 2023). LAB has resistance activity against pathogenic bacteria especially food-borne pathogens. It has beneficial activity, signified as a non-pathogenic starter in the production of yogurt, cheese, and butter (Karaduman et al., 2017; Marchwińska & Gwiazdowska, 2022; Yerlikaya et al., 2021). Lactococcus spp. have critical involvement in fermented dairy products and also play a role as antimicrobial chemicals that have probiotic activities such as decreasing serum cholesterol, stabilizing gut microflora, alleviating lactose intolerance, and stimulating the immune system. Because during fermentation, lactic acid bacteria produce substances in lower pH and release compounds such as hydrogen peroxide, carbon dioxide, bacteriocins, and diacetyl that prevent other harmful bacterial growth in food (Fernandes et al., 2021). During milk fermentation, some lactic acid bacteria strains produced exopolysaccharides and presumed effects of mannitol, which improve milk viscosity and smoothness, and mannitol effect is helpful in promoting health (Ruas-Madiedo et al., 2002). The microbial population of milk is essential for assessing its quality (Safak et al., 2021). A recent study reveals that *Lactococcus lactis* is the most researchable species in the milk industry due to its fermentation applications and probiotic characteristics. Probiotics are live bacteria or yeast that provide health benefits to the host body in improving health problems (Taye et al., 2021). With the increase in knowledge of one health problem the concept of probiotics is keenly interesting day by day. Lactococcus spp. have a large number of probiotic genera, such as *Lactobacillus.* Other probiotic microorganisms of lactic acid bacteria are *Streptococcus, Enterococcus, Bifidobacterium, Propionibacterium,* and the yeast *Saccharomyces* (Gareau et al., 2010; Velez et al., 2007). LAB also has the ability to give aroma and flavor to fermented products due to its organic activity.

Isolation of *Lactococcus spp.*

The isolation of Lactococcus spp. involves the serial dilution of samples spread on sterilized media, including de Man, Rogosa, and Sharp agar; M17 broth + agar powder of Lactococcus isolation (HiMedia, India, Mumbai); and skim milk agar, Rogasa SL agar for *Streptococcus thermophilus* and MRS agar + Cysteine (0.5%) for *Bifidobacterium* (Taye et al., 2021). Raw cow milk samples are collected aseptically and incorporated on selective enrichment media, agar plates. The agar plates were incubated for 48-72 hours at 30°C and 37°C under aerobic and anaerobic conditions to favor *Lactococcus* growth. The isolation of Lactococcus spp. done by small, white, and creamy morphology characteristics (colony and cell colony) are selected for further study (Lin et al., 2006).

Recent studies explore that isolation techniques can enhance their accuracy and efficiency by using selective media markers and microfluidic devices.

Identification of *Lactococcus spp.*

Identification of *Lactococcus spp.* is observed phenotypically by biochemical tests: gram staining, sugar fermentation profile, production of acid from glucose, catalase test, and growth at different temperatures. For confirmation of the identification, molecular techniques are used; PCR-based methods targeting 16S rRNA and species-specific genes, metagenomics and Whole Genome Sequencing (WGS) and Proteomics techniques (Matrix assisted laser desorption/ Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Modern technologies have improved the strain level accuracy of identification tool such as bioinformatics and sequencing (Taye et al., 2021).

Fermentation application of *Lactococcus spp.*

Lactococcus spp. has lactic acid bacteria which are essentials in the fermentation process to produce fermented food and has probiotic nature. LAB has significant role in producing bioactive substances during fermentation process such as lactic acids, enzymes, exopolysaccharides (Eps), peptides, bacteriocins (nisin) and others. In dairy food fermentation industry, *Lactococcus lactis* is the well-known lactic acid bacteria used as a functional starter culture that can contribute the food products and play as a vital microorganism due to its organized characteristics such as organoleptic, food flavoring, acidification, and in the creation of various fermented dairy products such as sour cream, cheese, butter and more (Li et al., 2020; Riberio et al., 2016).

Bioactive compound produced by Lactococcus spp. Genera *L. lactis*

L. lactic has the capacity to produce bioactive compounds, have food quality and safety parameters for humans' health (Perez et al., 2022). Bioactive compounds that produced by *L. lactis* are bacteriocins, bioactive peptides, lactic acids, conjugated linoleic acid (CLA), enzymes, vitamins, exopolysaccharides (EPs) and gamma-amino butyric acids (GABA) (Tiwari et al., 2020).

Table 1. Advantageous properties of bioactive compounds produced by *L. lactis* in food quality as well as in better human health.

Bioactive Compounds	**Composition**	**Advantageous properties**	**References**
Gamma Amino Butyric Acid (GAMMA)	Eukaryotes and prokaryotes have four carbons and non-protein amino acids that are naturally distributed.	Maintain viability under acidic condition (pH is 5 under aerobic condition) Serve as crucial inhibitory neurotransmitter	(Ham et al., 2022; Santos-Espinosa et al., 2020)
Exopolysaccharides (EPs)	Are plant and animal origin polymers which	Improved low fat cheese yeast	(Nehal et al., 2019;

	are homopolysaccharides or heteropolysaccharides that composed of mannose, rhamnose and glucose, excreted by LAB.	Fermented dairy products like cheddar cheese, mozzarella cheese, and yogurt	Jeong et al., 2023; Wening et al., 2022)
Bacteriocins	Antimicrobial proteins synthesize by ribosomes such as Nisin	Has antimicrobial activity against gram-positive bacteria	(Takala et al., 2023; Acedo et al., 2018)
Lactic acid and Citrate	During metabolic activity, two organic isomers formed D-Lactic acid and L-Lactic acid	Leading to shelf life due to the big source of prospective advantageous microorganisms Helpful in organoleptic characteristics Specificity in food nutrition improvement	(Abdul Hakim et al., 2023)
Bioactive Peptides	Consist of 2-20 amino acids which originated by residues, have unique and low molecular weight and peptide sequence	Have antimicrobial, antioxidant, antihypertensive activities, anti-obesity, opioid role, blood lowering role, ability to bind minerals, anti-diabetic effects	(Rendón-Rosales et al., 2022)
Conjugated Linoleic acid (CLA)	Omega-6-fatty acid	Have positive impact on health such as Decreasing cancer incidence	Nasrollahzadeh et al., 2023; Dachev et al., 2021)

Vitamins	Essential micronutrients	for the smooth functioning healthy food metabolized immune system	(González et al., 2022)
Enzymes	Broad spectrum catalytic activity	Fermented milk products	(Konkit et al., 2016)

Challenges and Future Perspectives

Globally the antimicrobial resistance is the biggest challenge of using any product as a probiotic. Lactic acid bacteria and its bioactive compound commercially used in fermentation process. Ensuring its safety and suitability for consumption in dairy food products before its use is important. Extreme safety measures required while using LAB and bioactive compounds such as bacteriocins, nisin, etc. Most regulatory frameworks give quite comprehensive guidelines. Furthermore, the researches reveal that the energetic nature of microbial genetics and food interaction complexity increase our food systems' safety as a probiotic. LAB has B-strains which drastically damage the flavor, texture, and safety of fermented dairy products (Bhardwaj et al., 2022; Ganesan et al., 2016). This, we have to provide a holistic approach to safety assessment.

Conclusion

In the development of the dairy food industry *L. lactis* is an effective probiotic, living organism and the most important food fermenter and bioactive compound. LAB is the first microorganism used in the production of antimicrobial compounds like bioactive, used in preservation, technology production and human health advantages to consumers. This study explore that the most common isolated bacteria form cow milk, yogurt and cheese is *Lactobacillus* spp. however *Lactococcus* and *Bifidobacteria* spp. However, the health and economic benefits of the identified LAB should be given more attention in order to improve community health.

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