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A Review on Lactic Acid Bacteria Identification by Molecular and Metagenomics Approaches

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A B S T R A C T

This review aims to understand the new technology used in microbiology and identify various constraints to the development and commercialization of naturally fermented milk products. Lactic acid bacteria mostly determine the advantages of fermented milk and related products for health. Most of them are predicated on targeting a chosen gene or a variable section of the selected gene and amplifying bulk bacterial DNA directly taken from a sample. Therefore, a combination of molecular methods for locating lactic acid bacteria in dairy products is important; utilizing culture-independent techniques has illuminated the diversity in fresh ways until now, foods naturally fermented by unidentified and non-cultural bacteria are focused. The place of origin and varieties of fermented milk products influenced bacterial diversity in samples of spontaneously fermented dairy products. The recognition and characterization of microbiological species are being revolutionized through metagenomic order of sequencing, and a large range of software tools are available to perform taxonomic categorization of this data. The use of culturally independent next-generation sequence and the ways to research that foods have been fermented have rich microbial ecology importance in figuring out the products.

Introduction

Various microorganisms that generate lactic acid are major molecules produced during sugar metabolism and make up the Lactic Acid Bacteria (LAB) (Davis, 1986). *Lactobacilli* naturally thrive and exhibit probiotic activity in the gastrointestinal system (GIT) using dairy items such as cheese and yogurt made from raw milk (Vernoux, 2003). Researchers are more interested in examining the probiotic bacteria's performance in the food sectors and research centers because of its qualities and effectiveness in boosting fermented products (Hooshang *et al.*, 2014). Regarding natural human health, the genera *Lactobacillus* and *Bifidobacterium* receive the most attention

(Fernandez *et al.,* 2003). Phenotypical tests and molecular-based methods are also used to find them (Terzic Vidojevic *et al.,* 2014). Sequencing deep metagenomic data has demonstrated an effective way of giving refinement of taxonomy down to the species and even intensities of strain, estimating the functional potential of the metagenome, and analyzing the microbiota of fermented foods (Niccum *et al.*, 2020). Old dairy products that have spontaneously fermented include specific LAB that can be exploited to produce new fermented dishes and useful dairy products (De Melo Pereira *et al.*, 2020). Recent research has revealed that deep sequencing can enhance our comprehension of how microbes work in fermented dairy products. Using metagenomic-based techniques, such as metabarcoding, shotgun metagenomics, and a key element of the upcoming disruptive revolution in diagnostics for food safety is long-read metagenomics. These technologies have a likelihood and significantly improve the observation and modification of foods and products made by fermentation providing information regarding microbial diversity, population dynamics, and population structures. They should enhance their security as well. Next Generation Sequencing (NGS) was typically used to identify dominant species discovered through culture. For a systematic investigation of gene functions, the metagenome pipeline prediction programme was used (Ye and Doak, 2009). The microbial population of fermented dairy products has been better understood because of high-throughput sequencing (Leech, 2020). The wide range of microorganisms has been examined using techniques such as 16S rRNA gene sequencing is more trustworthy for molecular ecology and diversity analysis studies (Kim and Chun, 2005).

Molecular Identification of Lactic Acid Bacteria

Characterising LAB species has made extensive use of molecular methods. Every technology has advantages, disadvantages, and benefits of its own. Interestingly, no single technique can fully capture the details of intra- and inter-species difference. Consequently, the current tactic is to appropriately identify and characterise LAB strains using a multiphasic method.

Finding the enzymatic processes in LAB that break down biogenic amines was the goal of a random amplified polymorphic DNA investigation (Zheng *et al.*, 2020). Type II restriction fragment length polymorphism utilised to evaluate the variety of the LAB as an alternative to the traditional RFLP approach (Baniyah *et al.*, 2018). The field of microbial typing has seen an increase in the use of amplified fragment length polymorphism (AFLP) over time. A only one primer and restriction enzyme are present in one of the two AFLP variants, while two distinct primers for PCR amplification and restriction endonucleases included. (Zheng *et al.*, 2020). A significant reduction in testing time is achieved with ribotyping, which may be carried out more quickly and easily with an automated riboprinter that identifies microbial species in roughly eight hours (Zheng *et al.,* 2020). One kind separating big DNA molecules using gel electrophoresis in a pulsed field is called pulse field gel electrophoresis (Chen and Gu, 2018). However, the process of analysing the fingerprinting patterns is labor-intensive and complex, requiring workers with specialised training (Neoh *et al.*, 2019). To clarify their distinct functions in different biological processes, non-conventional LAB must be identified. Rep-PCR stands for repetitive extragenic palindromic PCR. It generates highly precise genetic identifiers by joining primers that align repeating specific DNA segments that are scattered throughout the LAB genome at different places (Kaur *et al.*, 2018). Using mass spectrometry The three primary MS platforms for bacterial characterization, matrix assisted laser desorption/ionization time of flight (MALDI-TOF) MS Laser radiations are applied to the target microorganism and propagate through the matrix, liquid chromatography (LC)- MS/MS fingerprinting method that is founded on peptide analysis., and targeted LC–MS/MS employs stable isotope-labeled standards to identify and measure the target peptide (Vinusha *et al.*, 2018). Information on posttranslational changes in proteins,

new amino acid sequence data, and peptide mass are all obtained using the MS technique. French Maroilles cheese made from raw and pasteurised milk, respectively, has had its LAB identified using the MALDI-TOF MS technology (Zheng *et al.*, 2020), further LAB species derived from uncooked sheep and goat milk (Gantzias *et al.*, 2020). The first use of unique peptide markers for each species at the taxon, species, and subcategories levels in the nano-LC-ESI-MS/MS methodology to recognise LAB (Russo *et al.*, 2019). The amplified ribosomal DNA restriction analysis, Uses specific restriction endonucleases to disassemble the amplified ribosomal DNA, this method is used to identify Lactobacillus species (O¨ ztu¨rk and Meterelliyo¨z, 2015).

Automated DNA sequencing is utilised in multilocus sequence typing (MLST) to characterise alleles by sequencing internal segments of bacterial strains' housekeeping gene loci (Maiden *et al.*, 2013). Genetic variations have been identified using the MLST approach (Feng *et al.*, 2018),

Viral load or precise DNA level is measured using real-time PCR and the standard curve technique (Stachelska and Foligni, 2018). The LAB biodiversity in fermented dairy items was evaluated using instantaneous PCR or live action PCR (Mo *et al.*, 2019a). Microarrays can be used to examine how different microorganisms' genomes differ or are similar in terms of gene expression profiles across them (Miller, 2011). A potent method for precisely characterising strains and deciphering LAB activities at the genome level is whole-genome sequencing (WGS) (Buron-Moles *et al.*, 2019). WGS technology is gradually displacing conventional techniques for microbiological classification and characterization, delivering information more quickly and accurately results (Jagadeesan *et al.*, 2019). Currently, single nucleotide polymorphism and gene based methodologies can be employed to analyse WGS data to get results like phylogenetic relationships between the LAB strains and allele matrices (Schu¨rch *et al.*, 2018). The primary technique used to illustrate the variations in DNA denaturation techniques is gradient gel electrophoresis controlled by temperature (T) and denaturing (D) which is non-cultural. A target bacterial population's total DNA is taken, and the hypervariable portions of the 16S rDNA gene are amplified using PCR (Dimitrov, 2019). Probiotic strains were verified to be present in lactic acid compounds that are spontaneously fermenting using this technique (Liang *et al.*, 2018). Moreover, non-conventional LAB species have been identified using this method (Zheng *et al.*, 2020).

Metagenomics

In addition to various human microbiota, metagenomics is evidence that a powerful method for investigating many different natural habitats, including air, soil, water, and plants (e.g., digestive tract, lungs, skin). A growing number of people are using DNA sequencing procedures, which are also getting cheaper. There is enormous development potential for such technologies in food microbiology, considering that high-volume DNA sequencing methodologies have only recently begun to be utilized to understand food microbiological communities (Hajigholizadeh *et al.*, 2020). The cost and difficulty of sequencing an entire microbial genome have been great thanks to Next Generation Sequencing (NGS) technology developments.

A solitary sample of a food, substance, or environmental factor may be used to directly identify whole microbial populations using metagenomics-based techniques, such as metabarcoding, shotgun metagenomics, and extended reading metagenomics, a section of the upcoming revolutionary shift in diagnostics for food safety. This method is changing the characterization of microbiological pathogens found in food, enabling quick identification and containment of food-borne illness breakouts, enhancing public medical care, and reducing expensive recalls. Essential factors for conducting metagenomic assays and data processing are also introduced and situate metagenomic-based approaches concerning traditional detection and diagnostic methods.

Table.1 Tool used in the identification of the microorganisms that produce lactic acid bacteria

DNA Fingerprinting Method	
Random Amplified Polymorphic DNA	*Lactobacillus brevis, Lactobacillus fermentum*
Restriction Fragment Length Polymorphism	*Lactobacillus plantarum, Lactobacillus rhamnosus*
Ribotyping	*Lactobacillus paracasei subsp. paracasei*
Pulse Field Gel Electrophoresis	*Lacticaseibacillus rhamnosus, Lactobacillus delbrueckii subsp. bulgaricus*
Denatured Gradient Gel Electrophoresis	*Lactobacillus helveticus, Leuconostoc mesenteroides,*
Polyphasic Approaches	
16s rRNA gene sequencing	*Lactobacillus delbrueckii ssp. indicus,* and *Lactobacillus delbrueckii ssp. lactics,*
Repetitive Element PCR (rep-PCR)	*Lactococcus lactis ssp. Lactis, Lactobacillus paracasei* subsp. *paracasei*
Fingerprinting	*Lactobacillus casei, Lactobacillus pentosus*
Metagenomics	*Streptococcaceae, Lactobacillaceae*
Whole Genome Sequencing	*Lentilactobacillus rapi subsp. dabitei subsp. nov.*

New prospects from this technique are reviewed along with current limits and knowledge gaps, and metagenomics has recently been used for food safety applications (Mo *et al.,* 2019b). High throughput sequencing, a recent development, has gained importance in food microbiology. Using whole genome sequencing, HTS enables detailed characterization of microbial isolates connected to food, and metagenomic sequencing allows mixed microbial populations in foods to be analyzed without regard to culture. Sequencing of the metagenome can be used to follow specific microorganisms along the food production chain and to shed light on the activity of the microbes at each stage, as shown (Yang *et al.,* 2016).

Metagenomic sequencing is revolutionizing microbiology by examining the makeup of the community directly, quickening the finding of new species, and lowering reliance on culturally specific techniques (Loman *et al.,* 2013). The bacteria population engaged in naturally occurring milk fermentation has been accurately depicted by metagenomic investigations of various fermented milk goods, including buttermilk, cheeses, kefir, and others (Liu *et al.,* 2015). Here is the initial study on the biodiversity of microbes found in Himalayan NFM products utilizing apprehensive metagenomic evaluation. Illumina barcodes 16S rRNAMiSeqamplicon sequencing genes carried out through bacterial community characterization (Romi *et al.,* 2015). For sample multiplexing, the forward primer and barcoded reverse primer were utilized to prepare sequencing libraries, and after that, sequencing carried out was on the IlluminaMiSeq platform. Recently, various food ecosystem has been studied using NGS approaches, which are transforming the study of ecology of microbes.

NGS techniques have become accustomed to research food fermentations and food microbiology. By providing information on the diversity of microbes, population composition, and population movement, these technologies have the potential to dramatically advance the ability to monitor and control food and fermented food products (Dão Pedro de CarvalhoNeto *et al.,* 2020). Alternate strain-level identification approaches are required because the bioinformatics techniques utilized in the investigation are based on metagenome assembly, a computationally taxing procedure. Various software programs have recently been created to taxonomize,

categorize data from metagenomics and calculate taxa levels of abundance (Zolfo *et al.*, 2017). Although metagenomic tools have been compared by numerous groups in the past (Meyer *et al.*, 2019).

The complexity and advantages of the bacterium found in spontaneously fermented milk products have been better understood through a blend of culture-dependent and culture-independent strategies. Naturally, fermented milk product is a part of the diet in human health. Molecular technologies based on 16S rRNA sequencing are becoming more widely available, and high-throughput sequencing revolutionized the understanding of the immense diversity of milk products that naturally ferment and contain lactic acid bacteria. Additional research on dominating bacteria and the advancement of probiotic starting cultures may result in the industrialization of food products.

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