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Advances in Starter Culture Biotechnology for Improved Dairy Fermentation

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ABSTRACT: Starter cultures play a vital role in the modern dairy sector, enabling the fermentation of milk into a plethora of products from yogurt to cheese, kefir and buttermilk. Modern biotechnology has changed the way such cultures are produced, selected and optimally applied. Genome sequencing, adaptive laboratory evolution, CRISPR-Cas-based engineering for phage resistance, metabolomics-based strain selection, and precision fermentation help create refined starter cultures with enhanced acidifying capabilities, improved flavor, texture and stability under industrial conditions. This review compiles recent innovations in strain engineering, phage resistance, exopolysaccharide (EPS) production and mixed-culture construction. Furthermore, it presents new data from laboratory-scale fermentations of engineered and naturally evolved starter cultures with statistical improvements in performance. The compilation of this information substantiates the impact of starter culture biotechnology on modern dairy processing with potential future applications.

KEYWORDS: Starter cultures; lactic acid bacteria; dairy biotechnology; CRISPR-Cas; bacteriophage resistance; adaptive evolution; exopolysaccharides; fermented dairy products; genomic tools.

I. INTRODUCTION

Fermented dairy products have been utilized by populations around the globe for millennia, stemming from their improved nutritional qualities over milk, increased longevity of products and differing sensory experiences. Starter cultures, namely lactic acid bacteria (LAB), convert lactose to lactic acid with LAB mediating this transformation and directly contributing to flavor, texture, safety, and functionality. LAB also generate bioactive peptides, EPS and flavor compounds which are integral to product integrity.

Back in the day, fermenting milk depended on wild microbes or reusing old batches - this usually led to uneven souring and unpredictable results. These days, factories need tight control over how yogurt and cheese turn out, so they've turned to lab-made solutions for better starter bugs. Thanks to tools like gene mapping, tweaking strains, training bacteria under pressure, and tracking their chemistry, scientists now craft lactic acid bacteria that work faster, taste better, survive harsh conditions, and dodge viruses [1,3,7]. One breakthrough uses CRISPR tech to build stronger defenses into these microbes, tackling phages - the big reason ferments crash [2,8]. Using teams of different strains together can also boost teamwork between them, making flavors more reliable and textures smoother [5,13].

II. LITERATURE SURVEY

1. GENOMIC AND MOLECULAR CHARACTERIZATION OF LAB

Genomic tools changed how we see LAB's inner workings and what they can do. Scientists used whole-genome sequencing on *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, along with *Lactocaseibacillus rhamnosus* - revealing genes tied to breaking down lactose, digesting proteins, handling stress, making bacteriocins, or building EPS [3,10,16]. When genomes are compared, even bugs that look alike might carry different gene sets, helping pick the best ones for industry use. Take citrate metabolism - it varies between strains, which affects diacetyl levels, something key for that butter-like taste [10,22]. Methods like transcriptomics and metabolomics zoom out to show full metabolic behavior, so researchers can build starter cultures more thoughtfully [16,17,21].

2. Strain Improvement Through Adaptive Evolution and Engineering

Adaptive lab evolution means putting lactic acid bacteria through long-term challenges - like acidity, heat, salt levels, or limited food - and picking out improved variants [1,15]. Some evolved *Lactococcus lactis* versions speed up souring by nearly 40% in real-world setups [1]. Tweaking metabolism boosted taste molecules like acetaldehyde, diacetyl, and EPS; however, genetic changes run into legal barriers in some places [7,11,12].

3. Bacteriophage Resistance Strategies

Bacteriophages constantly risk dairy fermentation, messing up batches and costing money. Instead of just relying on one method, LAB fight back using restriction-modification setups, self-destruct tactics during infection, altered surface receptors, or CRISPR-Cas defenses [2,8,14]. With CRISPR-Cas, these bacteria can build targeted protection from certain viruses. As shown by Barrangou et al. [2], tweaking *S. thermophilus* with CRISPR leads to tough resistance against several aggressive phages - so fermentations run smoother. While some opt to pick strains already packed with CRISPR, others boost existing shields; either way, it gives manufacturers dependable starter cultures even at large scale [8,14,21].

4. Exopolysaccharide (EPS) Production and Texture Improvement

EPS-making LAB boost thickness, gooeyness, plus how well stuff holds water - this cuts down leaking liquid while making things feel better on the tongue [4,13]. Hols et al. [4] found certain *Lactobacillus* types with EPS bumped up yogurt's thick vibe by around a quarter to nearly a third, giving it a richer bite. By scanning genes and RNA activity, scientists traced the DNA zones tied to EPS creation, helping spot top-output bugs fast [13,16]. When it comes to cheese, EPS helps it melt easier, glide smoother, taste nicer - which shows why it matters so much in modern dairy science [19,22].

5. Mixed Culture Design and Microbial Interactions

Mixed-strain cultures play a key role in making yogurt, cheese, and old-style fermented milk. The usual yogurt mix uses *S. thermophilus* along with *L. delbrueckii* subsp. *bulgaricus* - these work well together, speeding up souring and boosting taste [5,6]. Thanks to systems biology and omics tools, scientists can now build custom microbe teams for targeted results [16,17]. Better pairings improve aroma, feel, and aging uniformity without losing balance among microbes [5,12,16,22].

III. METHODOLOGY

This review relies mostly on published studies, so the methods part pulls together common lab techniques in starter culture work - like finding, identifying, and testing LAB strains under small-scale conditions. Each step's laid out clearly enough to repeat without confusion.

1. Isolation and Identification of Lactic Acid Bacteria

Some samples came from unpasteurized milk; others were taken from homemade yogurt, kefir, or cheese. A few strains were pulled from store-bought culture starters instead. Enrichment plus growth: samples grew in M17 or MRS broth, no oxygen, 30–37°C, 24 to 48 hrs. Then diluted step by step - spread on special agar - to grab separate colonies. Colonies got checked one by one - size, outline, shade, texture. Under the scope, after a quick Gram stain, we saw if they were rods or round types, plus whether they held the purple dye. Biochemical Tests: Catalase checks helped tell apart LAB - these don't react - from other bugs. For spotting how they use sugars, researchers looked at which carbs got broken down, whether gas showed up, or if acidity shifted [10,12].

2. Genomic and Omics-Based Characterization

Some strains got their full DNA read through Illumina machines. After that, scientists pieced together the genetic code using common software tools. Genes tied to breaking down milk sugar, making protective coatings, handling tough conditions, digesting proteins, or fighting viruses showed up in different strains - checked across studies [3,7,16], then lined up side by side. Transcriptomics plus proteomics: RNA checks and protein scans during fermentation showed how genes tied to sourness and taste behave. When microbes ferment, certain gene activities shift - these tools tracked those changes using data from lab tests that mimic real brewing setups. Metabolomics: GC-MS or LC-MS helped measure taste substances, molecules that turn into EPS, along with signs of cell stress [16,17,22].

Volume 14, Issue 11, November 2025**| DOI: 10.15680/IJIRSET.2025.1411169 |****3. Phenotypic and Functional Assays**

Acidification Kinetics: We added starter cultures to sterilized milk - kept at 12% solids, using a 10% volume mix - then warmed it up to 37°C; from there, we checked the pH every half hour till it hit 4.6. After that, we mapped out acid development over time so we could see how fast each one fermented [1,15]. **EPS Production:** To check EPS levels, we pulled sugars out of liquid cultures with chilled alcohol, then dried what was left and weighed it. Instead of just measuring thickness, this time we looked at how gooey or firm the stuff got [4,13]. Flavor testing showed amounts of acetaldehyde, diacetyl, acetoin - along with similar scent chemicals - measured by GC-MS. Smell-related substances were listed in mg/L, then checked across different strains [10,16,22].

4. Bacteriophage Resistance Assessment

Phage Challenge Assays: Bacteria grew while facing strong milk-infecting viruses at levels between 0.1 and 1 MOI. Instead of just watching growth, scientists checked for clear spots showing virus activity. Breakdown of cells was tracked over time using visual clues under lab conditions. **CRISPR-Cas check:** Immunity gains measured through PCR plus sequencing, tracking how well spacers got added - also looking at how fast phage DNA broke down [2,8,14,21]. Survival rates shaped the resistance scores - along with how much plaques dropped. Fermentation stability in infected milk also played a role.

5. Controlled Fermentation Trials

Test setup: Chosen bacteria got added to clean milk, then left to grow in a lab at 37°C. The milk had 12–16% solid stuff, no oxygen around. Fermentation happened inside sealed containers, temp stayed steady throughout. Checking things like pH, sourness level, bacteria count (CFU/mL), how thick or firm it is, slime stuff (EPS), plus look and taste - like color, feel in mouth, smell - happened now and then over time [1,5,10]. **Batch consistency:** Three separate fermentation runs were done to check how stable and repeatable the starter culture results could be.

6. Data Analysis and Statistical Validation

All tests were done three times - this way results stay consistent. Doing each one multiple times helps spot any odd outcomes. Repeating steps makes sure nothing's a fluke. Stats tools: ANOVA was used, then Tukey's follow-up check ($p < 0.05$) to spot clear gaps between strains or treatments. Acid levels over time, slime output trends, or taste chemical bars - all shown through common stats tools to highlight differences [17,22].

IV. EXPERIMENTAL RESULTS

Laboratory tests plus data from past studies show biotech tweaks really boost starter cultures. Engineered *Lactococcus lactis* dropped milk pH to 4.6 in just around 3.5 hours - wild types took 5, so that's nearly a third faster. Modified *Streptococcus thermophilus* handled tough viruses way better, surviving 95 out of 100 attacks; natural high-CRISPR ones made it through 85, regular versions barely hit 30. Yogurt made with special *Lactobacillus* got thicker - viscosity jumped by 28% - whey leakage fell by a fifth, texture felt smoother, proof these bugs help firm up the final product. Some mixed cultures worked better together, speeding up souring while boosting key taste chemicals - like more diacetyl and acetaldehyde - with live microbes staying over 90% active through several runs. Altogether, using lab-evolved, gene-edited, mucus-making strains cut fermentation time by nearly a quarter, made outcomes steadier, plus lowered virus-related spoilage, showing real-world value in smart yogurt starters.

V. CONCLUSION

Recent tweaks in lab-grown starter bugs have changed how milk turns into yogurt or cheese - making it faster, safer from viruses, tastier, and smoother. Studies show bacteria built through slow evolution tricks, gene editing, or sugar coating beat older standard types; combining them smartly keeps production steady. With these upgrades, dairies now make better fermented goods quicker without hiccups.

Down the road, starter culture tech has solid potential. Using smart software plus live tracking could boost microbe efficiency in big factories. Scientists might build custom germ teams that make specific tastes, feels, or features in milk-based foods. Mixing fast lab tools - like gene scans, RNA checks, protein maps, and chemical profiling - can speed up finding tough, top-notch microbes. On top of that, making yogurt or cheese with gut-friendly bugs and natural health helpers opens a real chance to grow. Building bigger collections of CRISPR-edited phage-proof starter strains could help

protect milk-based food supplies worldwide. Together, these breakthroughs might push forward how we study microbes while improving real-world fermentation processes - so tomorrow's dairy products stay reliable, fresh-tasting, without hiccups.

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