Current Research in Food Science 12 (2026) 101318

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Preserving fermented-foods microbial diversity through systematic culturomics for the discovery of multi-strain probiotic candidates

Eskindir Getachew Fentie [a,b,c], Kyeongmo Lim [a], Yohannes Ebabuye Andargie [b], Sihyun Park [a], Jae-Ho Shin [a,b,*]

[a] Department of Applied Biosciences, Kyungpook National University, Daegu 41566, South Korea
[b] NGS Center, Kyungpook National University, Daegu, 41566, South Korea
[c] Department of Chemical Engineering, Addis Ababa Science and Technology University, P.O.Box 16417, Addis Ababa, Ethiopia

ARTICLE INFO

Keywords:
Fermented foods
Probiotics
Microbiome
Metagenomics
Culturomics
Kimchi

ABSTRACT

Fermented foods (FFs) represent complex living ecosystems that deliver viable microbes and bioactive metabolites linked to human health benefits. However, many probiotic strains isolated from FFs fail to reproduce these effects *in vivo*, likely due to the disruption of their natural ecological synergy during isolation. Here, we employed a systematic, ecology-aware culturomics framework to transform the Kimchi microbiome into genome-vetted, multi-species probiotic candidates while preserving ecological fidelity. Specifically, 56 distinct enrichment culture conditions were established using six liquid media (In-situ, MRS, NB, TSB, BHI, BB) across varied redox states (aerobic, anaerobic, microaerophilic), incubation periods (12 h, 66 h), and selective suppressants (CHIR-090, nalidixic acid). Results indicated that In-situ and MRS media under microaerophilic conditions effectively preserved the lactobacilli core, whereas generalist media and aeration expanded taxonomic breadth to include rare taxa. Furthermore, extended incubation (66h) successfully unlocked 107 unique taxa compared to the limited diversity of short incubation (12h). Shotgun metagenomic mining further revealed promising functional properties, including acid tolerance, adhesion modules, and diverse bacteriocin-skewed biosynthetic gene clusters. Crucially, the collection exhibited a strong safety profile: only 1 % of identified risk factors were antibiotic resistance genes (ARGs) on mobile genetic elements (MGEs), and only 4 % represented colocalized ARGs, virulence factors, and MGEs. Systematic-culturomic isolation later yielded over 90 strains, including *Weissella*, *Bacillus*, and *Lactococcus*, significantly expanding beyond standard lactobacilli-centric portfolios. Overall, this study confirms that ecology-aware culturomics captures the functional diversity of the Kimchi microbiome, providing a scalable model for realizing the full therapeutic potential of FFs.

1. Introduction

Fermented foods (FFs) are among the earliest biotechnological applications by mankind and, still today, one of the most promising "whole-food" microbiome interventions (Ecklu-Mensah et al., 2024). Beyond viable microbial taxa of FFs, a matrix of bioactive metabolites also contributes to modulating host physiology (Diez-Ozaeta and Astiazaran, 2022; Tamang et al., 2025). Contemporary reviews and clinical work increasingly position FFs as diet-based levers for gut health. For example, a recent randomized dietary study showed that FFs rich diet increases gut microbial diversity and reduces multiple inflammatory biomarkers in adults (Wastyk et al., 2021). Comparative genomics further reveals that lactic acid bacteria (LAB) and related taxa in FFs share close strain-level relationships with human-associated microbes, placing FFs as potential repositories of gut microbiome strains (Pasolli et al., 2020). However, based on the ISAPP consensus definition, FFs are classified in a different category from probiotics or postbiotics, emphasizing the necessity of in-depth characterization of their microbial isolates (Marco et al., 2021). Nevertheless, the current targeted isolation practice from this source has been limited to some LAB and selected yeast species, thereby limiting the true health-promoting potential of FFs bioagents (Fentie et al., 2025; Rodzi and Lee, 2021).

High-throughput sequencing has demonstrated the remarkable taxonomic and functional depth of the microbiome of FFs, but sequencing alone does not yield deployable strains (Fentie et al., 2022; Valentino et al., 2024). Although targeted isolation from diverse FFs

* Corresponding author. Department of Applied Biosciences, Kyungpook National University, Daegu 41566, South Korea.
E-mail address: jhshin@knu.ac.kr (J.-H. Shin).

https://doi.org/10.1016/j.crfs.2026.101318
Received 12 October 2025; Received in revised form 26 December 2025; Accepted 18 January 2026
Available online 23 January 2026

produced probiotic strains long ago, these strains often failed to colonize the human gut and perform well *in vivo* despite excellent *in vitro* results, falling short of the benefits seen with the original FFs. One plausible explanation for this discrepancy could be the inherent microbial strains' harmony of action with their natural ecology. Thus, maintaining this environmental and ecological stability might improve the likelihood of success *in vivo*. A promising way to achieve this stability is through the application culturomics technique to FF samples (Fentie et al., 2025).

Culturomics integrates systematic, high-throughput, and condition-diverse culturing with rapid identification and genome mining, providing a powerful bridge between microbial ecology and practical applications. By converting metagenomic signals into isolate libraries and annotated genomes, it gives the possibility to detect and characterize organisms with specific functional potential (Y. Huang et al., 2023). To maximize the outcomes of food culturomics, it is essential to ground experimental design in an understanding of microbial community assembly in fermented foods (FFs), a process strongly shaped by environmental filtering and niche differentiation (Louw et al., 2023). Within this ecological framework, microbial succession in FFs emerges from the interplay between the physicochemical properties of the food matrix, changing redox conditions, time-dependent transformations, and inter-microbial interactions. The composition of the raw substrate acts as an initial selective landscape by supplying specific nutrients that preferentially support metabolically adapted, often recurring, core taxa (Caffrey et al., 2024). As fermentation progresses, metabolic activity rapidly alters oxygen availability, transitioning the system from an initially aerobic state toward microaerophilic and, in some cases, anaerobic conditions within the fermenting mass (Heo et al., 2024). These shifts unfold over time, during which early colonizers reshape the environment through substrate depletion and metabolite production, thereby enabling or constraining the establishment of subsequent microbial populations. Concurrently, the accumulation of organic acids and antimicrobial compounds such as bacteriocins imposes additional selective pressure, particularly against acid-sensitive Gram-negative bacteria, further refining community structure (Fentie et al., 2025).

Although culturomics technique has been well-documented in human gut microbiome research (H. Park et al., 2024), only a limited number of studies have been applied to recover microbial diversity from FFs samples. To our knowledge, only two published reports have used this technique to isolate microbial strains. Xu et al. (2020) employed 27 culture conditions, including multiple growth media, supply of oxygen gas, and additions of either modifier or suppressing agents to isolate diverse microbial strains from a traditional Chinese distilled spirit product (PM Baijiu). Similarly, Li et al. (2024) used two incubation temperatures, four microbial growth media, and prolonged enrichment times in their culturomics techniques to isolate diverse microbes from naturally fermented milk.

The dearth of available literature and the current routine reliance on LAB-selective media for probiotic strain isolation, coupled with the underuse of deliberate enrichment media that give a better resolution to rare and condition-dependent taxa, constrains discovery by favoring a narrow subset at the expense of microbial ecological fidelity and taxonomic breadth. Strain-level genomic diversity also remains underappreciated, even though strain-resolved analyses reveal functionally meaningful differences, particularly in relation to their efficacy in the host environment (Armetta et al., 2025). Furthermore, the genomic safety characterization even for isolates obtained through classical isolation techniques often stops at simple ARG/VF mere abundance by overlooking horizontal gene transfer risks. Thus, this study aims to: (1) identify the most significant culture condition that shapes community structure and isolation outcomes from Kimchi, our model FF sample; (2) assemble and annotate MAGs to profile probiotic mechanisms and safety, with specific attention to ARG/VF localization within mobile genetic elements (MGEs); (3) build a diverse culture collection and isolate LAB and co-occurring non-LAB microorganisms. In doing so, this study links ecology-aware enrichment to deployable, genome-vetted strain portfolios, advancing FFs from descriptive omics to translational, multi-species, multi-strain probiotic consortia.

2. Materials and methods

2.1. Sample collection

Samples, and respective raw materials of cabbage kimchi were collected from the four different locations in Daegu, South Korea. The samples were then transported to the point of analysis through an ice box and inoculated into the enrichment media, and isolation of genomic DNA was commenced immediately. The remaining samples were immediately frozen using liquid nitrogen with 20 % glycerol and stored at −20 °C for a period not exceeding two weeks for the isolation of microbial strains.

2.2. Enrichment growth media preparation and inoculation

To enrich the microbial community from kimchi samples, 56 distinct culture conditions were employed (Table 1). Liquid media included nutrient broth (NB), blood broth (BB), MRS, tryptic soy (TSB), brain–heart infusion (BHI), and In-situ broth was prepared by homogenizing fresh kimchi ingredients comprising 70 g napa cabbage, 10 g radish, 5 g green onion, 5 g garlic, 2 g ginger, 3 g red pepper powder, 3 g salt, and 2 g fish sauce per 100 g of total vegetable mass (g per 100 g basis). This vegetable mixture was homogenized with an equal mass of sterile distilled water (1:1 w/w vegetable:water) (Patra et al., 2016). The resulting slurry was filtered through sterile cheesecloth to remove solids, supplemented with sucrose at 2 g per 100 mL of broth (2 % w/v), and the pH was adjusted to 6.5 prior to sterilization at 121 °C for 15 m. Cultures were incubated under anaerobic, microaerophilic, or aerobic conditions. For anaerobiosis, media containing 0.05 % cysteine or sodium sulfide, were nitrogen gas (N_2) sparged (SFig. 1), sealed, sterilized (121 °C, 15 min), and cooled before inoculation (Wagner et al., 2019). Microaerophilic cultures were left to passively deplete oxygen, whereas aerobic cultures were maintained under ambient air. To suppress fast growers, CHIR-090 or nalidixic acid was included as needed. The concentration of nalidixic acid (25 μg/mL) and CHIR-090 (0.1 μM) were selected based on prior reports showing selective inhibition of fast-growing Gram-negative bacteria at these concentrations without broadly suppressing Gram-positive bacteria in complex communities (Claverías et al., 2015; Tejeda-Garibay et al., 2024; Zhao et al., 2023). Temporal effects were assessed by sampling at 12 h and 66 h. All enrichments were inoculated with serially diluted samples prepared in sterile saline.

2.3. DNA extraction and amplicon sequencing

Genomic DNA was extracted from kimchi samples and their respective enrichment media using the DNeasy Fecal® Pro Kit (Qiagen, Hilden, Germany) and quantified using Qubit™ 2.0. For amplicon library preparation, the V4 region of the 16S rRNA gene was amplified from the extracted DNA using the primer pair of 515F/806R. Amplicons were cleaned, indexed, and assessed for size and quality (Agilent 2100 Bioanalyzer). The final amplicon libraries were pooled in equimolar concentrations and re-quantified using the Qubit™ 2.0. Finally, sequencing was conducted on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) with the MiSeq Reagent Kit v3 configured for 300 bp single-end reads at the Next Generation Sequencing (NGS) Core Facility, Kyungpook National University (KNU), Daegu, South Korea.

2.4. Amplicon sequencing bioinformatics analysis

Raw single-end reads were processed using Quantitative Insights into Microbial Ecology 2 (QIIME2, v2024.11) (Bolyen et al., 2019) with the DADA2 plugin (Callahan et al., 2016). Reads were truncated at 280 bp to

Table 1
Diverse culture conditions of liquid enriched growth media.

Sample-Id	Culture_condition	Media	Treatment	Time
InSiAN12	microaerophilic	insitu_media	none	12hr
BHAN12	anaerobic	brain_heart_infusion_broth	sodium_sulfide	12hr
MRAN12	anaerobic	mrs_media	sodium_sulfide	12hr
TSAN12	anaerobic	tryptic_soy_broth	sodium_sulfide	12hr
NA6	aerobic	nutrient_broth	none	12hr
TS6	aerobic	tryptic_soy_broth	none	12hr
BH6	aerobic	brain_heart_infusion_broth	none	12hr
BA6	aerobic	blood_broth	none	12hr
NA6C	aerobic	nutrient_broth	CHIR-090	12hr
TS6C	aerobic	tryptic_soy_broth	CHIR-090	12hr
BH6C	aerobic	brain_heart_infusion_broth	CHIR-090	12hr
BA6C	aerobic	blood_broth	CHIR-090	12hr
NA36	aerobic	nutrient_broth	none	12hr
TS36	aerobic	tryptic_soy_broth	none	66hr
BH36	aerobic	brain_heart_infusion_broth	none	66hr
BA36	aerobic	blood_broth	none	66hr
NA42C	aerobic	nutrient_broth	CHIR-090	66hr
TS42C	aerobic	tryptic_soy_broth	CHIR-090	66hr
BH42C	aerobic	brain_heart_infusion_broth	CHIR-090	66hr
BA42C	aerobic	blood_broth	CHIR-090	66hr
KimA	control	control	control	control
KimB	control	control	control	control
KimC	control	control	control	control
olKim	control	control	control	control
NAM36	microaerophilic	nutrient_broth	cystein	66hr
TSM66	microaerophilic	tryptic_soy_broth	cystein	66hr
BHM36	microaerophilic	brain_heart_infusion_broth	cystein	66hr
BAM36	microaerophilic	blood_broth	cystein	66hr
MRM36	microaerophilic	mrs_media	cystein	66hr
NAAN36	anaerobic	nutrient_broth	cystein	66hr
TSAN36	anaerobic	tryptic_soy_broth	cystein	66hr
BHAN36	anaerobic	brain_heart_infusion_broth	cystein	66hr
BAAN36	anaerobic	blood_broth	cystein	66hr
MRAN36	anaerobic	mrs_media	cystein	66hr
BA30N	aerobic	blood_broth	nalidixic_acid	66hr
NA30N	aerobic	nutrient_broth	nalidixic_acid	66hr
BH30N	aerobic	brain_heart_infusion_broth	nalidixic_acid	66hr
NA42N	aerobic	nutrient_broth	nalidixic_acid	66hr
BA42N	aerobic	blood_broth	nalidixic_acid	66hr
TS36N	aerobic	tryptic_soy_broth	nalidixic_acid	66hr
BH36N	aerobic	brain_heart_infusion_broth	nalidixic_acid	66hr
MR36C	aerobic	mrs_media	CHIR-090	66hr
MR30N	aerobic	mrs_media	nalidixic_acid	66hr
MR36	aerobic	mrs_media	none	66hr
InS12	aerobic	insitu_media	none	12hr
InS12N	aerobic	insitu_media	nalidixic_acid	12hr
InS12C	aerobic	insitu_media	CHIR-090	12hr
MR12	aerobic	mrs_media	none	12hr
MR12C	aerobic	mrs_media	CHIR-090	12hr
MR12N	aerobic	mrs_media	nalidixic_acid	12hr
TS12N	aerobic	tryptic_soy_broth	nalidixic_acid	12hr
NAAN66	anaerobic	nutrient_broth	sodium_sulfide	66hr
BAAN66	anaerobic	blood_broth	sodium_sulfide	66hr
Insitu66C	aerobic	insitu_media	CHIR-090	66hr
Insitu66	aerobic	insitu_media	none	66hr
Insitu66N	aerobic	insitu_media	nalidixic_acid	66hr
InsitumAA	microaerophilic	insitu_media	none	66hr
BHAN66	anaerobic	brain_heart_infusion_broth	sodium_sulfide	66hr
MRAN66	anaerobic	mrs_media	sodium_sulfide	66hr
TSAN66	anaerobic	tryptic_soy_broth	sodium_sulfide	66hr

remove low-quality trailing bases while maintaining full coverage of the V4 amplicon (~253 bp). To normalize sampling effort, the resulting high-resolution amplicon sequence variants (ASVs) were rarefied to a depth of 1031 reads per sample. This threshold was validated by rarefaction curves (SFig. 2), which indicated that ASV richness reached a plateau at this depth for the majority of samples. The raw sequencing depth per sample ranged from 1008 to 6682 reads (median: 3211 reads), and rarefaction resulted in the exclusion of one sample falling below the cutoff. Chloroplast, mitochondrial, and unassigned ASVs were removed prior to taxonomic classification. Finally, taxonomic assignments were performed using the classify-sklearn classifier trained on the SILVA 138-99-NB diverse-weighted reference database at 99 % sequence similarity, and phylogenetic trees were constructed using the SEPP (SATé-enabled phylogenetic placement) algorithm.

2.5. Shotgun metagenomics sequencing

The quality and concentration of the DNA were assessed by NanoDrop and Qubit 3.0 (dsDNA HS). Libraries were prepared using the DNBSEQ-G400RS high-throughput sequencing kit (MGI Tech, China). Approximately 100 ng DNA was enzymatically fragmented to ~300–500 bp (MGIEasy FS), size-selected with beads, end-repaired,

adapter-ligated, PCR-amplified, and purified. The amplified products were then subjected to single-stranded circularization and digestion to generate DNA nanoballs (DNBs). Sequencing libraries were then quantified and quality-checked using the Qubit dsDNA HS Assay Kit and Agilent 2100 Bioanalyzer. Paired-end (PE150) sequencing was performed on the DNBSEQ-G400RS platform (MGI, Shenzhen, China) at KNU, NGS Core Facility (Daegu, Korea).

2.6. Shotgun data bioinformatics analysis

Raw reads were first quality filtered and adapter-trimmed using Trimmomatic v0.39 with a sliding window approach (Bolger et al., 2014). High-quality reads were assembled de novo using MEGAHIT v1.2 (D. Li et al., 2015). Reads were mapped back to assemblies with Bowtie2 to calculate contig coverage. Genome binning was conducted with MetaBAT2, VAMB, and MaxBin2.0, and bins were refined using MetaWRAP. The completeness and contamination of metagenome-assembled genomes (MAGs) were assessed with CheckM. MAGs with $\geq$90 % completeness and <5 % contamination were retained as high-quality MAGs. Taxonomic assignment was then performed using GTDB-Tk (v2.1). MAGs obtained from GTDB-Tk were then used to construct a phylogenetic tree by utilizing iTOL platform. Gene prediction was conducted with Prodigal, and redundant proteins were clustered at 95 % identity using CD-HIT. Probiotic property functional annotation of predicted proteins was then performed using eggNOG-mapper, and dbCAN for CAZyme identification. Moreover, secondary metabolite biosynthetic gene clusters were predicted using antiSMASH (v6.1). Safety-related genomic features were predicted by aligning predicted protein sequences against the CARD (Alcock et al., 2019) and VFDB (Liu et al., 2022) databases to identify ARGs and VFs, respectively. To assess the potential for horizontal gene transfer, MGEs were identified by aligning sequences against the MobileOG (Brown et al., 2022) database. This MGE alignment was performed using DIAMOND blastp with a maximum e-value of $1e^{-5}$. To ensure robust identification, raw hits were rigorously filtered to retain only alignments exhibiting a minimum amino acid identity of 40 % and a query coverage of at least 70 %.

2.7. Isolation of strains and respective genomic identification

Freeze-stored samples were thawed, serially diluted (10^{-6}) in sterile saline, and 100 μL of each dilution was spread on solid media and incubated at diverse culture conditions to isolate the strains. Then, about 15–20 colonies with distinct morphology were selected and purified multiple times till a single isolate was obtained by utilizing the same media. The isolates were then stored in multiple 25 % glycerol stock solutions. The genomic DNA of the isolated strains was then extracted and submitted to Macrogen (Seoul, Korea) for Sanger–based sequencing. Nearly full-length 16S rRNA gene was amplified using universal primers (27F/1492R), followed by bidirectional internal sequencing with primers (785F/907R) on an ABI 3730xl platform, generating a contiguous consensus sequence of $\geq$1350 bp that was finally blasted to the NCBI database for identification.

2.8. Statistical analysis and visualization

All statistical analyses and visualization of metagenomics data were conducted in R software (v4.4.0) using the packages *phyloseq* (McMurdie and Holmes, 2013) and *microeco* (Liu et al., 2021). Alpha diversity was assessed based on species richness (Chao1 index) and diversity (Shannon index). To ensure statistical robustness, group comparisons were performed using the Kruskal–Wallis rank-sum test, followed by Dunn's post-hoc test with Benjamini-Hochberg (FDR) correction to control the false discovery rate. Beta diversity was evaluated through principal coordinates analysis (PCoA) based on the Bray–Curtis dissimilarity index to visualize microbial community composition. The statistical significance of between-group differences in community structure was assessed using Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations. Redundancy analysis (RDA) was further applied to explore the relationships between enrichment culture conditions and microbial community structure. However, it should be noted that due to the exploratory nature of the enrichment design, statistical comparisons were performed on aggregated groups rather than between individual unique conditions. Therefore, reported P-values should be interpreted as indicative of broad ecological patterns guiding isolation strategies, rather than confirmatory variance analysis.

3. Results and discussion

3.1. Effect of enrichment growth media and suppressant

To capture a comprehensive picture of microbial diversity, including rare taxa, multiple liquid growth media were used for enrichment prior to the isolation of individual strains on solid media from kimchi samples. MRS, NB, TSB, BB, BHI, and an In–situ matrix were the enrichment media utilized to achieve this objective. The term “control” was applied to the four kimchi samples as they don't pass through any isolation or enrichment step. In the context of growing the kimchi's core microbiome, mostly lactobacilli, with substantial *Leuconostoc* and *Weissella*, the In–situ medium largely mimics the control sample, according to amplicon sequencing data (Fig. 1A and B, SFig. 3). This may be attributed to the dominant microbes being well adapted to the food matrix and its associated metabolites, enabling them to grow more rapidly in this culture medium than in synthetic media (Torres et al., 2020). MRS medium also promoted the growth of dominating bacteria, with the community composition somewhat different from the control (Fig. 1A and B). Given its innate selectivity of MRS for LAB, and LAB's natural dominance in kimchi samples, this outcome was predictable. However, while these two media effectively represented the dominating taxa, they had limits in portraying the less abundant taxa found in the control sample (Fig. 1B). Generalist enrichment media like the above two also favored the growth of LAB but these media exceptionally provided greater resolution of low-abundance taxa not captured in metagenomic profiles. For example, BHI increased *Lactococcus* and *Enterococcus*, BB expanded non-LAB fractions including *Enterococcus* and *Bacillus*, while TSB and NB enriched *Gammaproteobacteria* such as *Acinetobacter* and *Hafnia–Obesumbacterium*, which are opportunistic pathogens (Fig. 1A and B, SFig. 3).

The flower venn diagram showed a core of 14 taxa (52.2 %) shared across all enrichment media, reflecting a robust kimchi core microbiome. Each medium also contributed unique taxa, most notably BB (36), NB (16), TSB (13), BHI (12), Insitu (11), and MRS (10), while control samples contained 27 exclusive taxa absent from culture-based methods (Fig. 1C). This suggests generalist media can unlock suppressed taxa compared to selective media. Beta diversity (PCoA, 45.1 % variance) showed distinct clustering by medium (Fig. 1D–STable 2), which showed In–situ samples overlapped with controls, indicating microbial ecological fidelity; NB and BB diverged from the lactobacilli-centric cluster; and TSB lay between LAB- and *Proteobacteria*-rich regions. In contrast, alpha diversity (Chao1 and Shannon) displayed comparable levels across media (Fig. 1E and D), a pattern potentially reflecting core microbiome dominance, selective enrichment effects, or distinct medium-dependent conditions (Armetta et al., 2025; Kers and Saccenti, 2022).

In addition to employing different enrichment growth media, selective agents, both suppressants and growth promoters, were introduced to enhance the enrichment potential of the culture conditions. Specifically, we applied CHIR-090 and nalidixic acid to suppress fast-growing Gram-negative bacteria, thereby facilitating the recovery of more rare fastidious taxa. These agents had little visible effect on MRS and In–situ media, since both of which were dominated by LAB, but they clearly reduced Gram-negative proliferation in generalist media, particularly with nalidixic acid (Fig. 1A–Table 1). The observed restriction of Gram-

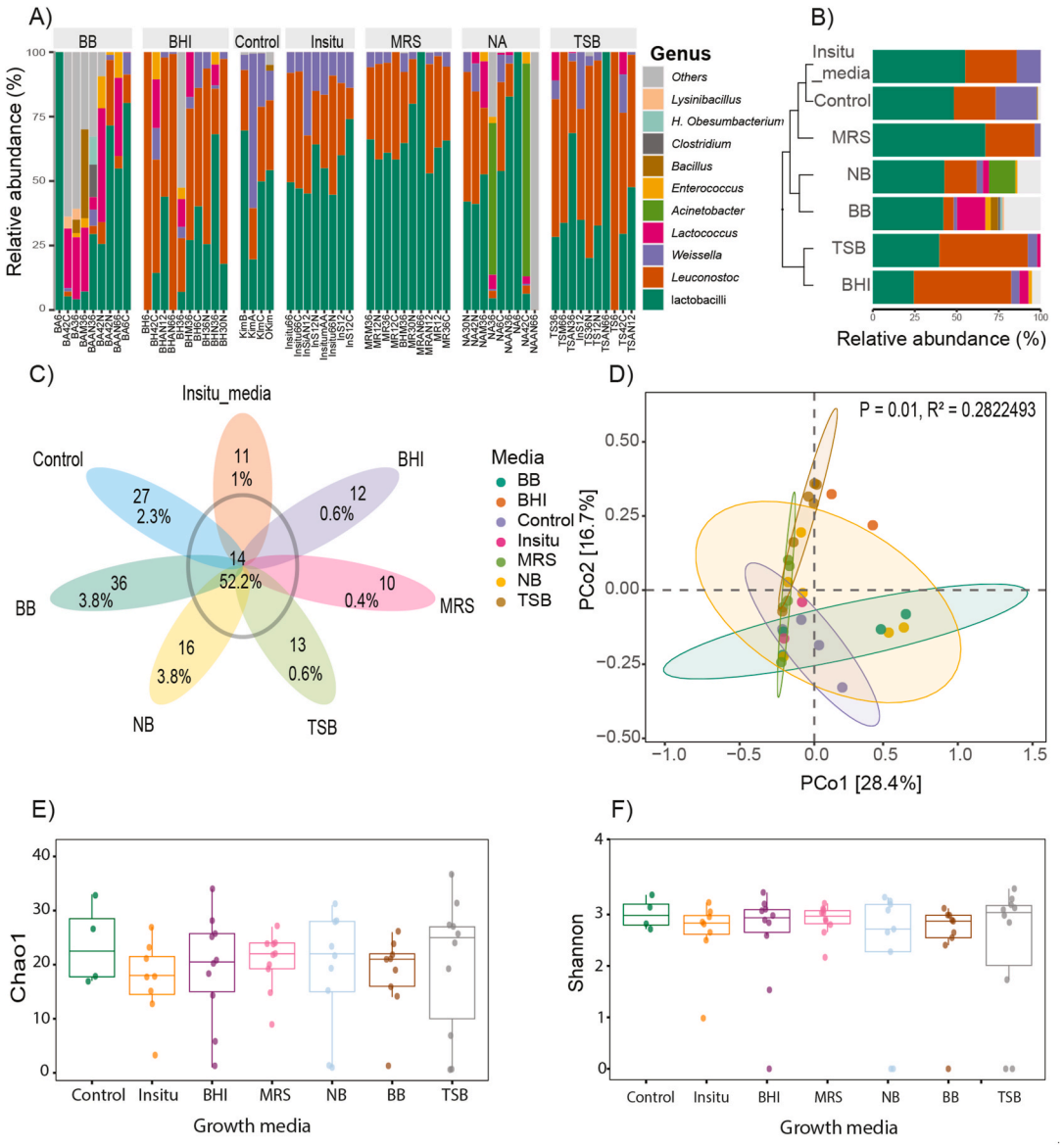

Fig. 1. Effects of enrichment culture media on the microbial community structure. (A) Relative abundance of taxa at the genus level, (B) Mean genus-level relative abundance by growth medium with hierarchical clustering, (C) Venn diagram showing shared and unique genera across media, (D) Principal coordinates analysis (PCoA, Bray–Curtis) illustrating beta diversity patterns, (E–F) Alpha diversity indices (Chao1 and Shannon) across media. Media abbreviations: BB, blood broth; BHI, brain heart infusion; In–situ, kimchi-derived medium; MRS, de Man–Rogosa–Sharpe; NB, nutrient broth; TSB, tryptic soy broth.

negative bacteria can be attributed to the distinct modes of action of the inhibitors: nalidixic acid interferes with DNA gyrase and topoisomerase IV, whereas CHIR-090 inhibits LpxC, a key enzyme in lipid A biosynthesis. Together, these mechanisms are known to preferentially constrain the proliferation of rapidly growing Gram-negative bacteria (Barb et al., 2007; Spencer and Panda, 2023). However, within the limitations of the present dataset and in the absence of detailed time-series measurements, only CHIR-090 exhibited a clear initial suppressive effect on Gram-negative growth in the generalist medium, and this inhibitory effect diminished with prolonged incubation.

Overall, the media selection is the most important factor influencing the recovery of microbial strains from fermented food samples. In–situ media offer the most ecological authenticity, followed by MRS, which best retains the kimchi LAB core. In–situ media, in particular, may be capable of reproducing kimchi microbial consortia that preserve intra- and inter-microbial diversity. BHI and BB promote greater *Firmicute* diversity beyond lactobacilli, whereas TSB and NB create habitats for aerobic and facultative *Proteobacteria*. In practice, a combinatorial plating method that combines In–situ/MRS for microbial ecological fidelity and BHI/BB/TSB for larger taxonomic coverage provides the greatest isolation frontier. These enrichment media, especially when coupled with selecting agents, may be a useful technique for cultivating functionally relevant microbial communities while maintaining species- and strain-level diversity, without the urgent need for single-strain isolation.

3.2. *Effect of oxygen supply and scavenging agents*

The level of oxygen availability is not only critical for the growth of microbial strains but also influences their potential applications. It is especially important to confirm the capacity of probiotic strains to function in the very hypoxic conditions of the human gastrointestinal tract (Khan et al., 2023). To assess the impact of oxygen content on our enrichment growth media, three conditions were established: aerobic, anaerobic, and microaerophilic. Across these settings, the core LAB species remained significant, although their relative abundance varied

according to oxygen availability (Fig. 2A). Notably, the microaerophilic state was most similar to the LAB-dominant community, indicating that lowered redox conditions are better for preserving the original kimchi microbiome. *Lactococcus* and *Bacillus* were among the low-abundance species recovered under these conditions. Anaerobic conditions maintained LAB dominance but had lower evenness and fewer contributions from other *Firmicutes*. In contrast, aerobic conditions resulted in a significant non-LAB abundance, predominantly Enterococcus and Acinetobacter. This reflects the tendency of Gram-negative, fast-growing, opportunistic organisms present in low abundance in the food matrix to flourish when oxygen is accessible (Birmeta et al., 2019).

A core of around 20 taxa (57.6 %) was found in all four oxygen-manipulated growth conditions, confirming the presence of oxygen-insensitive members of the kimchi microbiome. This is to be expected given that LAB dominate the core community, with the majority of them being facultative anaerobes capable of growing in both the presence and absence of oxygen (Heo et al., 2024). Each oxygen state also produced distinct lineages, with aerobic conditions contributing the most (82 taxa; 12 %), followed by anaerobic (21 taxa; 1.2 %), and microaerophilic (10 taxa; 0.7 %) (Fig. 2B). Although aerobic settings increased total diversity, microaerophilic and anaerobic environments were more effective at capturing core taxa as well as the most technologically relevant strains. This was verified by alpha diversity analysis, where aerobic cultures had higher richness but produced uneven communities dominated by rapidly growing microbes (Fig. 2C and D). Conversely, stringent anaerobiosis coincided with lower richness and evenness, potentially reflecting a selection for facultative anaerobes. The microaerophilic condition, on the other hand, appeared to maintain an ecological balance, displaying diversity and richness levels comparable to the control samples. Beta diversity analysis visualized these distinct compositional trends: microaerophilic samples were positioned near the controls, aerobic samples exhibited divergence, and anaerobic samples formed a distinct, tightly grouped cluster (Fig. 2E). Similarly, the RDA plot highlighted a tendency for aerobic conditions to co-occur with *Acinetobacter* and *Lactococcus*, whereas lactobacilli appeared to align more strongly with microaerophilic and anaerobic conditions (Fig. 2F).

In addition to reducing oxygen supply, two well-known oxygen scavengers, cysteine and sodium sulfide, were used to improve

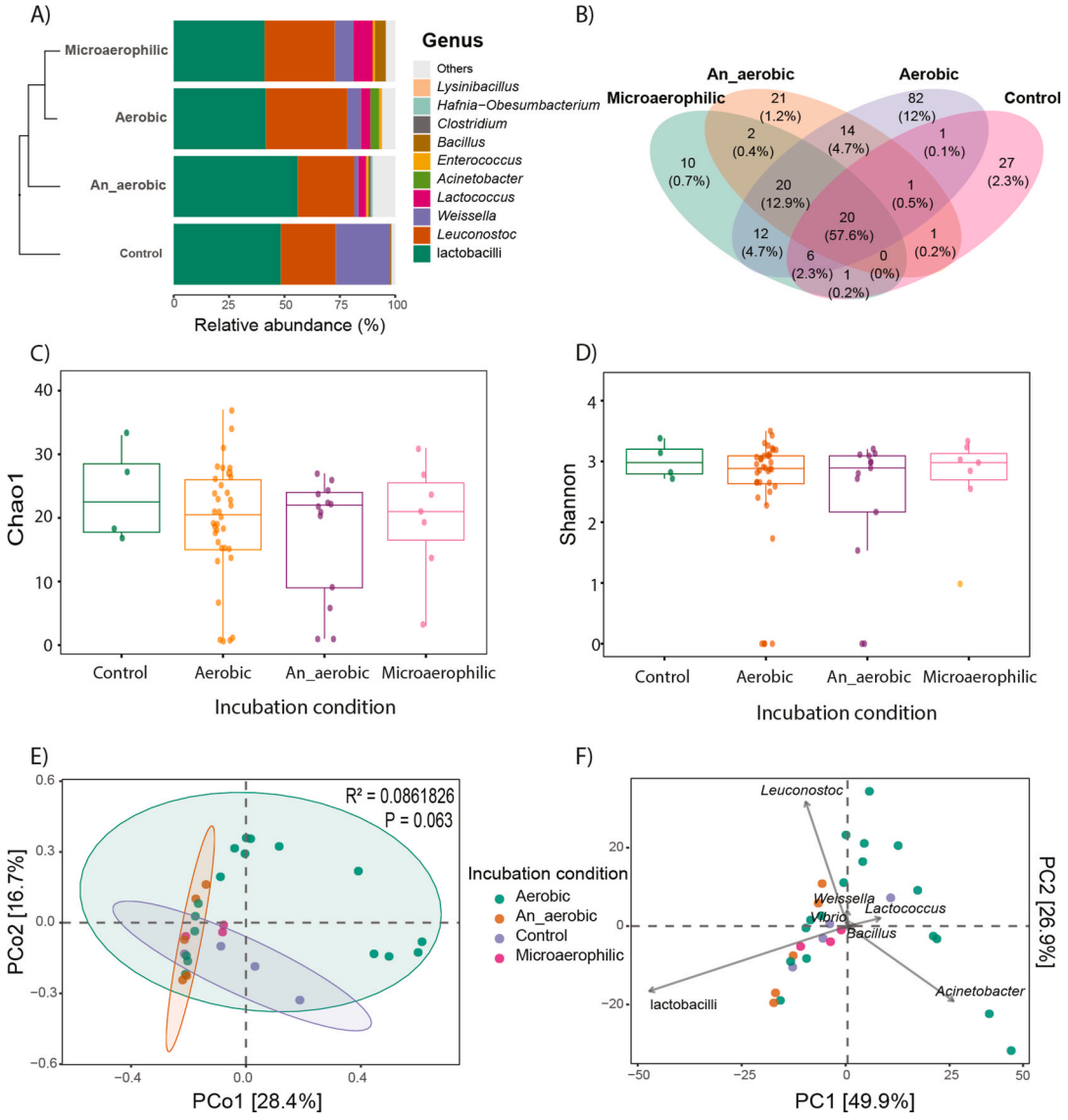

Fig. 2. Influence of oxygen supply on enrichment microbial community composition. (A) Mean genus-level profiles to show microbial composition, (B) Venn diagram to demonstrate shared and unique genera, (C–D) Alpha diversity indices (Chao1 and Shannon) to show inter sample differences, (E) Principal coordinates analysis (PCoA, Bray–Curtis) illustrating beta diversity, (F) Constrained ordination bi–plot (RDA) to show taxon–oxygen supply associations. Media abbreviations: BB, blood broth; BHI, brain heart infusion; In–situ, kimchi-derived medium; MRS, de Man–Rogosa–Sharpe; NB, nutrient broth; TSB, tryptic soy broth.

anaerobic conditions. In these tests, it was determined that the additives' impact went beyond simply causing anaerobiosis; they also influenced the microbial community structure of the enrichment media (Fig. 1A). Notably, sodium sulfide effectively inhibited Gram-negative bacteria while retaining anaerobic taxa. This action is most likely due to the generation of hydrogen sulfide, which inhibits cytochromes and other metal-based enzymes that are especially sensitive in many Gram-negative bacteria (Korshunov et al., 2016). Thus, sodium sulfide's selective influence stems from both its ability to produce anaerobic conditions and its direct inhibitory activity on Gram-negative bacteria. Overall, oxygen availability determines both which microorganisms grow and how evenly they are distributed. To capture the whole range of bacteria from fermented functional foods, a balanced mixed-oxygen method is proposed to produce representative probiotic and post–biotic candidates. For instance, the best culture conditions for Kimchi samples depend on the desired outcome: microaerophilic settings maintain microbial ecological fidelity, aerobic conditions extend taxonomic coverage, and anaerobic conditions enrich for LAB. Similarly, oxygen scavenging agents shape microbial structure under strict anaerobiosis: cysteine gently lowers redox potential, supporting a diverse lactobacilli-centered community, whereas sodium sulfide imposes a stronger reduction, suppressing aerobic Gram-negatives and concentrating LAB, resulting in the most distinct anaerobic signature in both composition and diversity.

3.3. Effect of inoculation time

The microbial community composition was also affected by the enrichment time. After 12 h of incubation time, the microbial profiles remain lactobacilli-centric and closely resemble the control sample (Fig. 3A). However, after 66h of incubation period, the composition shifted from lactobacilli-centric to an inclusion of more non-LAB species, most notably *Acinetobacter*, *Bacillus*, and *Lactococcus*. This strong time-based skewness was also observed in the showing about 107 unique taxa, which contribute about 18.9 % the total taxa composition (Fig. 3B). This is mainly due to the prolonged incubation period, which promotes late-appearing spore forms, and facultative non-LAB that are rare or suppressed at the early stage of the incubation period. The

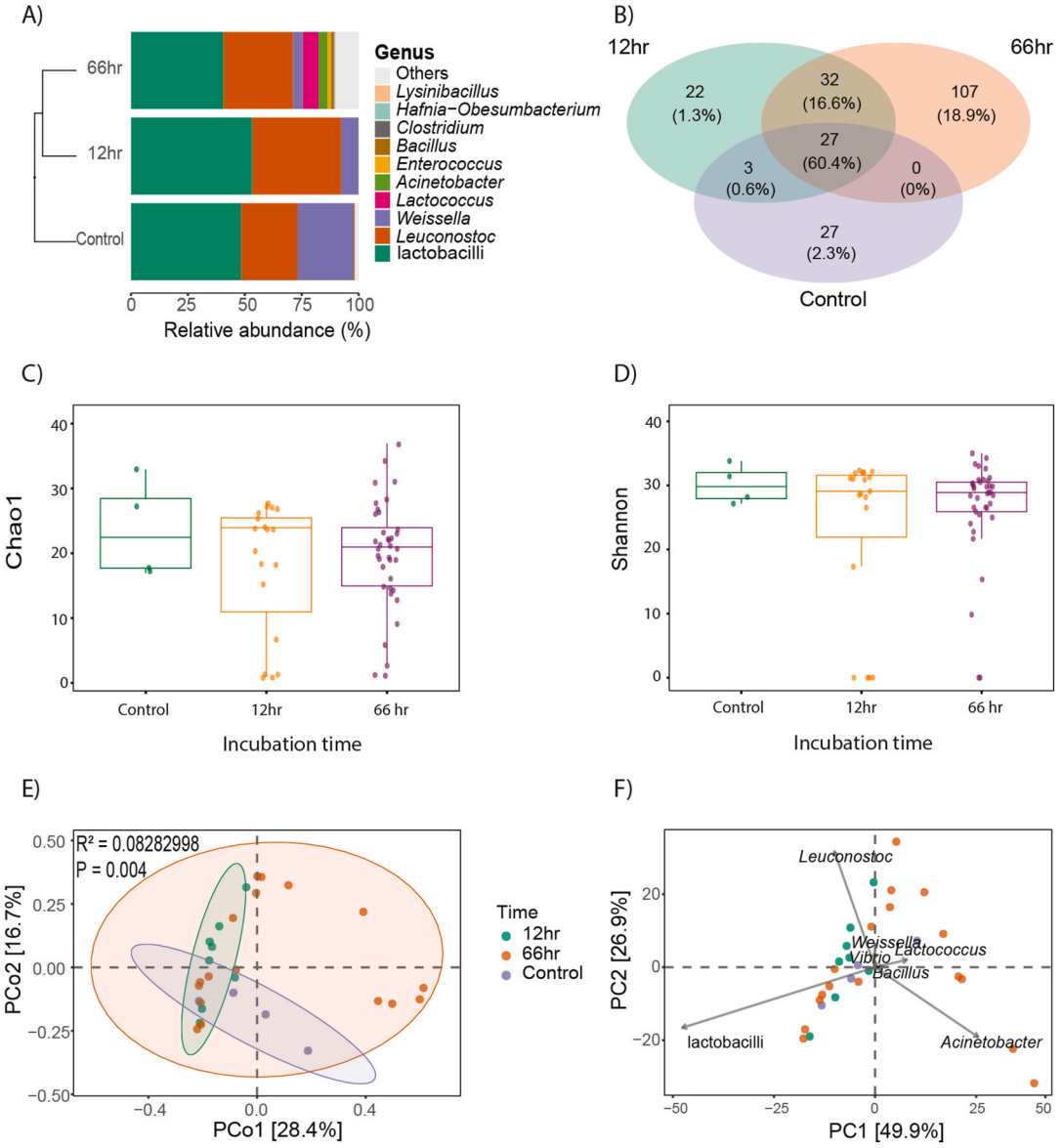

Fig. 3. The impact of the incubation time (12 h and 66h) on the recoverable diversity. (A) Mean genus microbial profiles across incubation time, (B) Venn diagram showing unique and shared taxa, (C–D) Alpha diversity (Chao1, Shannon) inter-sample microbial diversity, (E) Principal coordinates analysis (PCoA, Bray–Curtis) showing beta diversity, (F) Ordination bi–plot (RDA) showing the association between incubation time and microbial taxa.

underlying mechanism of the outcome would be the "competitive release" notion, which deals with the gradual elimination of inhibition and resource re-partitioning (Lund et al., 2020). Moreover, an observed trend of higher richness and evenness was noted at the extended incubation time (Fig. 3C and D). Consistent with this, samples from the 66 h incubation period exhibited distinct separation from the control and 12 h samples in the beta diversity analysis (Fig. 3E–STable1). This shift appeared to coincide with the relative enrichment of *Acinetobacter*, *Bacillus*, and *Lactococcus* communities (Fig. 3F).

Generally, incubation time affects both which microbes grow and how evenly they coexist. Short incubations maintain the kimchi LAB signature, but if the objective is to get a better resolution, extended incubations diverge compositionally yet unlock a substantially broader culturable repertoire. Nevertheless, it is also important to acknowledge that while extended incubation (66 h) unlocks microbial diversity, it also enriches opportunistic pathogens such as *Acinetobacter*. Consequently, if the major objective is to isolate classical probiotics from FFs, avoiding excessively long incubation times may be advisable to mitigate these safety risks and maintain a lactobacilli-dominant core. However, in the context of culturomic discovery, avoiding these conditions would inadvertently exclude rare but potentially beneficial taxa (e.g., *Bacillus* and *Lactococcus*). Furthermore, microbial growth and its associated

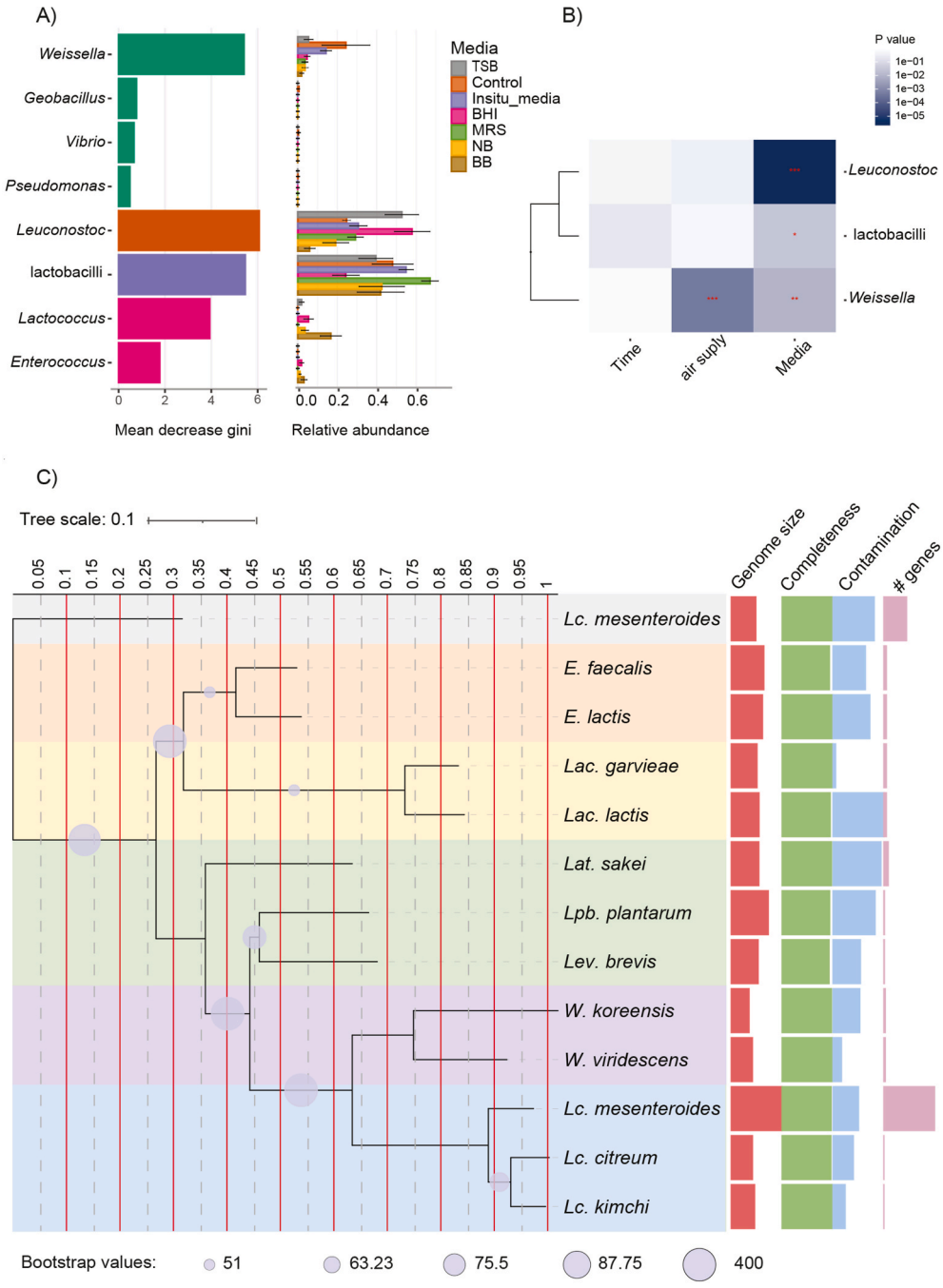

Fig. 4. Ecological drivers and strain resolved high quality metagenome assembled genome (HQ-MAGs). (A) Random-forest markers distinguishing the growth media together with corresponding relative abundance distributions, (B) Heat map showing a considered factor importance (p-values) on core–LAB genera, (C) Phylogenomic tree of representative HQ-MAGs with per-genome metrics of genome size, completeness, contamination, and gene counts. Bootstrap support is indicated by node symbols. Media abbreviations: BB, blood broth; BHI, brain heart infusion; In–situ, kimchi-derived medium; MRS, de Man–Rogosa–Sharpe; NB, nutrient broth; TSB, tryptic soy broth.

metabolic product dynamics in enrichments were not monitored in this study due to the high-throughput scale of the enrichment matrix. Future work combining time-resolved metabolite measurements with metagenomics will be essential to mechanistically connect the ecological transitions observed here to underlying fermentation chemistry.

3.4. Functional properties of high–quality metagenomics assembled genome (MAG)

Differences among growth media were most strongly marked by species of *Weissella* and *Leuconostoc*, followed by lactobacilli (Fig. 4A). Practically, this means that the core microbiome can be captured in almost every enrichment media tested under this investigation. Importantly, the type of medium proved to be the strongest determinant in cultivating these taxa, with aeration exerting a secondary while incubation time contributed only a minor additional effect (Fig. 4B). Utilizing this information, 32 culture conditions were selected for further shotgun metagenomics sequencing to enhance strain-level resolution and facilitate functional genome mining for the assessment of probiotic and safety attributes (STable 1). The MAG set was predominately composed of genera associated with Lactic Acid Bacteria (LAB), encompassing key functional groups identified in the enrichment samples. *Leuconostoc* species, specifically *Lc. citreum*, *Lc. kimchi*, and a strain of *Lc. mesenteroides*, formed a cohesive clade supported by high bootstrap values (Fig. 4C). Similarly, members of the family *Lactobacillaceae*, represented here by *Lactiplantibacillus plantarum (Lpb. plantarum)*, *Latilactobacillus sakei (Lat. sakei)*, and *Levilactobacillus brevis (Lev. brevis)*, mirrored the core LAB diversity previously identified by amplicon sequencing. Distinct phylogenetic branches were also resolved for *Weissella* (*W. koreensis*, *W. viridescens*), *Lactococcus* (*Lac. lactis*, *Lac. garvieae*), and *Enterococcus* (*E. faecalis*, *E. lactis*), confirming the recovery of a broad range of lactic acid producers.

Most bins satisfied high-quality MAG requirements (>90 % completeness and <5 % contamination), though distinct variations in genomic architecture were observed (Fig. 4C). Unlike the relatively uniform genome sizes among the *Weissella* and *Lactobacillaceae* representatives, substantial genomic expansion was noted within the *Leuconostoc* clade. In particular, one *Lc. mesenteroides* MAG exhibited an

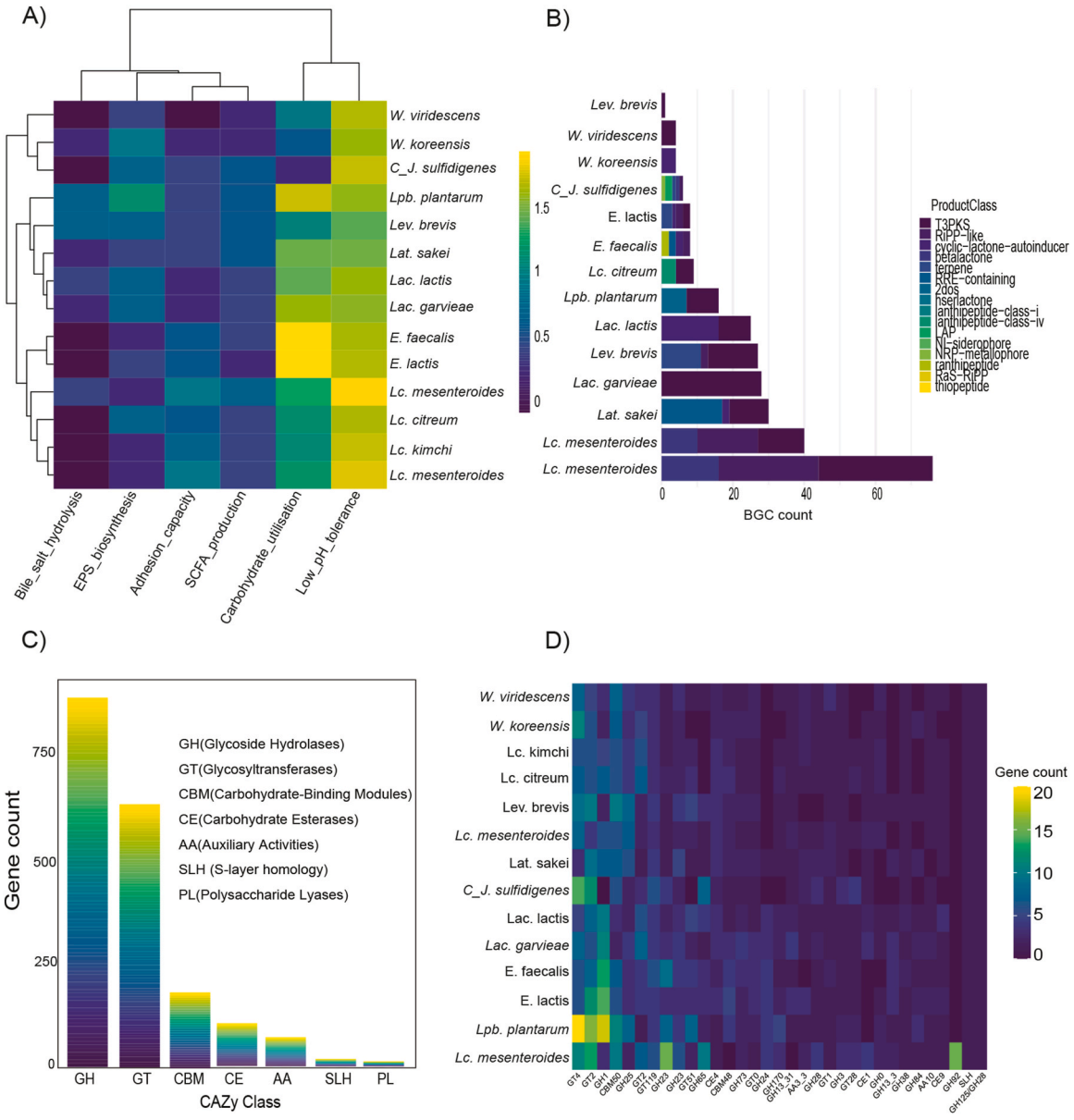

Fig. 5. Functional potential of HQ-MAGs. (A) Heatmap of probiotic-related features per MAG, (B) Secondary-metabolite biosynthetic gene clusters (BGCs) per genome by class, (C) A bar chart representing CAZy class totals, (D) Heatmap depicting the CAZy family's diverse glycan-use repertoires.

exceptionally large genome size and gene count compared to its counterparts. This genomic plasticity may indicate the acquisition of accessory genes facilitating metabolic adaptation or environmental flexibility within the fermentation matrix (Candeliere et al., 2024). Overall, the high resolution of the assembly combined with robust phylogenetic placement confirms that the recovered MAGs represent the ecologically dominant and biotechnologically significant fraction of the kimchi microbiome.

3.4.1. Probiotic properties of strains

Candidate probiotic strains are generally expected to exhibit functional properties such as adhesion capacity, pH tolerance, short-chain fatty acid (SCFA) production, exopolysaccharide (EPS) biosynthesis, bile salt hydrolysis (BSH), and metabolic versatility regarding carbohydrate sources (Fentie et al., 2024; Wang et al., 2024). While *in vitro* validation is essential, genome-wide functional profiling provides a robust framework for selecting putative probiotic strains (Castro-López et al., 2021). The probiotic potential of the high-quality MAGs (HQ-MAGs) was evaluated by mining functional genes against the EggNOG, antiSMASH, and dbCAN databases (Fig. 5). Consistent with their isolation from a fermented food environment, nearly all identified bins exhibited high genomic potential for low pH tolerance and carbohydrate utilization. This aligns with the selective pressures of the fermentation niche, where adaptation to acidic conditions and complex substrate metabolism is critical for survival. *Enterococcus* species (*E. faecalis* and *E. lactis*) and *Leuconostoc* species (*Lc. mesenteroides* and *Lc. kimchi*) displayed particularly strong scores for carbohydrate utilization and acid tolerance, reflecting their active metabolic roles in vegetable fermentation.

In contrast to the widespread resistance to acid stress, the capacity for bile salt hydrolysis (BSH) appeared limited across the dataset. With the exception of minor signals in *Weissella* spp., most bins displayed low functional completeness for BSH pathways (Fig. 5A). This suggests that while these strains are well-adapted to the fermentation environment, their ability to hydrolyze bile salts in the gastrointestinal tract may be strain-specific or less pronounced than their acid tolerance. Regarding colonization factors, distinct variation was observed among the lactic acid bacteria (LAB). *Lpb. plantarum* emerged as a standout candidate, exhibiting a unique combination of moderate-to-high scores for both adhesion capacity and EPS biosynthesis, alongside robust carbohydrate metabolism. This profile distinguishes *Lpb. plantarum* from other LAB in this cohort, such as *Lat. sakei* and *Lev. brevis*, which showed comparatively lower genomic potential for adhesion and EPS production. The presence of these genes in *Lpb. plantarum* supports its potential to be considered as probiotic candidate capable of temporary gastrointestinal colonization and mucosal interaction (Goh, 2009; Senanayake et al., 2025). While *Enterococcus* bins displayed robust metabolic toolkits and stress resistance, their application requires stringent safety assessments given the genus's association with opportunistic pathogenicity.

The genomic potential for secondary metabolite production within the identified metagenome-assembled genomes (MAGs) was assessed using antiSMASH. The analysis predicted a diverse array of putative biosynthetic gene clusters (BGCs) across the recovered taxa. Based on the *in silico* annotations, the most BGC-rich genomes were assigned to *Lc. mesenteroides*, followed by *Lat. sakei*, *Lac. garvieae*, and *Lev brevis*. Additional clusters were identified in *Lpb. plantarum*, *Lac. lactis*, *Lc. citreum*, *E. faecalis*., *Weissella* spp., and *C_J. sulfidigenes* (Fig. 5B). The predicted BGC composition appeared to be dominated by clusters associated with Type III polyketide synthases (T3PKS), RiPP-like (Ribosomally synthesized and Post-translationally modified Peptides), and cyclic-lactone-autoinducer classes. Notably, putative lanthipeptide (class I and IV) and other bacteriocin-associated clusters (e.g., LAP, thiopeptide) were also detected, particularly within the *Lactobacillaceae* and *Leuconostocaceae* families. If expressed, these gene clusters suggest a potential for bacteriocin synthesis, a trait frequently observed in LAB strains that contributes to antagonism against foodborne pathogens and colonization resistance (Zhang et al., 2023).

Furthermore, recurring fractions of clusters annotated as terpenes, betalactones, and (metallo)-siderophores were identified across the dataset (Fig. 5B). The presence of putative T3PKS and terpene elements is of particular interest, as these classes have been previously linked to the production of lipophilic metabolites that may support membrane integrity and stress responses (Shah et al., 2025); however, experimental validation is required to confirm these functions in the specific MAGs described here. Additionally, several genomes contained "cyclic-lactone autoinducer" predictions. These sequences may represent distant homologs of quorum-sensing (QS) systems, potentially hinting at cell-to-cell communication capabilities within these Gram-positive backgrounds (Fig. 5B). Overall, the antiSMASH landscape suggests a functional potential in which LAB-dominated MAGs could contribute to a multi-layered antimicrobial metabolite network. This predicted genomic architecture provides a mechanistic hypothesis for the probiotic value of these strains, identifying high-priority targets for future isolation and phenotypic characterization.

The carbohydrate-active enzyme (CAZyme) profile of the identified HQ-MAGs was dominated by glycoside hydrolases (GH) and glycosyltransferases (GT), followed by a substantial contribution from carbohydrate-binding modules (CBM) and carbohydrate esterases (CE) (Fig. 5C). Family-level resolution revealed that GT2 and GT4 were the most abundant glycosyltransferases across the majority of identified MAGs, with particularly high counts observed in *Lpb. plantarum* (Fig. 5D). These families support exopolysaccharide synthesis, a trait essential for stress tolerance, biofilm formation, and immune modulation within the gut environment (Z. Huang et al., 2021). Among the glycoside hydrolases, the GH1 family was ubiquitously enriched, particularly in *Lpb. plantarum*, *E. lactis*, and *Lc. mesenteroides*. Together with minor contributions from GH13 and GH3, this enzymatic repertoire enables the utilization of vegetable-derived oligo- and polysaccharides (such as β-glucosides), promoting colonization and potential cross-feeding activities (Møller et al., 2014). Furthermore, the heatmap highlights a distinct enrichment of peptidoglycan-associated families, specifically GH25, GH23, and CBM50 (LysM domains). The high abundance of GH25 and CBM50 implies active cell-wall turnover and autolysis capabilities; these processes release peptidoglycan and teichoate fragments with documented immunomodulatory effects and may enhance co-aggregation with pathogens to promote competitive exclusion (Dik et al., 2017). Additionally, the presence of S-layer homology (SLH) domains in select strains (e.g., *Lpb. plantarum*) further supports surface adhesion potential. Mucin-targeting families (such as GH101/109) were notably absent from the dominant features, reinforcing the safety profile regarding host mucus barrier integrity (Fig. 5D). Overall, the eggNOG, dbCAN, and antiSMASH profiles complement each other, indicating that the MAGs possess a robust probiotic signature well-suited for gut-ecosystem support.

3.4.2. Safety properties of probiotic strains

The mere existence of antibiotic resistance genes (ARGs) and virulence factors (VFs) in probiotic candidates is not inherently alarming, provided these elements are intrinsic to the chromosomal DNA and lack mobility (Xu et al., 2025). Significant safety concerns arise only when these determinants colocalize with mobile genetic elements (MGEs), thereby posing a risk of horizontal gene transfer (Merenstein et al., 2023). In this study, the intersection of these genomic features revealed that the majority of identified elements were isolated MGEs (59 %) or VFs (20 %) unrelated to mobility mechanisms. Crucially, only 1 % of the total pool represented ARGs located on MGEs, while 4 % represented a colocalization of ARGs, VFs, and MGEs (Fig. 6A).

The phylogenetic distribution of these risks was highly taxon-specific. While *Lat. sakei* accounted for the highest volume of total identified elements (Fig. 6A), the specific association of risk factors with mobile elements was most pronounced in *E. faecalis*, *C._J. sulfidigenes*, and *Lc. mesenteroides*. These taxa exhibited the highest density of

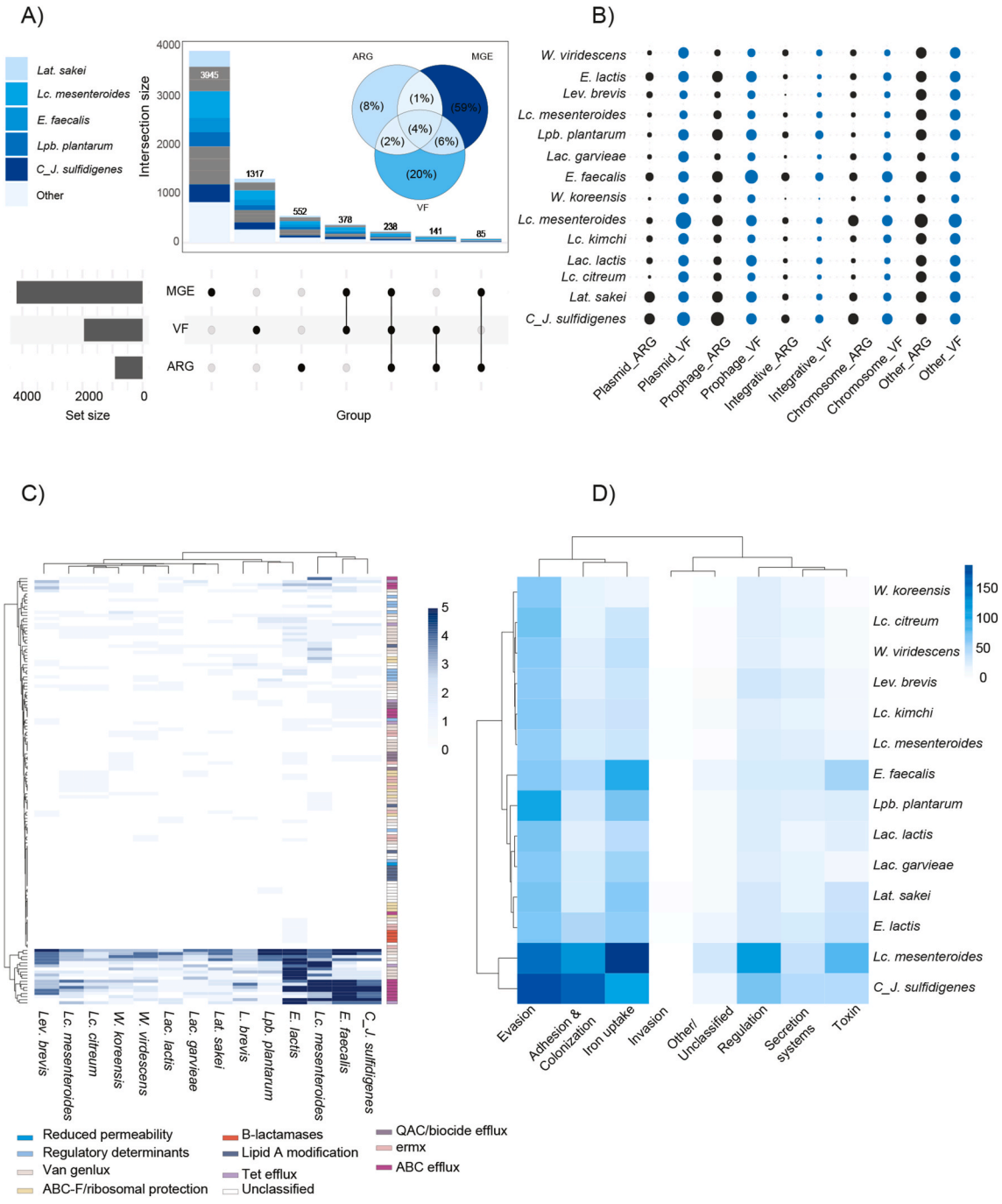

Fig. 6. Safety landscape of HQ-MAGs (A) UpSet plot of intersections among antibiotic-resistance genes (ARG), virulence factors (VF), and mobile genetic elements (MGE) with group contributions, (B) Bubble chart partitioning ARG/VF counts by genomic context across taxa, (C) Heatmap showing the ARG mechanism of action, (D) Heatmap demonstrating the VF category.

plasmid- and prophage-associated VFs and ARGs (Fig. 6B). In contrast, bins classified as *Lev. brevis*, *Lpb. plantarum*, and *W. viridescens* displayed a favorable safety profile, characterized by a distinct lack of plasmid- or prophage-borne resistance determinants.

To assess the functional risks associated with these mobile determinants, we performed a mechanism-level screening of the resistome (Fig. 6C). The analysis revealed a stark dichotomy between potential pathogens and food-associated LAB. *E. faecalis* and *C._J. sulfidigenes* exhibited high-intensity signals for high-risk mechanisms, including β-lactamases, vancomycin resistance clusters (*van* genlux), and diverse efflux pumps (Tet and QAC/biocide). Notably, *Lc. mesenteroides* also displayed a substantial burden of mobile resistance genes, specifically β-lactamases and ABC efflux systems, diverging from the typically low-risk profile associated with the genus. Conversely, the majority of other LAB MAGs (e.g., *Lpb. plantarum*, *Lat. sakei*) showed sparse, mechanism-limited profiles dominated by intrinsic features such as regulatory determinants and reduced permeability, consistent with housekeeping-level resistance rather than acquired transmissible traits (Forster et al., 2022).

Finally, the virulence landscape was evaluated to distinguish between pathogenic potential and commensal adaptation (Fig. 6D). While adhesion and colonization factors were ubiquitously detected across most bins, reflecting necessary traits for gut residence, the presence of aggressive virulence markers was rare. High-risk categories, such as toxins and specialized secretion systems, were conspicuously absent in *Lpb. plantarum*, *Lat. sakei*, and *W. koreensis*. However, *Lc. mesenteroides* and *C._J. sulfidigenes* were exceptions, showing elevated intensities for toxin production and secretion systems (Fig. 6D). Collectively, these results confirm that with the exception of specific *Enterococcus*, and *Clostridium*-related bins, the identified LAB candidates possess a genomic architecture consistent with safe, food-grade organisms, devoid of the mobile resistome and toxigenic potential that would preclude their use as probiotics.

3.5. Systematic culturomics based strain isolation

The allocation of plates and culture conditions was deliberately designed based on microbiome and functional analysis results of the enriched samples to optimize the recovery of expected taxa. Nevertheless, due to the known limitation of metagenomics in detecting the "rare biosphere", it was also critical to preserve flexibility in isolating unexpected taxa that may develop under unconventional or optimum settings (Lagier et al., 2018). This work ultimately recovered more than 90 bacterial isolates representing six major clades, ranging from core lactic acid bacteria (LAB) to diverse non-LAB lineages characteristic of kimchi fermentation (Fig. 7A), consistent with the findings of metagenomic analysis (Figs. 1–4). Among all examined media, the strains of *Lat. sakei* (n = 20) and *Lc. mesenteroides* (n = 12) were predominant in the collection (Fig. 7B). This prevalence, regardless of cultured media and conditions, originates from the dominance in the original samples, as previously indicated in classical kimchi succession models and targeted isolation experiments (Lee et al., 2020; J. Park et al., 2024). However, other than the above two strains, the majority of lactobacilli and *Leuconostoc* strains were isolated in the In–situ and/or MRS growth media (Fig. 7B). More specifically, strains of *Lc. carnosum*, *Lc. curvatus*, *Lev. brevis*, *Lpb. plantarum*, and *Lc. gelidum* were isolated from the above-mentioned growth media most of the time in microaerophilic environment. However, a strain of *Lc. lactis* was surprisingly isolated from a non-selective BA growth medium by overcoming the struggle with the other rapidly growing microorganisms. Overall, the dominance of LAB isolates in the majority of the growth media and culture conditions is expected from the FF sample, including alcoholic beverages (Fentie et al., 2022).

A strain of *W. virdescens*, *W. confusa*, *W. kandleri* were also dominantly isolated in the In–situ and/or MRS growth media in microaerophilic culture conditions. *Enterococcus lactis,* on the other hand, was peculiarly isolated from In–situ growth media. Comparative genomic study indicates that numerous strains of this species have demonstrated safety record, warranting their consideration as next-generation probiotics (Lu et al., 2023; Tadesse et al., 2024). Moreover, strains of *Bacillus* species, particularly those that might have health-promoting properties, such as *B. subtilis*, *B. cereus*, and *B. amyloliquefaciens*, as well as opportunistic pathogens such as *B. velezensis*, *B. halotolerans*, *B. pumilus*, and *B. safensis*, were isolated mainly from BA and TSA growth media, mainly at aerobic culture conditions (Fig. 7A). These strains are one of the showcases of the extended isolated microbial taxa outside LAB recoveries, showing ecological niches that are commonly neglected when depending exclusively on selective LAB media and focusing on the dominating taxonomic (Fig. 7A–STable 3).

Among the isolated strains *Lc. gelidum*, *Lev. brevis*, *W. kandleri*, *E. lactics,* and some *Bacillus* species were the isolates recovered from kimchi samples that had not been previously documented. Additionally, the majority of the strains had microgenomic (sub-strain) diversity, highlighting sub-strain level differences that may contribute to

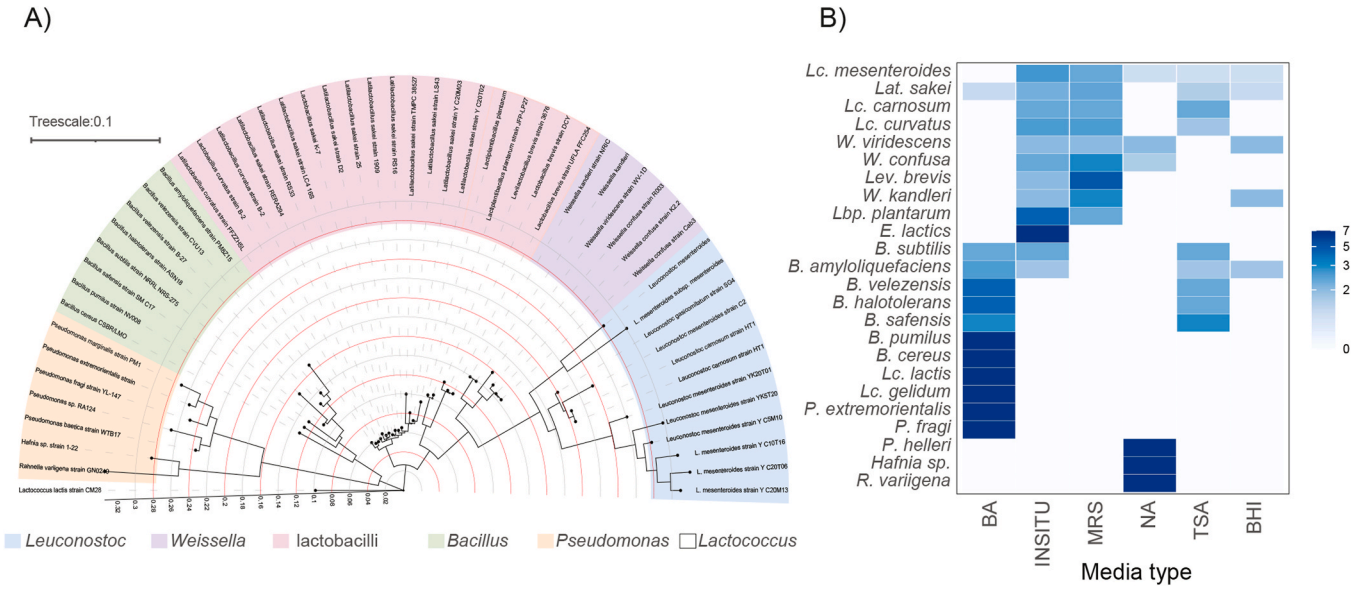

Fig. 7. Solid media isolation outcomes. (A) Phylogeny of cultured isolates colored by genus, (B) Heat map showing the isolation and respective growth media matrix.

significant functional properties. By contrast, taxa reported in other kimchi studies, mainly *Pediococcus*, were not recovered here (Ko et al., 2023; Mun et al., 2024). The most plausible explanations might be: (i) limitations in experimental design, particularly the lack of deliberate temperature modulation as a culture-condition variable, and/or (ii) true absence of *Pediococcus* in the original sample. Meanwhile, a few pathogenic bacteria, including strains of *Pseudomonas*, were also co-isolated alongside presumed functional strains (Fig. 7A). Taken together with functional and safety profiles, most, though not all, isolates of *W. Lactococcus*, *Enterococcus*, *Leuconostoc*, and *Bacillus* show potential as putative next-generation probiotics, pending deeper downstream characterization. Furthermore, in-situ media prepared from raw food ingredients with an appropriate energy source closely replicated the original community, making them particularly useful for isolating dominant taxa from other fermented-food samples as well.

Collectively, although there is a high taxonomic similarity between the isolated strains and the high-quality MAGs, the robust probiotic and safety profiles observed in the MAGs, characterized by low and primarily non-mobile ARG/VF burdens, do not automatically extend to the isolated strains, despite their shared species identity. We acknowledge the inherent limitations of functional and safety characterizations derived from MAGs, as these represent consensus genomic reconstructions of the microbial community. Given the prevalence of strain-level microdiversity, these consensus genomes may not fully capture the specific gene content of every individual isolate recovered. To definitively bridge this gap, future work will prioritize Whole Genome Sequencing (WGS) of the isolated strains, allowing for direct verification of the *in silico* predictions against the actual genomic architecture of the isolates. Furthermore, recognizing that genomic potential does not guarantee phenotypic expression, this collection will serve as a foundational library for comprehensive *in vitro* validation, including assays for adhesion, acid tolerance, bile salt hydrolysis, and antimicrobial activity, followed by *in vivo* efficacy trials. In this context, the MAG atlas generated here serves as a high-resolution ecological and genomic scaffold, guiding the targeted selection of isolates for rigorous downstream validation.

4. Conclusions

This study employed a comprehensive systematic culturomics pipeline, from liquid-enriched growth media to ecological-aware solid media isolation to unlock the true health-promoting potential of FFs, using cabbage kimchi as a model. Among all variables tested, growth media selection was the most critical factor for enriching and isolating microbial strains, outweighing oxygen supply, incubation time, and addition of promoter or suppressant agents. In–situ and MRS media preserve the lactobacilli-core, while generalist media expand diversity to *Firmicute* non-LAB and selected *Proteobacteria*. Particularly, In–situ media yielded microbial profiles most closely resembling the control sample microbial community, underscoring their potential to develop a multi-species, multi-strain native consortia from FFs. Shotgun metagenome genome mining further showed that the majority of identified MAGs carry the most desirable probiotic and bacteriocin-producing features, and low or largely non-mobile ARG/VF burdens. Using this metagenome-informed strategy, more than 90 functional strains composed of six genera were isolated on solid growth media. Overall, this study underscores that maintaining the microbial ecological fidelity of functional strains within FFs is crucial for harnessing their potential as next-generation probiotics and achieving the genuine health-promoting effects of these foods.

CRediT authorship contribution statement

Eskindir Getachew Fentie: conceptualization; investigation; formal analysis; data curation; writing-original draft; writing-review and editing; Kyeongmo Lim: software; investigation; Yohannes Ebabuye Andargie: investigation; software; Sihyun Park: investigation, software; Jae-Ho Shin: conceptualization; writing-review and editing; supervision; fund acquisition.

Funding statement

This work was supported by Korea Basic Science Institute (National Research Facilities and Equipment Center, 2021R1A6C101A416) funded by the Ministry of Education, the project to train professional personnel in biological materials by the Ministry of Environment, Republic of Korea, and the Regional Innovation System & Education (RISE) Glocal 30 program through the Daegu RISE Center, funded by the Ministry of Education (MOE) and the Daegu, Republic of Korea (2025-RISE-03-001).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

All large data analyses in this study were carried out by utilizing the Next Generation Sequencing (NGS) Center server located at Kyungpook National University (Daegu, Republic of Korea).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2026.101318.

Data availability

The raw sequencing data generated in this study have been deposited in the NCBI public repository under BioProject accession number PRJNA1337317. Additional datasets supporting the findings of this study are available from the corresponding author upon reasonable request.

References

Alcock, B.P., Raphenya, A.R., Lau, T.T.Y., Tsang, K.K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A.-L.V., Cheng, A.A., Liu, S., Min, S.Y., Miroshnichenko, A., Tran, H.-K., Werfalli, R.E., Nasir, J.A., Oloni, M., Speicher, D.J., Florescu, A., Singh, B., et al., 2019. Card 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. NAR. https://doi.org/10.1093/nar/gkz935 gkz935.

Armetta, J., Li, S.S., Vaaben, T.H., Vazquez-Uribe, R., Sommer, M.O.A., 2025. Metagenome-guided culturomics for the targeted enrichment of gut microbes. Nat. Commun. 16 (1), 663. https://doi.org/10.1038/s41467-024-55668-y.

Barb, A.W., McClerren, A.L., Snehelatha, K., Reynolds, C.M., Zhou, P., Raetz, C.R.H., 2007. Inhibition of lipid a biosynthesis as the primary mechanism of CHIR-090 antibiotic activity in *Escherichia coli*. Biochemistry 46 (12), 3793–3802. https://doi.org/10.1021/bi6025165.

Birmeta, G., Bakeeva, A., Passoth, V., 2019. Yeasts and bacteria associated with kocho, an Ethiopian fermented food produced from enset (*Ensete ventricosum*). Antonie Leeuwenhoek 112 (4), 651–659. https://doi.org/10.1007/s10482-018-1192-8.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinform 30 (15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 (8), 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Brown, C.L., Mullet, J., Hindi, F., Stoll, J.E., Gupta, S., Choi, M., Keenum, I., Vikesland, P., Pruden, A., Zhang, L., 2022. mobileOG-db: a manually curated database of protein families mediating the life cycle of bacterial mobile genetic elements. Appl. Environ. Microbiol. 88 (18). https://doi.org/10.1128/aem.00991-22 e00991-22.

Caffrey, E.B., Sonnenburg, J.L., Devkota, S., 2024. Our extended microbiome: the human-relevant metabolites and biology of fermented foods. Cell Metab. 36 (4), 684–701. https://doi.org/10.1016/j.cmet.2024.03.007.
Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869.
Candeliere, F., Sola, L., Busi, E., Rossi, M., Amaretti, A., Raimondi, S., 2024. The metabolism of Leuconostoc genus decoded by comparative genomics. Microorganisms 12 (7), 1487. https://doi.org/10.3390/microorganisms12071487.
Castro-López, C., García, H.S., Guadalupe Martínez-Ávila, G.C., González-Córdova, A.F., Vallejo-Cordoba, B., Hernández-Mendoza, A., 2021. Genomics-based approaches to identify and predict the health-promoting and safety activities of promising probiotic strains – a probiogenomics review. Trends Food Sci. Technol. 108, 148–163. https://doi.org/10.1016/j.tifs.2020.12.017.
Claverías, F.P., Undabarrena, A., González, M., Seeger, M., Cámara, B., 2015. Culturable diversity and antimicrobial activity of Actinobacteria from marine sediments in Valparaíso bay, Chile. Front. Microbiol. 6. https://doi.org/10.3389/fmicb.2015.00737.
Diez-Ozaeta, I., Astiazaran, O.J., 2022. Fermented foods: an update on evidence-based health benefits and future perspectives. Food Res. Int. 156, 111133. https://doi.org/10.1016/j.foodres.2022.111133.
Dik, D.A., Marous, D.R., Fisher, J.F., Mobashery, S., 2017. Lytic transglycosylases: concinnity in concision of the bacterial cell wall. Crit. Rev. Biochem. Mol. Biol. 52 (5), 503–542. https://doi.org/10.1080/10409238.2017.1337705.
Ecklu-Mensah, G., Miller, R., Maseng, M.G., Hawes, V., Hinz, D., Kim, C., Gilbert, J.A., 2024. Modulating the human gut microbiome and health markers through kombucha consumption: a controlled clinical study. Sci. Rep. 14 (1), 31647. https://doi.org/10.1038/s41598-024-80281-w.
Fentie, E.G., Jeong, M., Emire, S.A., Demsash, H.D., Kim, M.-C., Lim, K., Shin, J.-H., 2022b. Development of mixed starter culture for the fermentation of Ethiopian honey wine. Tej. Sci. Rep. 12 (1), 13431. https://doi.org/10.1038/s41598-022-17594-1.
Fentie, E.G., Jeong, M., Emire, S.A., Demsash, H.D., Kim, M.A., Jeon, H.-J., Lee, S.-E., Tagele, S.B., Park, Y.-J., Shin, J.-H., 2022a. Physicochemical properties, antioxidant activities and microbial communities of Ethiopian honey wine. Tej. Food Res. Int. 152, 110765. https://doi.org/10.1016/j.foodres.2021.110765.
Fentie, E.G., Lim, K., Andargie, Y.E., Azizoglu, U., Shin, J.-H., 2025. Globalizing the health-promoting potential of fermented foods: a culturomics pathway to probiotics. Trends Food Sci. Technol. 163, 105119. https://doi.org/10.1016/j.tifs.2025.105119.
Fentie, E.G., Lim, K., Jeong, M., Shin, J., 2024. A comprehensive review of the characterization, host interactions, and stabilization advancements on probiotics: addressing the challenges in functional food diversification. Compr. Rev. Food Sci. Food Saf. 23 (5), e13424. https://doi.org/10.1111/1541-4337.13424.
Forster, S.C., Liu, J., Kumar, N., Gulliver, E.L., Gould, J.A., Escobar-Zepeda, A., Mkandawire, T., Pike, L.J., Shao, Y., Stares, M.D., Browne, H.P., Neville, B.A., Lawley, T.D., 2022. Strain-level characterization of broad host range mobile genetic elements transferring antibiotic resistance from the human microbiome. Nat. Commun. 13 (1), 1445. https://doi.org/10.1038/s41467-022-29096-9.
Goh, Y.J., 2009. Genomic features of Lactobacillus species. Front. Biosci. 14 (14), 1362. https://doi.org/10.2741/3313.
Heo, S., Jung, E.J., Park, M.-K., Sung, M.-H., Jeong, D.-W., 2024. Evolution and competitive struggles of Lactiplantibacillus plantarum under different oxygen contents. Int. J. Mol. Sci. 25 (16), 8861. https://doi.org/10.3390/ijms25168861.
Huang, Y., Sheth, R.U., Zhao, S., Cohen, L.A., Dabaghi, K., Moody, T., Sun, Y., Ricaurte, D., Richardson, M., Velez-Cortes, F., Blazejewski, T., Kaufman, A., Ronda, C., Wang, H.H., 2023. High-throughput microbial culturomics using automation and machine learning. Nat. Biotechnol. 41 (10), 1424–1433. https://doi.org/10.1038/s41587-023-01674-2.
Huang, Z., Zhou, X., Stanton, C., Ross, R.P., Zhao, J., Zhang, H., Yang, B., Chen, W., 2021. Comparative genomics and specific functional characteristics analysis of Lactobacillus acidophilus. Microorganisms 9 (9), 1992. https://doi.org/10.3390/microorganisms9091992.
Kers, J.G., Saccenti, E., 2022. The power of microbiome studies: some considerations on which alpha and beta metrics to use and how to report results. Front. Microbiol. 12, 796025. https://doi.org/10.3389/fmicb.2021.796025.
Khan, M.T., Dwibedi, C., Sundh, D., Pradhan, M., Kraft, J.D., Caesar, R., Tremaroli, V., Lorentzon, M., Bäckhed, F., 2023. Synergy and oxygen adaptation for development of next-generation probiotics. Nature 620 (7973), 381–385. https://doi.org/10.1038/s41586-023-06378-w.
Ko, H.I., Jeong, C.H., Park, S.-J., Kim, S.-R., Eun, J.-B., Kim, T.-W., 2023. Influence of isolation temperature on isolating diverse lactic acid bacteria from kimchi and cultural characteristics of psychrotrophs. J. Microbiol. Biotechnol. 33 (8), 1066–1075. https://doi.org/10.4014/jmb.2303.03047.
Korshunov, S., Imlay, K.R.C., Imlay, J.A., 2016. The cytochrome *bd* oxidase of *Escherichia coli* prevents respiratory inhibition by endogenous and exogenous hydrogen sulfide. Mol. Microbiol. 101 (1), 62–77. https://doi.org/10.1111/mmi.13372.
Lagier, J.-C., Dubourg, G., Million, M., Cadoret, F., Bilen, M., Fenollar, F., Levasseur, A., Rolain, J.-M., Fournier, P.-E., Raoult, D., 2018. Culturing the human microbiota and culturomics. Nat. Rev. Microbiol. 16 (9), 540–550. https://doi.org/10.1038/s41579-018-0041-0.
Lee, S.H., Whon, T.W., Roh, S.W., Jeon, C.O., 2020. Unraveling microbial fermentation features in kimchi: from classical to meta-omics approaches. Appl. Microbiol. Biotechnol. 104 (18), 7731–7744. https://doi.org/10.1007/s00253-020-10804-8.
Li, D., Liu, C.-M., Luo, R., Sadakane, K., Lam, T.-W., 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct *de Bruijn* graph. Bioinform 31 (10), 1674–1676. https://doi.org/10.1093/bioinformatics/btv033.
Li, Y., Guo, X., Peng, Q., Shen, T., Yao, J., Wei, Y., Duan, H., Liu, W., 2024. Culturomics: a promising approach for exploring bacterial diversity in natural fermented milk. Food Biosci. 62, 105383. https://doi.org/10.1016/j.fbio.2024.105383.
Liu, B., Zheng, D., Zhou, S., Chen, L., Yang, J., 2022. Vfdb 2022: a general classification scheme for bacterial virulence factors. NAR 50 (D1), D912–D917. https://doi.org/10.1093/nar/gkab1107.
Liu, C., Cui, Y., Li, X., Yao, M., 2021. microeco: an R package for data mining in microbial community ecology. FEMS Microbiol. Ecol. 97 (2), fiaa255. https://doi.org/10.1093/femsec/fiaa255.
Louw, N.L., Lele, K., Ye, R., Edwards, C.B., Wolfe, B.E., 2023. Microbiome assembly in fermented foods. Annu. Rev. Microbiol. 77 (1), 381–402. https://doi.org/10.1146/annurev-micro-032521-041956.
Lu, J., Shen, T., Zhang, Y., Ma, X., Xu, S., Awad, S., Du, M., Zhong, Z., 2023. Safety assessment of *Enterococcus lactis* based on comparative genomics and phenotypic analysis. Front. Microbiol. 14, 1196558. https://doi.org/10.3389/fmicb.2023.1196558.
Lund, P.A., De Biase, D., Liran, O., Scheler, O., Mira, N.P., Cetecioglu, Z., Fernández, E. N., Bover-Cid, S., Hall, R., Sauer, M., O'Byrne, C., 2020. Understanding how microorganisms respond to acid pH is central to their control and successful exploitation. Front. Microbiol. 11, 556140. https://doi.org/10.3389/fmicb.2020.556140.
Marco, M.L., Sanders, M.E., Gänzle, M., Arrieta, M.C., Cotter, P.D., De Vuyst, L., Hill, C., Holzapfel, W., Lebeer, S., Merenstein, D., Reid, G., Wolfe, B.E., Hutkins, R., 2021. The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on fermented foods. Nat. Rev. Gastroenterol. Hepatol. 18 (3), 196–208. https://doi.org/10.1038/s41575-020-00390-5.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8 (4), e61217. https://doi.org/10.1371/journal.pone.0.
Merenstein, D., Pot, B., Leyer, G., Ouwehand, A.C., Preidis, G.A., Elkins, C.A., Hill, C., Lewis, Z.T., Shane, A.L., Zmora, N., Petrova, M.I., Collado, M.C., Morelli, L., Montoya, G.A., Szajewska, H., Tancredi, D.J., Sanders, M.E., 2023. Emerging issues in probiotic safety: 2023 perspectives. Gut Microbes 15 (1), 2185034. https://doi.org/10.1080/19490976.2023.2185034.
Møller, M.S., Goh, Y.J., Viborg, A.H., Andersen, J.M., Klaenhammer, T.R., Svensson, B., Abou Hachem, M., 2014. Recent insight in α-glucan metabolism in probiotic bacteria. Biologia 69 (6), 713–721. https://doi.org/10.2478/s11756-014-0367-7.
Mun, S.Y., Lee, W., Lee, S.-Y., Chang, J.Y., Chang, H.C., 2024. Pediococcus inopinatus with a well-developed CRISPR-Cas system dominates in long-term fermented kimchi. Mukeunji. Food Microbiol. 117, 104385. https://doi.org/10.1016/j.fm.2023.104385.
Park, H., Yeo, S., Ryu, C.B., Huh, C.S., 2024. A streamlined culturomics case study for the human gut microbiota research. Sci. Rep. 14 (1), 20361. https://doi.org/10.1038/s41598-024-71370-x.
Park, J., Heo, S., Lee, G., Hong, S.W., Jeong, D.-W., 2024. Bacterial community of kimchi added with seafood based on culture-dependent investigations. Heliyon 10 (13), e34153. https://doi.org/10.1016/j.heliyon.2024.e34153.
Pasolli, E., De Filippis, F., Mauriello, I.E., Cumbo, F., Walsh, A.M., Leech, J., Cotter, P.D., Segata, N., Ercolini, D., 2020. Large-scale genome-wide analysis links lactic acid bacteria from food with the gut microbiome. Nat. Commun. 11 (1), 2610. https://doi.org/10.1038/s41467-020-16438-8.
Patra, J.K., Das, G., Paramithiotis, S., Shin, H.-S., 2016. Kimchi and other widely consumed traditional fermented foods of Korea: a review. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01493.
Rodzi, N.A.R.M., Lee, L.K., 2021. Traditional fermented foods as vehicle of non-dairy probiotics: perspectives in Southeast Asia countries. Food Res. Int. 150, 110814. https://doi.org/10.1016/j.foodres.2021.110814.
Senanayake, D., Ramarao-Milne, P., Pandey, G., Hlaing, M.M., Chandrapala, J., Torley, P.J., Terefe, N.S., 2025. Genomic insights into exopolysaccharide biosynthesis pathways in novel Lactiplantibacillus plantarum and Leuconostoc mesenteroides strains. Lebensm. Wiss. Technol. 225, 117863. https://doi.org/10.1016/j.lwt.2025.117863.
Shah, A.B., Cho, H., Shim, S.H., 2025. Exploring the bioactive landscape: Peptides and non-peptides from the human microbiota. Npj Biofilm. Microbiom. 11 (1), 76. https://doi.org/10.1038/s41522-025-00713-x.
Spencer, A.C., Panda, S.S., 2023. DNA gyrase as a target for quinolones. Biomedicines 11 (2), 371. https://doi.org/10.3390/biomedicines11020371.
Tadesse, B.T., Gu, L., Solem, C., Mijakovic, I., Jers, C., 2024. The probiotic *Enterococcus Lactis* SF68 as a potential food fermentation microorganism for safe food production. J. Agric. Food Chem. 72 (32), 18089–18099. https://doi.org/10.1021/acs.jafc.4c03644.
Tamang, J.P., Das, S., Lama, S., Utama, G.L., Safitri, R., Balia, R.L., Thapa, N., 2025. Analysis of meta-transcriptomics and identification of genes linked to bioactive peptides and vitamins in Indonesian tempe. Food Res. Int. 202, 115757. https://doi.org/10.1016/j.foodres.2025.115757.
Tejeda-Garibay, S., Zhao, L., Hum, N.R., Pimentel, M., Diep, A.L., Amiri, B., Sindi, S.S., Weilhammer, D.R., Loots, G.G., Hoyer, K.K., 2024. Host tracheal and intestinal microbiomes inhibit *Coccidioides* growth *in vitro*. Microbiol. Spectr. 12 (7). https://doi.org/10.1128/spectrum.02978-23 e02978-23.
Torres, S., Verón, H., Contreras, L., Isla, M.I., 2020. An overview of plant-autochthonous microorganisms and fermented vegetable foods. Food Sci. Hum. Wellness 9 (2), 112–123. https://doi.org/10.1016/j.fshw.2020.02.006.
Valentino, V., Magliulo, R., Farsi, D., Cotter, P.D., O'Sullivan, O., Ercolini, D., De Filippis, F., 2024. Fermented foods, their microbiome and its potential in boosting

human health. Microb. Biotechnol. 17 (2), e14428. https://doi.org/10.1111/1751-7915.14428.

Wagner, A.O., Markt, R., Mutschlechner, M., Lackner, N., Prem, E.M., Praeg, N., Illmer, P., 2019. Medium preparation for the cultivation of microorganisms under strictly anaerobic/anoxic conditions. J. Vis. Exp. 150, 60155. https://doi.org/10.3791/60155.

Wang, B., Rutherfurd-Markwick, K., Liu, N., Zhang, X.-X., Mutukumira, A.N., 2024. Evaluation of the probiotic potential of yeast isolated from kombucha in New Zealand. Curr. Res. Food Sci. 8, 100711. https://doi.org/10.1016/j.crfs.2024.100711.

Wastyk, H.C., Fragiadakis, G.K., Perelman, D., Dahan, D., Merrill, B.D., Yu, F.B., Topf, M., Gonzalez, C.G., Van Treuren, W., Han, S., Robinson, J.L., Elias, J.E., Sonnenburg, E.D., Gardner, C.D., Sonnenburg, J.L., 2021. Gut-microbiota-targeted diets modulate human immune status. Cell 184 (16), 4137–4153.e14. https://doi.org/10.1016/j.cell.2021.06.019.

Xu, J., Sun, L., Xing, X., Sun, Z., Gu, H., Lu, X., Li, Z., Ren, Q., 2020. Culturing bacteria from fermentation pit muds of baijiu with culturomics and amplicon-based metagenomic approaches. Front. Microbiol. 11, 1223. https://doi.org/10.3389/fmicb.2020.01223.

Xu, R., Yu, Y., Chen, T., 2025. Exploring the dark side of probiotics to pursue light: intrinsic and extrinsic risks to be opportunistic pathogens. Curr. Res. Food Sci. 10, 101044. https://doi.org/10.1016/j.crfs.2025.101044.

Zhang, D., Zhang, J., Kalimuthu, S., Liu, J., Song, Z.-M., He, B., Cai, P., Zhong, Z., Feng, C., Neelakantan, P., Li, Y.-X., 2023. A systematically biosynthetic investigation of lactic acid bacteria reveals diverse antagonistic bacteriocins that potentially shape the human microbiome. Microbiome 11 (1), 91. https://doi.org/10.1186/s40168-023-01540-y.

Zhao, J., Cochrane, C.S., Najeeb, J., Gooden, D., Sciandra, C., Fan, P., Lemaitre, N., Newns, K., Nicholas, R.A., Guan, Z., Thaden, J.T., Fowler, V.G., Spasojevic, I., Sebbane, F., Toone, E.J., Duncan, C., Gammans, R., Zhou, P., 2023. Preclinical safety and efficacy characterization of an LpxC inhibitor against Gram-negative pathogens. Sci. Transl. Med. 15 (708). https://doi.org/10.1126/scitranslmed.adf5668 eadf5668.