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Adopting omics-based approaches to facilitate the establishment of microbial consortia to generate reproducible fermented foods with desirable properties

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The quality of fermented foods is governed by the composition, function, and interactions of their microbial communities. However, fermentations carried out using traditional approaches are often variable with respect to their composition and are difficult to control, thereby limiting industrial reproducibility. Recent advances in omics technologies—including metagenomics, metatranscriptomics, metaproteomics, metabolomics, and culturomics—have greatly enhanced our ability to analyze and reconstruct the microbial ecosystems in fermented foods. This review first highlights the importance of omics analyses for characterizing microbial composition, metabolic potential, and functional interactions. It then discusses the bipartite structure of defined microbial consortia (DMCs), distinguishing between the core microbiome, comprising taxa consistently associated with fermentation performance, and the supplementary microbiome, consisting of variable species that influence flavor diversity and system stability. Finally, we describe a multi-omics–guided strategy for the design and refinement of DMCs, framed within the Assembly–Assessment–Redesign (A-A-R) workflow, which enables iterative optimization of microbial consortia for reproducible and desirable fermentation outcomes. Integrating omics insights with DMC engineering provides a systematic approach for precision fermentation, paving the way for next-generation fermented food production.

For centuries, fermentation has served as a means not only to preserve foods and beverages but also to enhance their sensory qualities, nutritional value, and health benefits. Traditional fermentations depended on empirical techniques such as backslopping or spontaneous fermentation. More specifically, spontaneous fermentation processes depend on the activities of naturally occurring microorganisms present in the raw ingredients, surrounding environment, and/or production equipment, while backslopping involves incorporating a portion from a previously successful fermentation into fresh substrate to serve as the starter culture for the next fermentation cycle[1]. However, those traditional fermentation methods rely on undefined microbial communities, which can lead to instability, inconsistency and, in some cases even food safety concerns[2]. To address these issues, modern fermented food production processes have increasingly relied on the use of a standardized starter strain or community of strains to control the microbial composition present during fermentation. In this case, the concept of defined microbial consortia (DMCs) has been proposed. DMCs are reconstructed consortia for which the microbial composition is defined and/or has been prepared with a view to the production of specific metabolites, flavour compounds or other end products.

However, designing and establishing DMCs still presents numerous challenges. The first challenge lies in the difficulty of screening and isolating the strains to produce optimal DMCs for a specific need[3]. Therefore, reliable information about the taxonomic classification of isolates and their specific functional potential is needed. Another challenge relates to the assembly strategy when designing/creating DMCs. Currently, two primary strategies are employed for design control to harness attributes from artisanal fermented foods: the top-down and bottom-up approaches. The top-down strategy entails guiding naturally occurring microbial communities toward simplified, representative consortia. The bottom-up approach instead focuses on constructing novel microbial communities, deliberately

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assembled based on defined objectives or principles[4]. Both benefit from having a comprehensive understanding of the specific roles played by microorganisms in driving key biochemical conversions and influencing the quality of traditionally fermented foods, together with genomic-based insights into their metabolic potential. However, regardless of the approach, using a reconstructed consortium to start the fermentation may still result in products that lack character and regional identity, have insufficient and/or monotonous flavors, and/or the absence of specific bioactive compounds[5]. This risk can be reduced by using multiple approaches to better understand the microbes, their metabolites and the mechanisms via which they can potentially impact, health, flavour or other attributes. More specifically, by capturing microbial and molecular complexity more effectively than single-omics, multi-omics analyses provide deeper insights into the underlying mechanisms of fermentation to facilitate the construction of consortia.

The successful application of multi-omics approaches, and accompanying bioinformatics analyses, hold the potential to provide a solid foundation for generating reproducible fermented foods with desirable properties. Despite this potential, there has, to date, been only relatively limited use of multi-omics approaches to: (i) obtain optimal microbial isolates from traditional fermented foods, (ii) construct functionally stable consortia, and (iii) ultimately produce fermented products with desired quality attributes. In this review, we outline how multi-omics techniques can be employed to establish DMCs and subsequently review recent advances in DMCs applications for fermented food production, culminating in a proposed standardized workflow for DMCs construction and implementation.

Omics analyses of great relevance to fermented food analysis and re-contruction

Metagenomics

The concept of 'metagenomics' was first introduced in 1998[4], referring to methods designed to analyze and understand the genomes of diverse microbial communities. Since then, advancements in sequencing technologies, coupled with a substantial decline in sequencing costs, have significantly mitigated previous limitations in microbial research. These developments now enable the acquisition of genomic information directly from microbial communities within their native environments. Consequently, instead of analyzing individual species in isolation, researchers can investigate entire microbial ecosystems, comprising thousands of species, simultaneously. The application of next-generation sequencing technologies in metagenomic research has led to the generation of vast sequence datasets from a variety of environments, including soil, the human body, and marine ecosystems[6–8]. Analysis of these datasets has provided unprecedented insight into the extensive taxonomic and functional diversity of microbial communities inhabiting these environments.

In recent years, a substantial number of studies utilizing metagenomic approaches to study fermented food microbiomes and their metabolic potentials have been published[9–11]. For example, the microbial composition and function of a collection of 69 different water kefir grains and their fermented products was revealed by Breselge et al. in their recent publication[12]. In this study, a metagenomic approach was employed to investigate the core genera with water kefir microbial community. Based on the occupancy of taxa, they established that the core microbiome consists of yeasts (*Saccharomyces*, *Zygotorulaspora*, *Pichia*, *Brettanomyces*), acetic acid bacteria (AAB; *Gluconobacter*, *Acetobacter*), lactic acid bacteria (LAB; *Liquorilactobacillus*, *Lacticasebacillus*, *Lentilactobacillus*, *Leuconostoc*, *Oenococcus*), as well as *Bifidobacterium* and *Zymomonas*. Their findings offer important references for the future development of reconstructed water kefir. In another study focused on kombucha microbiomes, researchers assigned *Komagataeibacter rhaeticus* and *Brettanomyces bruxellensis* as the core components of kombucha microbiomes based on their metagenomic sequencing results, reflecting the observation that these were the most prevalent species among bacteria and yeasts, respectively[13].

Although previous studies already have highlighted the potential to leverage metagenomic data to gain insights into the diverse and abundant microbial community in fermented foods, they still fall short in terms of accurately capturing microbial activities in situ. For instance, sequenced DNA may also derive from cells with limited activity during fermentation or post-ingestion, thus gene detection may not accurately reflect gene expression dynamics. Apart from this, it should be noted that the interpretation of metagenomic data in fermented food systems is influenced by multiple experimental and biological factors. Parameters such as sequencing depth, sampling strategy, and the choice between repeated sampling of the same product versus comparative sampling across different fermented foods can affect the apparent diversity and structure of microbial communities. In addition, different fermented foods may inherently differ in the flexibility or constraint of their core microbiomes due to matrix composition, processing conditions, and fermentation dynamics. Thus, to gain a more comprehensive understanding of the functional dynamics within complex microbial communities, it is therefore essential to integrate metagenomics with other molecular omics approaches, including metatranscriptomics, metaproteomics, and metabolomics[14].

Metatranscriptomics

Unlike metagenomics, which involves the collection of microbial DNA from a community to infer taxonomic composition and functional potential, metatranscriptomics targets the extraction of messenger RNA (mRNA), providing specific insights into the transcriptional activity and functional expression of the microbial community. In particular, it enables the elucidation of the detailed transcriptional activities of metabolically active microbial populations[15].

Although metatranscriptomics holds significant potential, its application in food and nutrition sciences remains relatively limited to date; however, interest and utilization in this field are steadily increasing. Recently, metatranscriptomic sequencing has been utilized to understand the microbial processes underlying flavor development in food fermentation, including various fermented foods based on different substrates, such as pickled vegetables[16–18], liquor[19] and soy sauce[20]. In a recent investigation concerning fermented noni (*Morinda citrifolia* L.) juice[21], metatranscriptomics was employed to comprehensively investigate the active microbial community and key metabolic functions involved in the natural fermentation of noni fruit. Metatranscriptomics results showed that during the initial phase of fermentation, the microbial community was primarily dominated by *Acetobacter sp*., whereas, in the later stages (beyond 30 days), *Acetobacter aceti* and *Gluconobacter sp*. emerged as the principal functional taxa driving the fermentation process. However, noni fruit can have an unpleasant odor due to presence of butanoic and hexanoic acids, and this problem persisted in the fermented product due to the absence of the genes associated with the degradation of these compounds. Subsequently, in another study published by the same laboratory, the researchers decreased the content of hexanoic acid, octanoic acid and butanoic acid by inoculating an *Acetobacter* strain isolated from naturally fermented noni juice[22]. This effectively demonstrates the potential of metatranscriptomics in generating reproducible fermented foods with desirable properties.

Despite its potential usefulness, this technique has multiple inherent limitations and deficiencies that warrant careful consideration. Firstly, obtaining high-quality mRNA, with corresponding depletion of the highly abundant ribosomal RNA (rRNA) from certain food samples can be challenging compared to DNA. Also, the limited stability of mRNA complicates the detection of rapid and transient responses to environmental changes. Another challenge can relate the analysis and interpretation of the resulting datasets, due to the lack of established reference genomes, computational tools and/or pipelines[23]. Finally, it should be noted that mRNA expression, protein levels, and related enzymatic or functional activity may not always show consistent relationships. Therefore, robust microbiome characterization ideally involves integrated analysis across different multi-omics datasets.

Metaproteomics

By the 1970s, two-dimensional gel electrophoresis laid the foundation for proteomics, and subsequent advances in mass spectrometry have greatly

increased the speed and sensitivity of protein detection[24]. In parallel, improvements in bioinformatics tools for 2-D gel and MS data analysis have enabled researchers to quickly identify proteins within databases[25]. Beyond simple protein identification, metaproteomics can also offer quantitative dynamic profiling of expressed proteins and their post-translational modifications. This capability proves particularly valuable for detecting stimulus-dependent temporal variations in protein composition.

In fermented food studies, metaproteomics has become a valuable omics tool for characterizing complex microbial interactions, fermentation kinetics, metabolic network dynamics, and functional diversity of microbial ecosystems[26]. In a reconstructed wine fermentation study, researchers employed metaproteomic approaches to investigate both monocultures and co-cultures of *Saccharomyces cerevisiae* with non-*Saccharomyces* yeasts (*Metschnikowia pulcherrima* and *Lachancea thermotolerans*)[27]. The results demonstrate that fermentation kinetics are influenced by yeast-specific protein secretion profiles. Comparative analysis revealed significant differences between the proteomes of monocultures versus co-cultures, providing direct evidence of microbial interactions through altered protein secretion patterns. The complex interplay between *S. cerevisiae*, non-*Saccharomyces* yeast species, and lactic acid bacteria forms the biochemical basis of wine flavor development[27]. Deciphering these microbial interactions and their collective metabolism represents a crucial step toward controlled quality improvement in winemaking.

Metaproteomics centers on examining the overall biological properties of microbial ecosystems, emphasizing the relationship between species composition and metabolic functions, as well as the interactions between microbial communities and their surrounding environments. However, some information cannot be revealed by metaproteomics, such as intermediate metabolic functions of RNA with poly-A tails[28] and the scaffolding capabilities of long noncoding RNA[29]. Moreover, metaproteomics alone cannot always predict the actual metabolic end products or their relative quantities. Therefore, integrating metabolomics data becomes essential to capture the final outcomes of microbial metabolism and to provide a more complete understanding of the functional dynamics within these ecosystems.

Metabolomics

By employing high-resolution mass spectrometry (MS) for accurate mass determination, advanced NMR spectrometers for structural elucidation, ultra-high-pressure liquid chromatography (UPLC/HPLC) for rapid compound separation, and advanced bioinformatics tools for spectral and chromatographic data processing, researchers can now detect and characterize dozens of small-molecule metabolites simultaneously within minutes, rather than analyzing just one or two compounds at a time[30]. The small-molecule metabolites analyzed include a wide variety of endogenous and exogenous chemical compounds, including peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids, minerals, and any other substance that a cell or organism can utilize, ingest, or synthesize[31]. Larger molecules, such as DNA, RNA and protein, are excluded from metabolomic datasets.

Food metabolites, such as sugars, organic acids and volatile compounds, can influence the taste and flavour of fermented foods. Most flavor compounds are typically secondary metabolites derived from food components, characterized by low molecular weight, low abundance, and high volatility. Metabolomics approaches enable comprehensive analysis of the composition and concentration of these volatile substances, thereby facilitating the optimization of food production and processing to enhance flavor quality in the final products[30]. A notable trend that has recently emerged in which researchers seek to elucidate the relationships between specific microbial communities and their associated metabolic outputs through integrative metagenomic and metabolomic approaches. For instance, Zhang and co-authors successfully identified correlations between the core microbial community of cheese and free amino acids (FAAs), volatile organic compounds (VOCs), and differential metabolites[32]. They found that *Lactococcus* and *Acinetobacter* actively participated in flavor compound and beneficial amino acid formation processes, whereas *Streptococcus* and *Serratia* mediated amino acid breakdown processes. In another study focused on the fermentation of a traditional Chinese red sour soup, metabolomic analysis and 16S rDNA amplicon sequencing of soup samples were performed to determine microbial diversity and metabolite contents[33]. Correlation analysis demonstrated significant positive associations between *Lacticaseibacillus rhamnosus* abundance and differentially enriched metabolites (lactic acid, ascorbic acid, and chlorogenic acid), known to positively influence red sour soup's flavor profile and nutritional quality.

However, relationships identified using correlation-based approaches (such as Pearson or Spearman) may not accurately capture biological significance and often fail to account for the complexity of microbial interactions[34]. Most studies lack experimental validation, failing to recapitulate observed/imputed findings through culture-based approaches. A comprehensive approach to validate the obtained relationship should include, for example, a reproduction of particular food properties after fermentation with specific isolated strains, relative to controls.

Culturomics

As outlined in the previous section, many potential metabolic features of fermented food microorganisms have not yet been tested by culture-based methods. To develop the synthetic microbial communities that can help to start a more controllable fermentation, we therefore need a high-precision and high-throughput technique to screen and isolate microorganisms in fermented food samples. Indeed, culturomics technology, a high-throughput method to culture and identify microorganisms at the strain level, possesses inherent potential to address this research challenge[35,36].

Culturomics was developed based on culture techniques and has, for example, been employed to isolate and identify previously uncharacterized bacteria residing in the human gut[35]. In terms of fermented food related studies, several studies have systematically isolated, characterized, and even commercialized strains derived from traditional fermented foods, with predominant emphasis on lactic acid bacteria and limited yeast species[37–39]. Xu and colleagues successfully isolated 19 novel strains from a traditional Chinese liquor, Baijiu, via 27 different culture conditions (media/gas/reagent additives), which were absent from prior isolations of this beverage[40]. Likewise, Li et al. employed culturomics techniques on naturally fermented milk, including different incubation temperatures and culture media[41]. This strategy resulted in the successful isolation of over four hundred strains, including four that had not been reported previously. The strains obtained via culturomics are expected to substantially expand the strain library available for the fermented food synthetic microbial communities.

While culturomics offers the advantage of obtaining pure and taxonomically well-defined isolates, thereby addressing some of the limitations inherent in metagenomic data, the isolation of rare or novel bacteria from natural fermented food is still a significant challenge. The presence of some microbes in a viable but non-culturable state can be very difficult to isolate by artificial media. Therefore, more culturomics strategies should be proposed, which rely on a more comprehensive understanding of the metabolic features of the different microbial composition in fermented food. Such understanding could be derived from comprehensive data mining of the metagenomics, metatranscriptomics, metaproteomics and metabolomics datasets.

Why and how can multi-omic approaches be combined?

To create both form and function, all biological systems rely on the interplay of information (genomics), function (transcriptomics and proteomics), and environment (with implications for metabolomics)[34]. Furthermore, to obtain a reproducible, controllable synthetic microbial community that can consistently produce final fermented products with desirable properties, it is important to use nucleic acid sequencing (both DNA and RNA) combined with metabolomics or metaproteomics to uncover composition, genomic features, and metabolic characteristics of the traditional fermented foods.

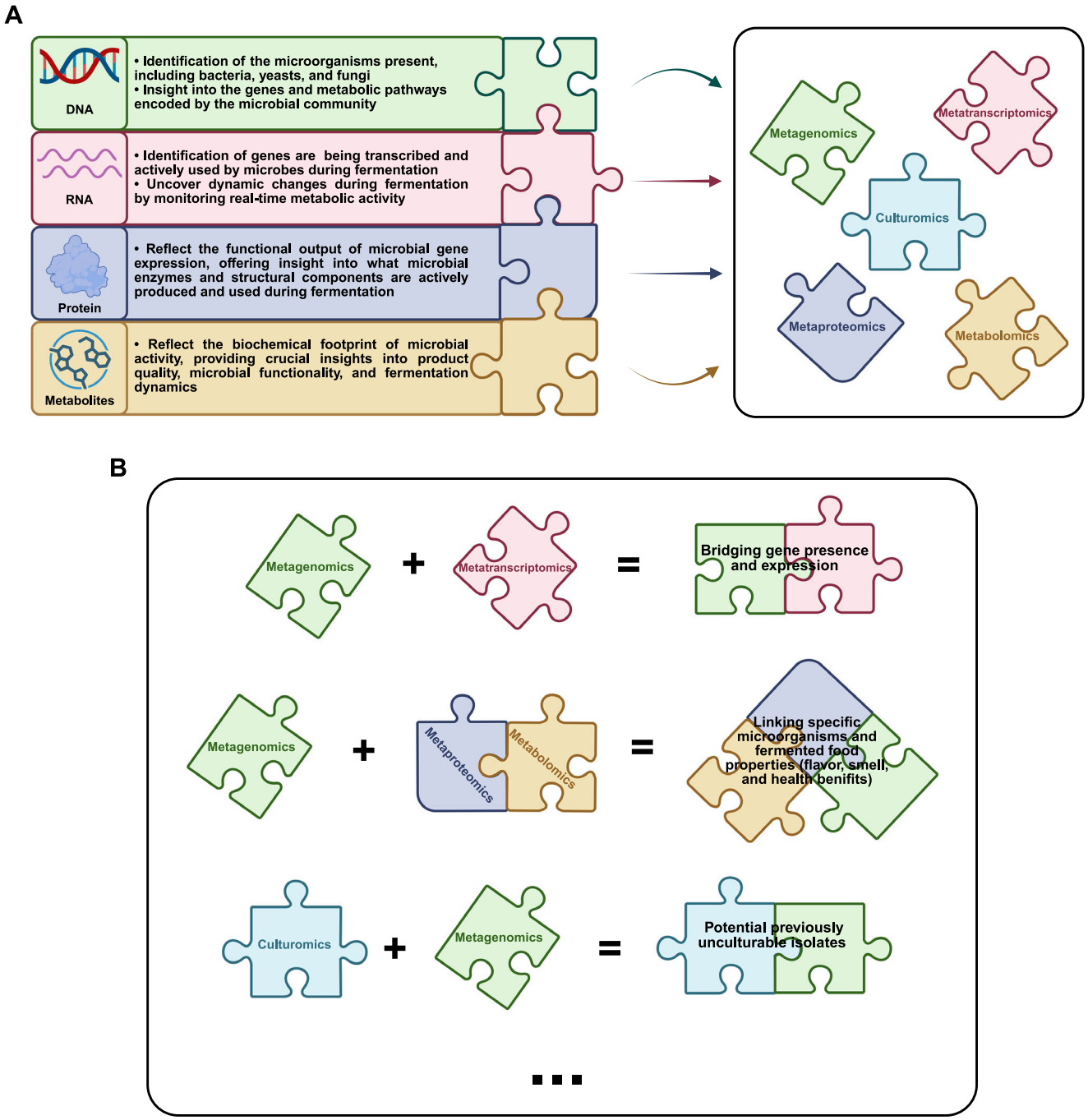


Fig. 1 | Multi-omics strategies used to facilitate the establishment of microbial consortia. The schematic highlights the types of information provided by individual omics approaches (**A**) and how their combined analysis can yield novel insights for the construction of DMCs (**B**). (Created in bioRender.com).

To be more specific, metagenomic sequencing is widely employed to characterize the taxonomic structure and potential functional pathways of fermented food microbial communities. In parallel, metatranscriptomics allows for the examination of active gene expression profiles via RNA sequencing, enabling researchers to identify which metabolic processes are actively occurring during fermentation. Furthermore, metaproteomics contributes by elucidating the proteomic landscape of the microbial community, thereby offering direct insights into the functional proteins involved and their metabolic roles. In this process, culturomics will also play a pivotal role, it can serve as an indispensable bridge between omics data and functional validation through culture-based experiments (Fig. 1).

In most relevant studies, shotgun metagenomics data have been conventionally employed to identify 'core microbiome' in microbial communities (Table 1). Their criteria for the 'core microbiome' is usually based on relative abundance data obtained from this metagenomic dataset. However, high abundance does not necessarily equate to functional dominance in fermented food ecosystems, as rare taxa can disproportionately influence community dynamics and metabolic outputs. To address this limitation, multi-omics integration offers a more comprehensive view. For example, integrated analysis of metagenomic and metatranscriptomic data can effectively distinguish the roles of various community strains. The analysis of spontaneous sourdough metagenomic and transcriptomic data uncovered a hierarchical microbial structure, comprising dominant, subdominant, and satellite species, each participating in different functional pathways[42]. Such results have facilitated their de novo reconstruction of a synthetic microbial community. Similarly, co-occurrence analysis at a

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Table 1 | Summary of latest five-year progress in multi-omics applications for defining core microbiomes in fermented foods

Type of food	Fermented food	Core microbiome candidates	Criteria for defining the core microbiome	Multi-omics techniques involved	Reference
Dairy	Milk kefir	*Lactobacillus kefiranofaciens ssp. kefiranofaciens* JCM6985, *Lactobacillus kefiranofaciens ssp. kefiranofaciens* JCM7446, *Lactobacillus kefiranofaciens ssp. kefirgranum* JCM8572, *Lactobacillus kefiranofaciens ssp. kefirgranum* JCM8570, *Lentilactobacillus kefiri* JCM5818, *Lentilactobacillus parakefiri* JCM8573, *Leuconostoc mesenteroides* GF04, *Lactococcus lactis* ssp. *Cremoris* YRC3780, *Lactococcus lactis* ssp. *lactis* biovar diacetylactis YRC3784, *Kazachstania exigua* TGY37	Microbial abundance and occurrence frequency	Culturomics	Motoshima et al. [77]
	Milk kefir	*Acetobacter pasteurianus* KC001, *Lactococcus lactis* KC002, *Leuconostoc mesenteroides* KC003, *Lentilactobacillus kefiri* KC004, *Lactobacillus kefiranofaciens* KC005, *Pichia fermentans* KC006, *Saccharomyces cerevisiae* KC007, *Kazachstania unispora* KC008, *and Kluyveromyces marxianus* KC009	Microbial abundance	Metabolomics	Bourrie et al. [64]
	Cheese	*Staphylococcus equorum*, *Brevibacterium auranticum*, and *Brachybacterium alimentarium*	Microbial abundance, occurrence frequency	Metagenomics, metabolomics	Niccum et al. [36]
Meat	Meat sausage	*Lactobacillus plantarum* LZ-4, *Staphylococcus saprophyticus* SZ-7, *Macrococcus caseolyticus* SM-2, *Streptococcus thermophilus* SL-17, and *Pediococcus pentosaceus* LP-1	Microbial abundance, occurrence frequency and microbial functionality	Metagenomics, metabolomics	Wu et al. [78]
	Meat sausage	*Latilactobacillus sakei* and *Debaryomyces hansenii*	Microbial abundance	Metagenomics	Degenhardt et al. [79]
Vinegar	Vinegar	*Acetobacter pasteurianus*, *Lactobacillus acetotolerans*, *L. helveticus*, and *Acetilactobacillus jinshanensis*	Microbial abundance, occurrence frequency	Metagenomics	Huang et al. [80]
Liquors	Chinese liquor	*Saccharomyces cerevisiae*, *Wickerhamomyces anomalus*, *Issatchenkia orientalis*, *Pichia manshurica*, *Kazachstania humilis*, *Bacillus amyloliquefaciens*, *Weissella cibaria*, *Acetobacter pasteurianus*, *Lactobacillus helveticus*, and *Rhizopus delemar*	Microbial abundance, occurrence frequency, inter-microbial interactions and microbial functionality	Culturomics, metagenomics and metabolomics	Wang et al. [70]
	Chinese liquor	*Lactobacillus*, *Thermoactinomyces*, *Aquabacterium*, *Aspergillus*, and *Kazachstania*	Microbial abundance and microbial functionality	Metagenomics, metabolomics	Jiao et al. [74]
Vegetable	Paocai	*Lactobacillus*, *Leuconostoc*, and *Weissella*	Microbial abundance, occurrence frequency, inter-microbial interactions and microbial functionality	Metagenomics, metabolomics and metatranscriptomics	Zhou et al. [81]
	Paocai	*Liquorilactobacillus nagelii* SAAS-B-MRS-20201023-12, *Pediococcus ethanolidurans* SAAS-B-MRS-20201030-3, *Lentilactobacillus buchneri* SAAS-B-MRS-20200909-11, and *Kazachstania exigua* SAAS-Y-YPD-20201023-3	Microbial abundance, occurrence frequency, inter-microbial interactions and microbial functionality	Metagenomics, metabolomics	Zhao et al. [82]
	Pickle	*Lactobacillus, Weissella* and yeast	Microbial abundance and occurrence frequency	Metagenomics	An et al. [83]
Others	Silage	*Lacticaseibacillus rhamnosus* M41, *Lactiplantibacillus paraplantarum* R6, *Pediococcus acidilactici* B2, *Lactiplantibacillus plantarum* B19, *Lactococcus garvieae* OS9, *Weissella cibaria* F1, *Lactiplantibacillus pentosus* A2, *Enterococcus faecalis* L1 and *Pediococcus pentosaceus* K3	Microbial abundance, occurrence frequency, inter-microbial interactions and microbial functionality	Culturomics, metabolomics	Zong et al. [84]
	Water kefir	*Saccharomyces*, *Zygotorulaspora*, *Pichia*, *Brettanomyces*, *Gluconobacter*, *Acetobacter*, *Liquorilactobacillus*, *Lacticaseibacillus*, *Lentilactobacillus*, *Leuconostoc*, *Oenococcus*, *Bifidobacterium* and *Zymomonas*	Microbial abundance, occurrence frequency and microbial functionality	Metagenomics, metabolomics	Breselge et al. [12]
	Sourdough	*Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Fructilactobacillus sanfranciscensis*, *Weissella confuse*, and *Saccharomyces cerevisiae*	Microbial abundance, occurrence frequency, inter-microbial interactions and microbial functionality	Culturomics, metagenomics and metabolomics	Calabrese et al. [42]
	Kombucha	*Komagataeibacter intermedius* and *Brettanomyces bruxellensis*	Microbial abundance, occurrence frequency, inter-microbial interactions and microbial functionality	Metagenomics, metabolomics	Huang et al. [71]
	Kombucha	*Komagataeibacter rhaeticus* and *Brettanomyces bruxellensis*	Microbial abundance, occurrence frequency	Metagenomics	Landis et al. [13]
	Kombucha	*Starmerella davenportii*, *Komagataeibacter saccharivorans*, and *Gluconacetobacter intermedius*	Microbial abundance, microbial functionality	Culturomics, metabolomics	Li et al. [65]

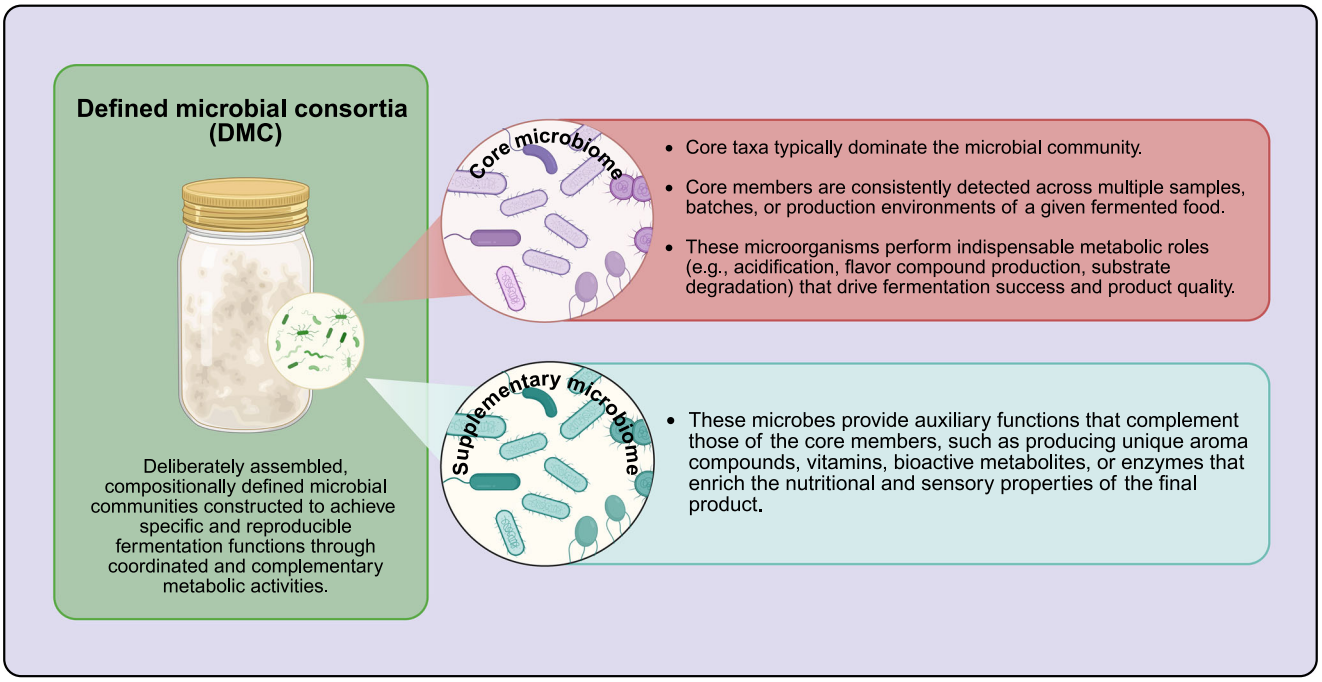


Fig. 2 | The bipartite structure and definition of DMCs (Created in bioRender.com).

metagenomic and metabolomic level also helps identify spontaneous food fermentation microbiota core species[43]. This integrative approach holds the potential to elucidate microbial dynamics and reveal the associations between specific microorganisms and flavor compound formation, which can provide guidance for the optimization process. Apart from this, the protein expression levels within a sample as well as microbial community relationships can be obtained through metagenomic-metaproteomic correlation analyses. Notably in this regard, Zhao and colleagues used such an approach to establish that *Aspergillus* was the dominant fungus and major source of identified proteins in the fermentation of Pu-erh tea[44].

The insights gained from these multi-omics integrations rely heavily on advanced bioinformatics approaches. Commonly utilized data analysis approaches include metagenome/transcriptome assembly and annotation, differential expression analysis, correlation analysis (Person correlation and Spearman correlation), regression (Chi-square, logistic regression, multinomial regression, and general linear model), and network analysis[45]. These findings can be visualized through PCoA plots, abundance plot of pathway, metabolic pathways network, PCA plots, bar plots, correlation heatmap and dendrograms. Meanwhile, there has been a growing adoption of machine learning (ML) in data analysis over recent years, emerging as a popular methodological paradigm. ML analysis approaches include unsupervised ML methods (clustering method, K-cluster), supervised ML methods (NB, SVM, k-NN, and RF), multivariate analysis (MVA) – including supervised MVA (OPLS-DA, PLS-DA, and PLSR) and unsupervised MVA (MANOVA, PCA, and PCoA), deep learning, and dimensional reduction/feature selection[46,47]. However, the application of these approaches in fermented food research remains limited. A recently published study used three ML models (Logistic Regression, KNN, and Random Forest) combined with multi-omics to successfully identify several microbial markers and flavor markers, to develop a classification model for abnormal fermentation of Baijiu[48]. Further application of ML can enhance predictive analytics in fermented food. Integrating machine learning with multi-omics data enables researchers to pinpoint key metabolites, detect subtle changes in metabolic profiles, and forecast the presence of specific compounds, thereby provide more insights to facilitate the building a reproducible synthetic microbial community of fermentation.

The bipartite structure of DMCs

A reconstructed consortium for fermented foods can be systematically categorized into two parts (Fig. 2). The first is 'core microbiome,' which is responsible for reconstructing the essential metabolic pathways required for fermented foods fermentation, such as carbohydrate/pyruvate and nitrogen metabolism. The second is 'supplementary microbiome', which can be introduced into the core microbiome to enhance the properties of the reconstructed fermented food, including flavor, nutritional value, and other desirable qualities. Recent work on the rational design of fermented-food microbiomes further supports this framework. For example, Arrigan et al. highlight the potential benefits of developing novel ferments through the integration of traditional fermentation practices with rational microbiome design, emphasizing community-level design principles that distinguish essential fermentation functions from auxiliary traits[49]. This perspective reinforces a function-driven definition of core microbiomes, in which a minimal set of microorganisms underpins the completion of fermentation, while supplementary microbiomes provide context-dependent modulation of product attributes. Such a framework offers a practical conceptual basis for the rational design of reproducible and adaptable microbial consortia in fermented food systems.

The 'core microbiome'

The core microbiome must be capable of completing the fermentation process; in other words, it can also be referred to as 'the minimal microbial community required to complete the fermentation of fermented foods'. Generally, fermentation is a microbial process that breaks down complex organic molecules into simpler compounds, while simultaneously serving as a natural means to enhance the nutritional profile—by increasing vitamins and essential amino acids, reducing anti-nutrients, and improving protein quality—as well as refine sensory attributes such as appearance, flavor, and aroma of foods. However, the specific processes and reactions may vary across different types of fermented foods (Fig. 3).

Depending on the predominant end products and the principal biochemical pathways involved, fermentation can be classified into lactic, acetic, alcoholic, and alkaline types[50]. Lactic acid fermentation involves the conversion of sugars into lactic acid and represents a defining process in the production of fermented foods such as yogurt, kimchi, and fermented

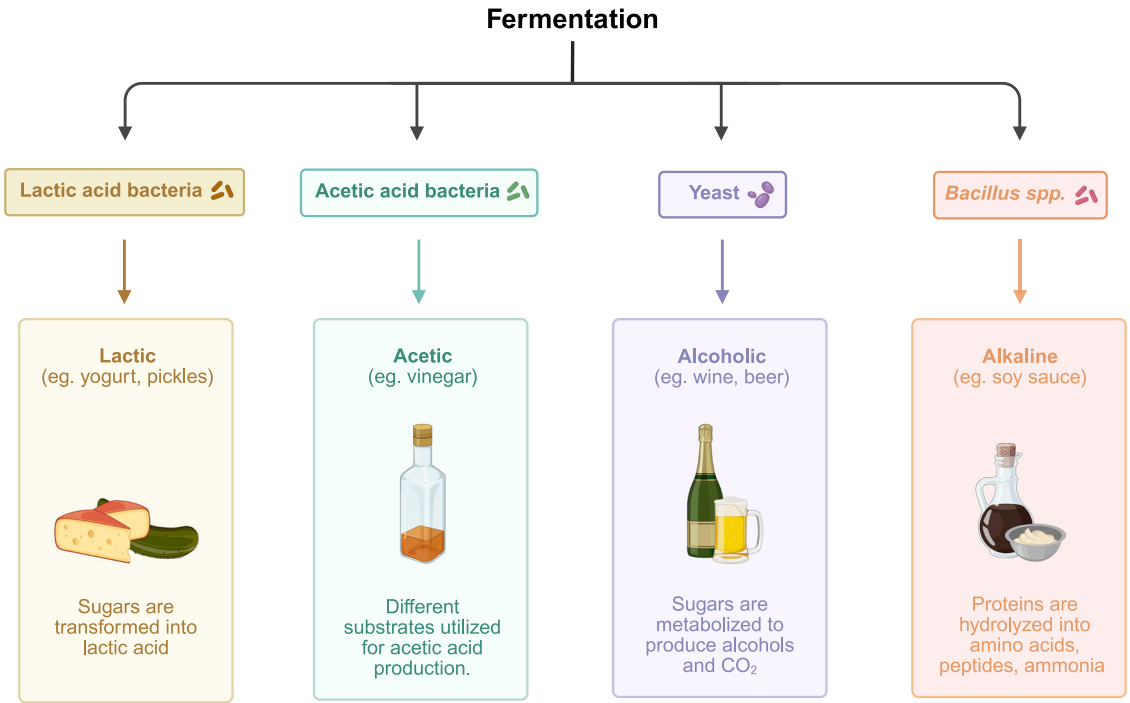


Fig. 3 | Overview of major fermentation processes, key microbial contributors, and their corresponding end products (Created in bioRender.com).

cereals. This fermentation pathway is predominantly mediated by LAB. In a similar manner, acetic fermentation is characterized by the oxidation of organic substrates, such as alcohols and sugars, into acetic acid. This process is predominantly carried out by acetic acid bacteria. As a key biochemical pathway, acetic fermentation underlies the production of a range of traditional fermented foods and beverages, including water kefir, kombucha, cocoa, and vinegar[51]. Alcoholic fermentation, by contrast, is primarily carried out by yeasts that transform sugars into ethanol and carbon dioxide, thereby underpinning the production of bread, beer, and wine. Distinct from these pathways, alkaline fermentation, typically mediated by *Bacillus* species and related microorganisms, results in the breakdown of proteins into ammonia and other alkaline compounds, contributing to the preservation and unique umami flavor of traditional foods such as natto, kinema, and ugba[42]. One or more of these key fermentation reactions are widely present during food fermentation. Together, these pathways indicate that the core microbiome of fermented foods generally comprises LAB, AAB, yeasts, and/or *Bacillus* species.

As a key component of the core microbiome in many fermented foods, LABs contribute to extended shelf life and enhanced nutritional value, flavor, and texture by fermenting available carbon sources in the native food matrix into lactic acid and other flavor compounds[52,53]. The production of lactic acid lowers the pH, inhibiting the growth of undesirable microorganisms. LABs also play a crucial role in flavor development, generating a wide range of aroma compounds during fermentation, including alcohols, aldehydes, ketones, fatty acids, esters, and sulfur-containing compounds. For example, acetoin, aldehydes, and ketones derived from LAB pyruvate metabolism impart characteristic aromas such as boiled cherries and rose[54]. In addition, many peptides and amino acids produced by LAB contribute distinctive tastes, including the umami of glutamic acid and the sweetness of proline[55]. AABs are also commonly found in fermented foods and beverages, although their specific functional roles and contributions to microbial interactions are less well characterized. A defining feature of AAB is their ability to directly oxidize carbohydrates and sugar alcohols, accumulating large quantities of the corresponding oxidation products while deriving energy from this process. This "oxidative fermentation", driven by membrane-bound dehydrogenases, is a hallmark of AAB physiology[56]. In many fermentations, AABs coexist with yeasts in a commensal relationship, relying on yeast-derived ethanol and sugars as substrates. Yeasts hydrolyze sucrose into glucose and fructose and preferentially convert fructose into ethanol, which is subsequently oxidized by AAB into acetic acid, contributing to the characteristic sourness of fermented products. In addition, yeasts play key roles in flavor development and gas production. For instance, during sourdough fermentation, yeast-derived ethanol and carbon dioxide are critical determinants of dough texture and structure[56]. Certain yeasts also generate succinic acid, carbonyl compounds, and esters, further enhancing flavor, texture, and overall sensory qualities in fermented foods[57]. In addition to LAB, AAB, and yeasts, *Bacillus* species are also important members of the core microbiome in many fermented foods. Fermented foods containing *Bacillus* species are typically prepared using legumes or tubers as substrates and are traditionally produced in regions of East Asia, Africa, and South America. During fermentation, *Bacillus* species secrete amylases and proteases, as well as extracellular polysaccharides, polypeptides, and lipopeptides with antimicrobial activity[58]. Our understanding of the functional roles of these core microbiome members is largely derived from systematic investigations using multi-omics approaches.

Beyond sustaining fermentation performance, the activity of core microbiome may also give rise to downstream outcomes, including the generation of metabolites with potential probiotic or health-promoting effects. Contributing functionalities such as modulation of gut microbiota, production of bioactive compounds, or enhancement of host health. At present, direct evidence supporting the intentional incorporation of such health-oriented core microbiomes in reconstructed fermented foods remains limited. Nevertheless, recent advances in gut microbiome research suggest the feasibility of defining functional microbial assemblages associated with host health. For example, Wu et al. proposed that a core microbiome signature can serve as an indicator of health, highlighting the potential to identify minimal, functionally relevant microbial sets linked to specific physiological outcomes[59]. Although these concepts have not yet been extensively translated to fermented food systems, they provide a conceptual framework for exploring health-oriented microbiomes in future studies.

Ultimately, within each functional group, only a limited subset of species consistently contributes to the core microbiome of a given fermented food, depending on the substrate, processing conditions, and stage of

fermentation. Building on the above understanding of fermentation, multi-omics approaches can be employed to identify the core microbiome of different fermented foods at species level, thereby enabling a comprehensive characterization of microbial community composition, functional potential, and gene expression patterns. Such integrative analyses are essential for revealing the key microorganisms and metabolic pathways that sustain the fermentation process and determine the quality and functionality of the final products, thereby facilitating construction of the DMCs. For example, Calabrese and co-authors characterized both the taxonomic composition and the functional redundancy of spontaneous sourdoughs using metagenomic and metatranscriptomic analyses[42]. Based on these datasets, the principal metabolic pathways underpinning key functional activities were reconstructed. This foundational knowledge was then applied to design specifically depleted DMCs, in which individual species were systematically removed from the consortium, thereby enabling the monitoring of transcriptomic changes associated with microbial interactions[42]. Numerous related studies have recently adopted a similar strategy, employing multi-omics approaches to define the core microbiomes of diverse fermented foods and, on this basis, constructing DMCs that enabled the development of preliminary reconstructed fermented foods (Table 1).

The 'supplementary microbiome'

As discussed above, the identified core microbiomes have been employed to develop reconstructed fermented products. These products, however, often exhibit various limitations, such as incomplete flavor profiles, suboptimal texture, or reduced nutritional functionality. For instance, authors found that a kombucha fermented with a traditional starter contained higher levels of esters, terpenes, and norisoprenoids compared to another made by DMCs[60]. These compounds play a central role in the aroma profile of fermented beverages, often imparting desirable floral and fruity odors[61,62]. In particular, esters often contribute to the enhanced perception of tea and white fruit aromas[63]. Additionally, in another study related to milk kefir, researchers constructed DMCs using nine different strains and found that a total of 51 compounds differed between the fermentations with traditional grains and reconstructed kefir consortia after 18 h of fermentation[64]. Fermentations initiated with kefir grains were distinguished by higher levels of ethanol and a greater relative abundance of ester compounds, particularly ethyl acetate.

To improve the characteristics of such reconstructed fermented foods, a variety of research efforts have explored different strategies. Wang et al. aimed to alter the ratio of acetic acid to gluconic acid in kombucha, as a higher proportion of gluconic acid has been shown to improve the taste of the beverage[65]. They established a reconstructed kombucha using the highest-yielding strains of key functional metabolites: *Starmerella davenportii*, *Komagataeibacter saccharivorans*, and *Gluconacetobacter intermedius*. The results demonstrated that the microbial community composed of these strains increased the gluconic acid content threefold and significantly improved sensory quality as well as higher concentrations of key functional metabolites[65]. Moreover, in a reconstructed vinegar study, Zhang et al. reported that intensive supplementation with *Pediococcus acidilactici* AAF1-5 into the microbial community stimulated the growth of *Acetobacter* and *Lactobacillus* and enhanced the production of flavor compounds[66]. Compared with the control, the concentrations of non-volatile acids, lactic acids, and amino acids in vinegar increased by 53%, 14%, and 32%, respectively[66]. In addition, the authors of a study on fermented fish reported that the introduction of *Weissella cibaria* M3, compared with fermentation carried out solely with *Lactococcus lactis* M10, led to a shorter time to complete fermentation[67].

Overall, these studies illustrate how building on the core microbial community can enhance the production of key metabolites and improve sensory quality. More specifically, additional microbial members—referred to as the 'supplementary microbiome'—can be introduced to further modulate fermentation outcomes, such as flavor complexity, nutritional value, and functional properties, beyond what is achievable by the core microbiome alone.

Multi-omics–guided design and refinement of the DMCs

The selection of core and supplementary microbiomes are critical steps in the rational design of reconstructed fermented foods. To achieve precise selection at species level, multi-omics approaches, including metagenomics, metatranscriptomics, metaproteomics, and metabolomics, provide comprehensive insights into microbial community structure, functional potential, and active metabolic pathways. These datasets enable the identification of keystone species and functional redundancies, which are crucial for determining both core and supplementary microbiomes. Furthermore, the iterative assembly–assessment–redesign cycle serves as a powerful framework to optimize these microbiomes: reconstructed communities are assembled, their fermentation performance is experimentally evaluated, and the results inform subsequent refinements (Fig. 4). Through repeated iterations, this process ensures the development of robust, predictable, and application-specific microbial consortia.

The identification of core and supplementary microbiomes

The selection of the core microbiome should adhere to multiple criteria, such as microbial abundance, occurrence frequency, inter-microbial interactions, and microbial functionality[68,69]. First, in the selection of a core microbiome, the abundance of microorganisms provides a measure of community richness, whereas their occurrence across different samples reflects the extent to which they are universally distributed within a given ecosystem. In this context, metagenomics plays a pivotal role, as it enables comprehensive profiling of microbial community composition and the discovery of taxa that might not be detectable through culture-dependent methods. In a study of Chinese traditional rice wine fermentation, the core microbiome was delineated based on both relative abundance and occurrence frequency. This analysis revealed that *Lactobacillus* (80.88%), *Acetobacter* (12.36%), *Weissella* (5.35%), and *Bacillus* (0.58%) constituted the principal bacterial taxa, which overall accounted for 99.17% of the total bacterial population[70]. However, defining the core microbiome solely based on abundance and occurrence has its limitations, and it is important that the inter-microbial interactions should also be taken into consideration. In fermentation environments, the formation and stabilization of community structures rely on continuous metabolic exchanges and signaling, which are facilitated by the close spatial organization of microorganisms. From a macro perspective, most candidate species within the core microbiome should exhibit positively correlated growth patterns. Amensalism, competition, commensalism, and mutualism are four basic motifs of microbial interactions in fermented foods. However, when considering the selection of the core microbiome, particular emphasis should be placed on commensalism and mutualism, as these interactions often underpin community stability and functional complementarity in fermentation ecosystems. In contrast, excessive competition or strong amensalistic effects may destabilize the community, reduce diversity, and impair the overall quality and reproducibility of the fermentation process. Commensalistic interactions have been reported in a proposed kombucha core microbiome, where *Brettanomyces bruxellensis* can stimulate *Komagataeibacter intermedius* growth, by breaking down sucrose to provide glucose and ethanol, which is consumed by *Komagataeibacter intermedius*[71]. Furthermore, in numerous fermented foods, the coexistence of yeasts and LAB is characterized by mutualism, where both groups of microbes benefit from one another's metabolic activities, driving community stability and product quality. For example, within the water kefir core microbiome, *Lactobacillus hordei* and *Lactobacillus nagelii* contribute to the environment by secreting lactic acid, which creates favorable conditions for the growth of *Zygotorulaspora florentina* and *Saccharomyces cerevisiae*. In a reciprocal manner, these yeasts provide essential metabolites such as amino acids and vitamin B6, thereby stimulating and sustaining the growth of *L. hordei* and *L. nagelii*[72]. Although species occurrence and interaction patterns shed light on the ecological basis of fermented food microbiomes, these features alone cannot fully capture the essential contributions of microbes to fermentation outcomes. Therefore, the selection of the core microbiome must also incorporate criteria of

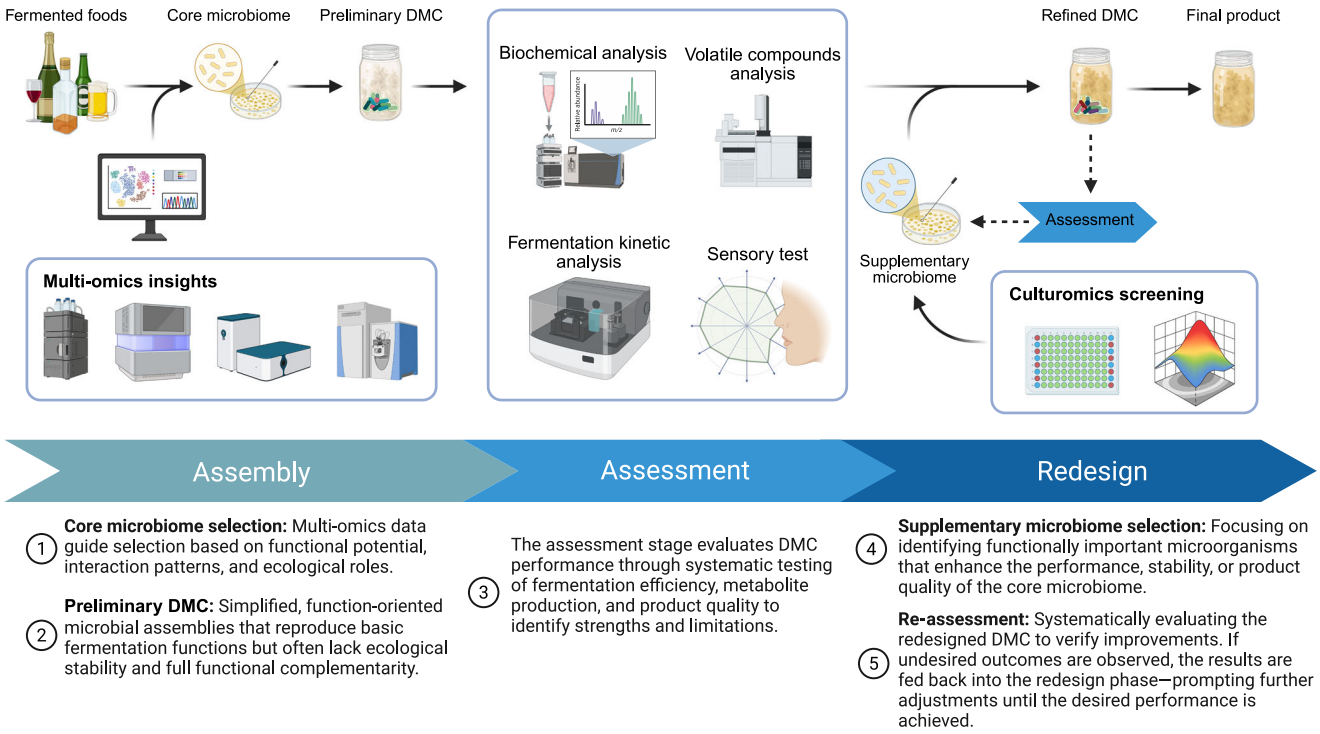


Fig. 4 | The "Assembly–Assessment–Redesign" approach of DMCs (Created in bioRender.com).

microbial functionality. In the previous section, we have discussed the contributions of the four major microbial groups in fermented foods; however, a more detailed species-level functionality profiling relies on the information provided by multi-omics approaches.

In contrast, the selection of the supplementary microbiome should be guided by different principles, as these microorganisms are not necessarily dominant or ubiquitous but instead provide auxiliary functions, enhance resilience, or contribute to specific product qualities under certain fermentation conditions. Generally, an ideal reconstructed fermentation process is one that combines efficiency and quality: it produces the target product rapidly and economically, while fully preserving its sensory characteristics, nutritional content, functional attributes, and hygienic standards. Such a process may also ensure enhanced stability and prolonged shelf life, thereby optimizing overall reconstructed fermented product integrity and value. Supplementary microbiomes are intended to assist the core microbiome in achieving these objectives. To identify candidate supplementary microbiomes, metagenomic analysis can first be used to predict the biochemical traits of isolates, followed by validation through culturomics screening, enabling the selection of strains with the desired functional characteristics. However, most current studies have primarily focused on using culturomics screening to identify strains with specific desirable traits. For example, Li et al. evaluated various *S. davenportii* and *G. intermedius* isolates for their ethanol and gluconic acid production capacities and subsequently selected suitable strains for microbial community reconstruction[65]. This approach effectively increased the proportion of gluconic acid, thereby improving the taste quality of kombucha. Apart from this, researchers also tried to screen for acid-producing strains in pickles microbiome, which was used in subsequent mustard leaves fermentation[73]. However, such culture-based approaches generally involve small sample sizes, limiting the diversity of strains that can be isolated and failing to fully capture the functional potential of the natural microbiome. At the same time, we note that a growing number of studies have integrated metagenomic and metabolomic datasets to uncover correlations between specific microbial species and distinct functional or sensory properties of fermented foods. For instance, by integrating metagenomic sequencing data with GC–MS metabolite profiling, researchers demonstrated that *Lactobacillus*, *Saccharomyces*, *Clavispora*, and *Candida* showed significant correlations with the majority of volatile compounds responsible for the flavor characteristics of Chinese Light-Aroma-Type liquor[43]. Similarly, in another study on Chinese Strong-Aroma-Type liquor, researchers identified that *Aquabacterium* exhibited a positive correlation with all detected desirable flavor compounds, further highlighting this genus as a key contributor to the brewing this liquor[74]. Nevertheless, while these omics-based approaches provide valuable insights into potential functional contributors, the lack of systematic wet-lab validation still hampers the confirmation of causal microbial roles in fermentation outcomes. Therefore, integrating culture-based screening with multi-omics analyses represents a promising strategy to both validate and harness the functional potential of candidate supplementary microbiomes, enabling more rational design of reconstructed fermentation communities.

While the above criteria provide a structured framework for selecting components of the core and supplementary microbiomes in reconstructed fermentation systems, their practical relevance may be influenced by process duration and ecological complexity. This is especially apparent in long-maturing fermentation systems, such as hard and semi-hard cheeses, where microbial contributions are distributed across multiple stages of product development. Moreover, microbial functions inferred from omics-derived correlations do not always translate directly into measurable phenotypic outcomes, particularly in systems characterized by strong metabolic coupling, spatial structuring, or dynamic niche partitioning. Under such circumstances, static community descriptors may oversimplify functional relationships within reconstructed consortia. Therefore, while multi-omics approaches provide a powerful foundation for microbiome selection, their effective application benefits from integration with targeted cultivation, stage-resolved experimentation, and hypothesis-driven validation. This integrative strategy enables a more nuanced interpretation of microbial functionality and supports the rational design of reconstructed fermentation communities across diverse fermentation contexts.

Assembly–Assessment–Redesign (A-A-R) framework for DMCs optimization

At present, most studies on fermented foods have remained at the stage of proposing or assembling preliminary DMCs; however, systematic

evaluation and iterative optimization of their functional performance, stability, and reproducibility are still largely lacking. These initial consortia therefore often fall short of being directly applicable to industrial-scale fermentation, since they may exhibit instability under varying conditions or fail to fully realize their functional potential. Hence, to achieve efficient and reproducible fermentation outcomes, the optimization of DMCs can be conceptualized within an assembly–assessment–redesign cycle (Fig. 4).

In the assembly stage, candidate strains drawn from the defined core microbiomes are systematically combined, with their selection guided by multi-omics insights to ensure functional complementarity and ecological compatibility. For example, functional complementarities, such as acidification by LABs to support AABs growth or ethanol production by yeasts to benefit AABs, are taken into account when designing the initial consortium.

During the assessment stage, the performance of the assembled DMCs is experimentally evaluated using comprehensive, high-throughput assays. Metrics include fermentation kinetics (substrate consumption, pH dynamics), metabolite profiles (flavor compounds, organic acids), community stability (species abundance and functional redundancy), and final product quality attributes, such as texture, flavor, and shelf life. At this stage, advanced tools such as microplate fermentations[75] or droplet microfluidics[76] can accelerate performance evaluation and provide detailed data on interspecies interactions.

Based on these experimental insights, the redesign stage involves iterative refinement of the consortium. Strategies may include adjusting strain ratios to enhance key functions, removing antagonistic or redundant members, introducing complementary taxa to improve flavor or nutritional properties, or even employing targeted strain engineering to strengthen specific metabolic pathways. Integration of multi-omics datasets with computational modeling or machine learning further informs these refinements, enabling prediction of community dynamics and functional outcomes.

Through repeated iterations of this cycle, DMCs can be progressively optimized toward stable, efficient, and predictable fermentation systems that consistently deliver high-quality products. Ultimately, the A–A–R cycle provides a systematic, data-driven approach to engineer robust, functional, and application-specific synthetic microbial communities for reconstructed fermented foods.

Conclusions and future prospects

Advances in multi-omics technologies have revolutionized our understanding of the microbial ecology that underpins traditional fermented foods. These approaches have enabled researchers to move beyond descriptive community analyses toward a more mechanistic comprehension of how microbial consortia function, interact, and collectively shape fermentation outcomes. By integrating metagenomic, metatranscriptomic, metaproteomic, and metabolomic data, scientists can now identify core microbiomes that are essential for fermentation stability and product consistency, as well as supplementary microbiomes that contribute auxiliary or condition-specific functionalities. This knowledge provides a robust foundation for the rational reconstruction of DMCs that can emulate or even improve upon natural fermentation processes.

Despite these advances, the practical implementation of DMCs in fermented food systems remains in its infancy. Most current studies remain at the conceptual or laboratory scale, and many DMCs have yet to be validated under industrial fermentation conditions. Looking forward, several key directions can help accelerate progress in this field. First, integrating multi-omics datasets with computational modeling and machine learning will allow the prediction of community behaviors and metabolic outcomes before physical testing, reducing experimental costs and time. Second, emerging high-throughput co-culture platforms will provide new avenues for systematically testing strain compatibility and optimizing metabolic interactions in real time. Finally, the establishment of standardized assessment metrics—such as community stability, metabolic efficiency, and sensory quality—for evaluating reconstructed fermentation products will be essential for translating laboratory success into industrial-scale reliability.

It is also important to recognize that many traditional fermented foods are produced under strict production specifications or disciplinary frameworks, such as PDO/PGI (Protected Designation of Origin/Protected Geographical Indication) schemes or national regulations, which may significantly constrain the practical deployment of DMCs. These regulatory frameworks are primarily intended to preserve the authenticity, typicity, and cultural heritage of traditional products, and therefore often require the maintenance of established raw materials, processing conditions, and fermentation practices. In this context, the introduction of selected, tailor-made, or engineered starter cultures may be regarded as an artificial intervention that could alter the characteristic microbial succession, metabolic profiles, and sensory attributes that define a protected product. Moreover, many PDO/PGI specifications explicitly mandate the use of spontaneous fermentation or indigenous microbiota, thereby limiting the legal feasibility of replacing native communities with reconstructed consortia, even when such consortia are rationally designed based on multi-omics insights. As a result, the immediate application of DMCs in real production systems may be limited, particularly for products that rely on spontaneous fermentation or legally protected traditional practices. Under these conditions, multi-omics technologies may currently play a more realistic role in the in-depth characterization and monitoring of autochthonous microbial communities, the identification of key functional members within naturally occurring consortia, and the support of process consistency and product authentication, rather than in the direct replacement or engineering of starter cultures. Acknowledging these regulatory and technological constraints is essential for aligning multi-omics-driven strategies with real-world fermentation practices.

To sum up, the convergence of multi-omics-guided microbiome selection, assembly–assessment–redesign optimization workflow, and data-driven engineering approaches will pave the way for the next generation of precision-designed fermented foods. Such innovations promise not only to preserve the cultural and sensory heritage of traditional fermentation but also to achieve unprecedented control, reproducibility, and functionality in modern food biotechnology.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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Author contributions

E.Z. and P.D.C. conceived the study idea and design. E.Z. wrote the manuscript with contributions from M.J.C. and P.D.C. P.D.C. supervised the project.

Competing interests

The authors declare no competing interests.

Additional information

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