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REVIEW

Recent advances in applying omic technologies for studying acetic acid bacteria in industrial vinegar production: A comprehensive review

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Abstract

Vinegar and related bioproducts containing acetic acid as the main component are among the most appreciated fermented foodstuffs in numerous European and Asian countries because of their exceptional organoleptic and bio-healthy properties. Regarding the acetification process and obtaining of final products, there is still a lack of knowledge on fundamental aspects, especially those related to the study of biodiversity and metabolism of the present microbiota. In this context, omic technologies currently allow for the massive analysis of macromolecules and metabolites for the identification and characterization of these microorganisms working in their natural media without the need for isolation. This review approaches comprehensive research on the application of omic tools for the identification of vinegar microbiota, mainly acetic acid bacteria, with subsequent emphasis on the study of the microbial diversity, behavior, and key molecular strategies used by the predominant groups throughout acetification. The current omics tools are enabling both the finding of new vinegar microbiota members and exploring underlying strategies during the elaboration process. The species *Komagataeibacter europaeus* may be a model organism for present and future research in this industry; moreover, the development of integrated metaomic analysis may facilitate the achievement of numerous of the proposed milestones. This work might provide useful guidance for the vinegar industry establishing the first steps towards the improvement of the acetification conditions and the development of new products with sensory and bio-healthy profiles adapted to the agri-food market.

KEYWORDS
acetic acid bacteria, fermented foods, metabolomics, metagenomics, metaproteomics, metatranscriptomics

1 | INTRODUCTION

Vinegar is one of the most appreciated and exclusive fermented foodstuffs in European and Asian countries. Because of their organoleptic and bio-healthy properties, vinegar and other bio-products containing acetic acid as the main component are increasingly in demand by consumers today. Certain advantages associated with the impact of acetic acid on the human body may encompass the stimulation of appetite, recuperation from exhaustion, antioxidative properties, reduction in lipid levels in the bloodstream, and the regulation of blood pressure. These effects may, in turn, have implications for biomarkers related to various diseases, including obesity, cancer, diabetes, and hypertension.[1–3]

Among multiple factors, the activity of complex microbiota, in which acetic acid bacteria (AAB) prevail over minor fractions of other microorganisms, is essential for obtaining these products in the industry.[4–6] This activity is mainly focused on the biotransformation of the ethanol from an alcoholic medium into acetic acid occurring in specific bioreactors (acetators).[7–9] Nevertheless, despite the well-established nature of this bioprocess from a technical standpoint, there remain numerous fundamental aspects that are still being researched or remain unknown. This is particularly true for aspects related to the biodiversity, behavior, and molecular mechanisms employed by the existing microbiota as they adapt to the medium during acetification. Numerous studies are currently facilitating the identification of the variety of microorganisms that conform this microbiota, not only within the predominant AAB group, but also other members of the Acetobacteraceae family, lactic acid bacteria, among other bacteria groups, and even archaea.[10–13] Although the key metabolism throughout acetification lies in the biotransformation of substrate (ethanol) into product (acetic acid), several metabolic pathways, mainly focused on the biosynthesis of cellular materials, energy metabolism, and membrane detoxification mechanisms, which are part of the microbiota molecular strategies for survival in these aggressive media, are increasingly the target of much research.[9,14–18]

To effectively address these research endeavors and as a viable alternative to overcome many of the conventional challenges associated with the isolation and characterization of microbiota members, mainly AAB, the omic technologies have emerged in the last years and are being applied in the fermented foods area.[19,20] In this context, omic tools address the massive analysis of the content of macromolecules such as genes (metagenomics), transcripts (metatranscriptomics), proteins (metaproteomics), and metabolites (metabolomics) when studying these microorganisms operating within their natural environments.[5,12,21–23] This approach allows us to attain a precise understanding of the processes occurring during crucial moments and under specific conditions of acetification. It also helps overcome numerous limitations associated with studying the richness and biodiversity of the microbiota inhabiting selective environments like vinegar.[8,20] The current state of omic sciences and molecular modification methods may be applied for increasing the understanding of physiological behavior, the characterization of new strains recovered from these complex media, as well as to exploit the full potential of AAB for producing vinegar and other related bioproducts.[20,24]

Considering all the aforementioned, this review starts with a comprehensive update on the application of diverse omic tools for the identification of vinegar microbiota, especially in the AAB area. Considering recent research, which includes a progressive increase in the number of new organisms and strategies existing throughout acetification, this review discusses the current state of knowledge about microbial biodiversity and key molecular strategies used; emphasis has been placed on *Komagataeibacter europaeus* as one of the main predominant species in industrial production of vinegar in Europe according to studies both their own and those of other authors. The main objective of this research is to offer valuable insights for the vinegar industry and other bioproduct sectors. Additionally, it seeks to establish operational methodologies for enhancing acetification conditions and the development of new products with sensory attributes and bio-healthy characteristics tailored to meet the demands of the agri-food market.

2 | OMIC TECHNOLOGIES FOR MOLECULAR IDENTIFICATION OF VINEGAR-PRODUCING MICROBIOTA

The unique growth conditions and metabolic traits of the most microbiota members make their isolation challenging through conventional techniques on solid media, especially when trying to replicate their full activity outside of their natural environments.[25,26] This is particularly evident in the context of fermented foods and beverages, such as produced vinegar within industrial bioreactors. In this setting, the microbiota predominantly consists of AAB, which need precise concentrations of substrate (ethanol) and product (acetic acid), a low pH environment, and a continuous oxygen supply achieved through medium aeration.[27] These challenges impose limitations on the exploration of microbial diversity and richness, potentially leading to the oversight of crucial species within this microbiota that thrive in such hostile environments.[26] Historically, the identification of AAB has relied on several morphological, biochemical, and physiological criteria. However, contemporary identification methods prioritize the comprehensive analysis of biological macromolecules, including DNA, RNA, proteins, and metabolites, within a cell, tissue, organism, or population, particularly during critical time points and under specific conditions.[20,24,28] As a result, omics tools are now emerging as a promising technology to address many of the challenges associated with traditional methods. In this section, we will provide an in-depth overview of the primary molecular techniques that have been employed over time and continue to be utilized today for the identification and classification of AAB. These techniques take into account both the taxonomic level and the specific macromolecule or metabolite analyzed. For a comprehensive compilation of these tools, please refer to Figure 1.

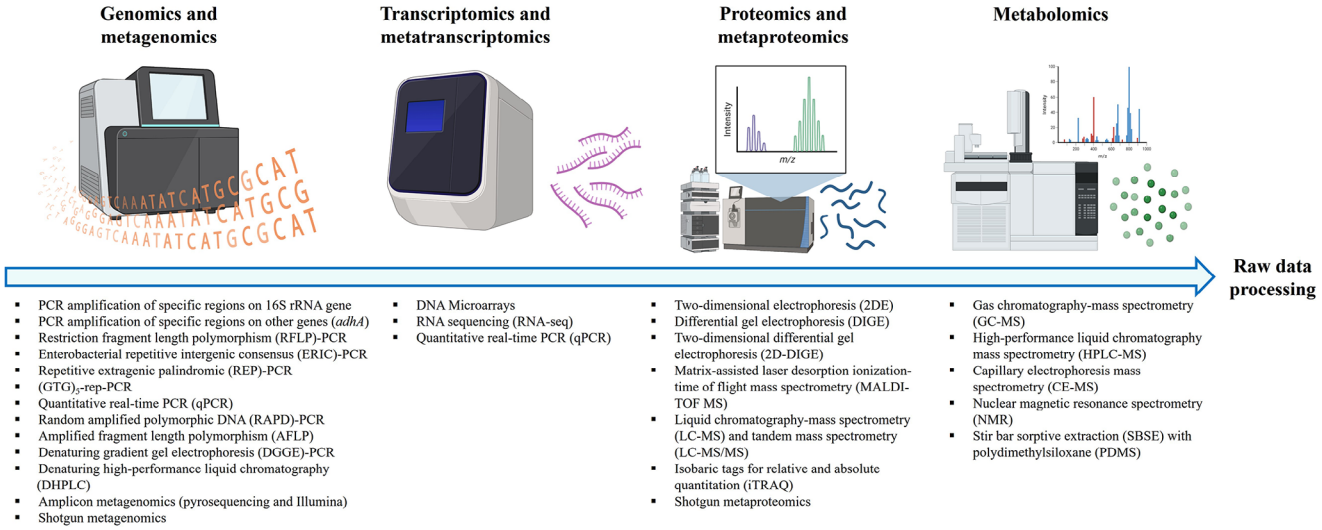

FIGURE 1 Compilation of the molecular tools for identification of the vinegar microbiota members for each (meta)omic discipline (meta(genomics), meta(transcriptomics), meta(proteomics), and metabolomics) from initials to the most current ones.

2.1 | Genomics and metagenomics

The application of molecular methods for microorganism identification in vinegar was initiated over 25 years ago, primarily through DNA-based methods. Initially, researchers employed assays centered on the selective extraction of nucleic acids, including plasmid profiling and DNA-DNA hybridization. Subsequently, PCR-based systems were developed.[29] These PCR methods involved the amplification of specific regions within the 16S rRNA gene and restriction fragment length polymorphism (RFLP)-PCR analysis of the same gene, enabling the identification of populations of microorganisms in vinegar at both the genus and species levels.[30,31] The use of restriction enzymes like *TaqI* and *RsaI* for digestion played a pivotal role in distinguishing between different genera of AAB. However, due to the relatively high conservation of the 16S rRNA gene, researchers found it necessary to turn to the 16S-23S rRNA intergenic spacer region (ITS) for their analysis. This region exhibits a greater degree of variability compared to the functional sequences, making it more suitable for differentiation at the species level.[32] Other PCR-based methods have also been employed in the identification and characterization of AAB. Firstly, researchers have amplified specific regions of other genes, such as *adhA* in *K. europaeus* and *nifH/nifD* in nitrogen-fixing AAB.[33,34] Secondly, a combination of various techniques has been used, including enterobacterial repetitive intergenic consensus (ERIC)-PCR, repetitive extragenic palindromic (REP)-PCR, (GTG)5-repetitive element-PCR, quantitative real-time PCR (qPCR), nested PCR, random amplified polymorphic DNA (RAPD)-PCR, and amplified fragment length polymorphism (AFLP). These methods have been applied by several researchers to identify, type, and sometimes quantify AAB species and strains, particularly within the microbiota of wine and vinegar.[25,30,35–38] While these techniques have been considered as rapid methods for taxonomically categorizing AAB, achieving accurate identification typically necessitates a polyphasic approach.[28] In this context, electrophoresis-based techniques come into play by separating amplified fragments of the 16S rRNA gene based on their mobility under denaturing conditions. Two commonly employed methods for this purpose are denaturing gradient gel electrophoresis (DGGE)-PCR and denaturing high-performance liquid chromatography (DHPLC). These techniques have been utilized to group the primary genera of AAB involved in both traditional[39] and submerged vinegar production.[11] Both of these approaches are valuable for monitoring structural changes in fermented food microbiota. However, due to the limited size of DNA fragments, they typically only allow identification at the genus level. Recognizing this limitation, Andrés-Barrao et al.[15] employed DGGE-PCR in conjunction with housekeeping gene sequencing (*dnaK*, *groEL*, *rpoB*) and multi-locus sequence typing (MLST) to construct a comprehensive phylogenetic tree of *Komagataeibacter* strains involved in the production of high-acid spirit vinegar. This approach enabled a more detailed and precise characterization of these strains.

Recently, metagenomics and high-throughput sequencing have emerged as innovative tools that facilitate the analysis of genomic DNA from all organisms within a microbial population.[20] These next-generation sequencing (NGS) tools offer a wealth of information concerning gene content and functionality, enabling researchers to identify and categorize microbiomes, reconstruct metabolic pathways, and compare experimental conditions to uncover differences in microbial composition, abundance, and functions.[40] Amplicon metagenomics, utilizing methods like pyrosequencing and Illumina sequencing, has been applied to identify and quantify metagenomes of AAB in several varieties of vinegar produced via both surface[41,42] and submerged culture.[10,11] In contrast, shotgun metagenomics employs sequencing data to deduce potential metabolic functions encoded by the genomes of the community members under investigation. This process involves the assembly of sequence reads, followed by gene prediction and even the discovery of whole genomes of VBNC (Viable

but Not Culturable) microorganisms.[43] In the context of vinegar, this technology was initially employed for analyzing the microbiota of cereal vinegar, revealing the metabolic network responsible for flavor, as depicted in Figure 1.[12] It is noteworthy that many of these tools are often used in conjunction with other technologies for the analysis of macromolecules (proteins, metabolites, etc.) to obtain comprehensive meta-omic profiles.[10,13]

2.2 | Transcriptomics and metatranscriptomics

Nowadays, transcriptomics has been obtaining more and more interest from researchers being considered as the link between genomics and proteomics, leading to the conduction of several relevant studies based on mRNAs. One of the first assays in this field was performed by Sakurai, Arai, Ishii, & Igarashi[44] consisting of a microarray transcriptomic study that analyzed the gene expression patterns of *A. aceti* NBRC 14818 when the cells were oxidizing ethanol. Later, Okamoto-Kainuma & Ishikawa[45] performed an RNA-Seq transcriptomic analysis based on acetic acid resistance mechanisms in *A. pasteurianus* NBRC 3283, with special attention to the role of molecular chaperones. Yang, Yu, Fu, & Chen[46] carried out an RNA-seq transcriptome study in *A. pasteurianus* CGMCC 1.41 under acetic acid fermentation conditions, proposing the 2-methyl citrate cycle as a potential acetic acid resistance-conferring pathway. Wang, Hong, Zhang, Huang, & Guo[47] conducted a transcriptomic study in *K. europaeus* CGMCC 20445 in an ethanol-oxidating medium to determine several mechanisms against high acid stress at different stages of acetic acid fermentation. More recently, the RNA-Seq transcriptomic analysis led by Yang et al.[22] has allowed studying gene regulation changes to find possible relationships with a high acidity level (8%–12%) in the medium, see Figure 1. Most of these studies aimed to determine mechanisms of resistance to acetic acid stress by the analysis of gene expression patterns.

Targeting the actively expressed genes, metatranscriptomics sequences the cDNA after reverse-transcription of community RNA, revealing the active population and actively expressed genes under specific conditions, which can be associated with ongoing variations in the metabolome as well as the flavor and taste formation in fermented foods; these are very useful tools for innovative multi-omic approaches.[24] In this sense, this methodology has been used in Shanxi-aged vinegar, obtained by the solid-state fermentation system, to explore the metabolic profile of main organic acids; carbon metabolism was the dominant pathway, as well as *Acetobacter* and *Lactobacillus* the main microbial taxa.[48] Huang et al.[49] used a metatranscriptomic approach in cereal vinegar, along with other omic tools, to identify the main species in an autochthonous vinegar starter culture (*A. pasteurianus*, *Lactobacillus acetotolerans*, *L. helveticus*, *Acetilactobacillus jinshanensis*), evaluate their interaction during acetic acid fermentation, and construct a defined starter with these organisms to favor the start-up of the process and improve the fermentation yield.

2.3 | Proteomics and metaproteomics

The DNA residing within cells serves as the comprehensive genetic blueprint of the entire organism. However, the cellular phenotype becomes apparent only through the examination of proteins. Proteins play pivotal roles in executing one or more specific functions within cells. Due to their remarkable activity and susceptibility to environmental fluctuations, the study of proteins throughout the dynamics of fermentation has generated considerable interest.[20,50] Proteomics involves the comprehensive examination of the complete set of proteins generated by a cell or organism. This approach enables the identification and quantification of these proteins, thereby providing a precise depiction of events unfolding during pivotal moments and under particular conditions in a biological process.[20,24] When dealing with complex microbiota, like AAB found in vinegar production, metaproteomic techniques come to the forefront. These methods facilitate the exploration of the composition and functionality of numerous proteomes originating from various species or strains, all in a high-throughput way.[51]

The initial protein-based technique employed for the examination of vinegar microbiota was two-dimensional electrophoresis (2DE). This method entails the separation of complex protein mixtures by first subjecting them to isoelectric focusing (IEF) and then sorting them based on their molecular weight using polyacrylamide gels (SDS-PAGE).[50] In a noteworthy study, Lasko, Schwerdel, Bailey, & Sauer[52] investigated protein patterns in response to acetate stress in two acetate-resistant species of *Acetobacter*. A few years later, researchers delved into the proteins associated with the response to acetic acid in *A. aceti* and their connection to the tricarboxylic acid (TCA) cycle as a strategy for its assimilation.[53,54] While 2DE brought significant molecular insights into enhancing vinegar production, it faced challenges regarding the inherent lack of reproducibility between gels. To address this issue, researchers developed differential gel electrophoresis (DIGE), which involves labeling two samples, each with a distinct fluorescent dye (Cy3-NHS and Cy5-NHS), before running them on the same gel. The implementation of two-dimensional-DIGE has enabled the identification of differentially quantified proteins within the proteome of *A. pasteurianus* and the metaproteome of *Komagataeibacter* spp. involved in spirit vinegar production.[15,55]

Given the labor-intensive nature of the molecular methods mentioned, there has been a drive to develop new approaches for the swift identification of bacteria.[56] Among these approaches, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) stands out. It enables the rapid and routine identification of a considerable volume of bacterial samples, producing a distinctive mass spectrum comprising multiple peaks associated with high-abundance soluble proteins. This, in turn, yields a unique protein profile for each bacterial group, facilitating differentiation at the levels of genera, species, and strains.[56] MALDI-TOF MS has garnered acclaim for its expeditious and dependable capabilities in the identification of acetic acid bacteria (AAB) involved in the industrial production of vinegar and its role in detecting beer spoilage.[10,57,58] In recent times, there

has been a fusion of mass spectrometry (MS) technology with liquid chromatography (LC), giving rise to LC-MS. This analytical method is characterized by its sensitivity, selectivity, and precision, as it involves the physical separation and mass-based detection of proteins, peptides, other macromolecules, and metabolites.[59] The incorporation of a second mass analyzer, operating in conjunction (LC-MS/MS), gave rise to shotgun metaproteomics technology, which has emerged as a potent tool for the rapid identification of thousands of proteins within a metaproteome. This is achieved by analyzing intricate mixtures of peptides generated through proteolytic digestion, all without the need for prior electrophoresis-based separation. Shotgun metaproteomics offers a broader dynamic range and enhanced protein coverage, making it a valuable approach in the field.[60] In recent years, a multitude of studies have homed in on various modalities of this technology within the realm of AAB. These modalities include free-label LC-MS/MS,[61] isobaric tags for relative and absolute quantitation (iTRAQ),[18] and the metaproteomics approach using LC-MS/MS.[5] These advancements have facilitated comprehensive investigations into the entire microbiota involved in vinegar production, see Figure 1.

2.4 | Metabolomics

The production of vinegar primarily hinges on a myriad of metabolic reactions in which the resident microbiota plays a pivotal role. This intricate process results in the generation of various metabolites, including sugars, sugar alcohols, amino acids, carboxylic acids, fatty acids, and volatile compounds. This assortment of metabolites forms a metabolomic profile that is instrumental in shaping the organoleptic characteristics of the final product.[21] Metabolomics is a relatively recent approach frequently employed in conjunction with (meta)genomics and/or (meta)proteomics. It aims to establish connections between various macromolecules such as genes and proteins derived from the microorganisms present in the environment and the corresponding metabolites they produce.[20,62] Metabolomic studies employ two primary approaches: untargeted and targeted analyses. In most cases, the involved instrumental techniques require the prior separation of metabolites from the samples through chromatography. Subsequently, the metabolites are identified and quantified using mass spectrometry (MS) based on their mass-to-charge (m/z) ratio.[21] For analyzing diverse metabolomic profiles in vinegar, particularly in products like balsamic and cereal vinegar, several techniques have been widely utilized, including gas chromatography-mass spectrometry (GC-MS)[13,21]; high-performance liquid chromatography-mass spectrometry (HPLC-MS)[63]; capillary electrophoresis-mass spectrometry (CE-MS)[64] and nuclear magnetic resonance (NMR) spectrometry.[65] Among these, GC-MS is considered one of the most mature technologies in metabolomics. It enables the simultaneous analysis and identification of hundreds of metabolites, offering high-resolution capabilities and sensitivity. This makes GC-MS an invaluable tool for generating comprehensive metabolite profiles of fermented products like vinegar. Indeed, while gas chromatography-mass spectrometry (GC-MS) is a powerful tool for metabolomic analysis, it often necessitates chemical derivatization for non-volatile metabolites.[66] However, for the extraction of volatile compounds, the use of stir bar sorptive extraction (SBSE) with a polydimethylsiloxane (PDMS) coating has proven advantageous, addressing some of the drawbacks associated with other extraction methods, see Figure 1. SBSE offers several advantages, including the absence of solvents, simplicity, speed, high sensitivity, and suitable limits for the quantification and detection of volatile compounds in vinegar.[67,68]

2.5 | Databases and software for raw data analysis in omic sciences

Once the various macromolecules or compounds originating from the microorganisms present in vinegar have been identified and/or quantified, understanding their microbial composition and behavior within their natural environment becomes essential. To achieve this, the raw data obtained must undergo processing using specific databases or software tailored to the respective omic tool being employed. This step is crucial in extracting meaningful insights from the complex data generated in omics studies.[24] The field of omic sciences continually sees advancements in technologies that support not only the experimental procedures but also the subsequent handling of raw data. These developments primarily focus on enhancing efficiency and accuracy. Selecting appropriate tools for processing raw data and conducting bioinformatic analyses is crucial for the success of experimental designs within omic sciences. Table 1 provides a compilation of some of the most commonly used databases and software for omic analyses in the realm of AAB. These tools and resources play a vital role in extracting valuable insights from omic data in the context of AAB research.

3 | MICROBIAL BIODIVERSITY THROUGHOUT THE ACETIFICATION PROCESS

The composition of microbiota inhabiting vinegar plays a pivotal role in shaping the organoleptic properties and overall quality of the vinegar. Omic tools have undeniably contributed to advancing our comprehension of microbial biodiversity during the acetification process. This has been achieved through various approaches, including metagenomics, allowing for a more comprehensive understanding of the microbial dynamics at play,[11,12,69,70] transcriptomics,[22,47] proteomics,[18,71,72] metaproteomics,[5,9,15] and metabolomics.[13,73,74] These studies have made it possible to identify a significant portion of the microorganisms participating in these biotransformations. Furthermore, they have described the behavior of these microorganisms under various operating conditions and within different media. As a result, they have played a crucial role in elucidating the essential functions and contributions of the vinegar-making microbiota.

The microbial composition of vinegar is indeed strongly influenced by factors such as the initial inoculum, raw materials used, and the production system employed.[4,6] Typically, *Acetobacter* and *Komagataeibacter* are the primary AAB responsible for the acetification pro-

TABLE 1 Compilation of the main databases, software, and tools used for raw data processing derived from omic technologies and mainly targeted to the analysis of bacterial groups.

Software/database	Accession link	Omic technology	Description	Reference
BLAST	https://blast.ncbi.nlm.nih.gov/Blast.cgi	(Meta)genomics and (meta)proteomics	Comparison of nucleotide or protein sequences to sequence databases	[104]
Quimee2	https://qiime2.org/	(Meta)genomics	Interactive visualization and treatment of metagenomic (amplicon sequencing) data	[105]
LPSN	https://lpsn.dsmz.de/	(Meta)genomics	Taxonomic classification of prokaryotic microorganisms	[106]
UniProt	https://www.uniprot.org/blast/	(Meta)proteomics	Freely accessible database of protein sequences and functional information. GO Term analysis	[107]
MaxQuant	https://www.maxquant.org/	(Meta)proteomics	Quantitative proteomics for analysis of high-resolution MS data	[108]
Perseus	https://maxquant.net/perseus/	(Meta)proteomics	Interpreting protein quantification, interaction, and post-translational modification data	[109]
STRING	https://string-db.org/	Proteomics	Protein-protein interactions, both physical and functional associations	[110]
KEGG	https://www.genome.jp/kegg/	Genomics	Collection of genome databases, enzymatic pathways, and biological chemicals	[111]
BioCyc	https://biocyc.org/	Genomics and proteomics	Collection of genome databases, metabolic pathways, and biological chemicals. Genomes and proteomes analyses	[112]
MetaboAnalyst	https://www.metaboanalyst.ca/	Metabolomics and multi-omics	Metabolomic data analysis (MS spectra processing, statistical and functional analysis) and other omic analysis	[113]
RStudio	https://www.rstudio.com/	Multi-omics	Raw data analysis through programming. Bioinformatics	
GenBank	https://www.ncbi.nlm.nih.gov/genbank/	Proteomics	Proteomics database with annotated collection of all available nucleotide sequences and their protein transitions	[114]
Reactome	https://reactome.org/	Multi-omics	Database for molecular details (signal transduction, transport, DNA replication, metabolism, and other cellular processes)	[115]
CellML	https://www.cellml.org/	Transcriptomics, proteomics, and metabolomics	Open-source language for biological cellular models	[116]
Escher	https://escher.github.io/#/	Multi-omics	Software for visualization and design of biological pathways and genome-scale models	[117]

cess due to their high oxidative capabilities, which enable them to efficiently convert ethanol into acetic acid. However, it is important to note that there may also be smaller fractions of other microorganisms coexisting alongside these well-adapted AAB strains.[8,10,69] Species within the *Acetobacter* genus typically become impaired when the acetic acid concentration reaches a range of 7%–8% (w/v), with a maximum tolerance level of around 9%–10% (w/v). Consequently, they are commonly found in traditional vinegar-making processes, as well as in the early stages of vinegar production using submerged culture methods or in the case of low-acid vinegar varieties such as cider vinegar [4.0%–9.0% (w/v)] and wine vinegar [4.5%–10% (w/v)]. Among the *Acetobacter* species, *A. pasteurianus* is often the most prevalent.[8,71] Nevertheless, *A. aceti*, *A. malorum*, and *A. pomorum* have also been identified in some of the aforementioned vinegar types, as indicated in Table 2.[39,57] In the case of low-acid vinegar, the microbiota often includes microorganisms beyond AAB. This includes various genera of lactic acid bacteria, such as *Lactobacillus*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, and *Weissella*, among others.[11,13,75] Additionally, small fractions of archaea and other microorganisms may also be present in the microbiota of low-acid vinegar.[12]

Species within the *Komagataeibacter* genus, many of which were formerly classified under *Gluconacetobacter*, exhibit a remarkable ability to withstand acetic acid concentrations ranging from 15%–20% (w/v).[76] These species are highly prevalent in submerged cultures, including the production of spirit vinegar and the later stages of most white and red wine vinegar.[5,8,15,25] Furthermore, due to their tolerance to lower acidity levels, typically in the range of 7%–9% (w/v), several strains of *Komagataeibacter* have been observed to contribute to the acetification of cider, various fruit vinegars, and even traditional vinegars.[11,25,77] Among the *Komagataeibacter* species, *K. europaeus* has been recognized as a key AAB for industrial vinegar production due to its specific growth requirements.[78,79] However, it is important to note that other *Gluconacetobacter* species (such as *Ga. entanii*) and additional *Komagataeibacter* species (including *K. hansenii*, *K. intermedius*, *K. medellinensis*, *K. oboediens*, *K. rhaeticus*, and *K. xylinus*) may also be present in various varieties of vinegar, as indicated in Table 2.[11,25,29,70,80]

The biodiversity of microbiota tends to decline as the concentration of acetic acid in the medium increases, particularly under controlled bioreactor operating conditions. These conditions render the media even more selective, as only a few species can thrive and adapt.[8,38] Nevertheless, recent studies have shed light on the presence of a minority fraction of microorganisms that attempt to coexist alongside the better-adapted species. These less-adapted microorganisms contribute to the stability of the overall microbial community, showcasing the complex interplay within these environments.[10,11,69]

4 | KEY MOLECULAR STRATEGIES THROUGHOUT THE ACETIFICATION PROCESS

The behavior of microorganisms involved in the industrial vinegar production process can be significantly influenced by various factors, including the chemical properties of the raw materials, the production system, and the operating conditions.[4,6] While the primary metabolic process in this complex microbiota is the incomplete oxidation of ethanol into acetic acid, there exist numerous other molecular strategies at both the cytoplasmic and membrane levels that enable the adaptation and survival of community members in response to the conditions imposed by the medium. This intricate interplay of metabolic pathways and adaptive mechanisms contributes to the resilience and stability of the microbial community in vinegar production.[18,69,81] Certainly, the incomplete oxidation reaction is a fundamental aspect of the assimilative metabolism of AAB in the context of vinegar production. This section aims to provide a comprehensive compilation of the primary processes and pathways associated with the central metabolism of acetic acid. These metabolic strategies are employed by the microbiota to ensure their survival and success in the vinegar production process, as illustrated in Figure 2. The analysis of these strategies, some of them partially or completely unknown, have been approached mainly from an omic perspective.

4.1 | Strategies on the cytoplasm: Biosynthetic and stress-related processes

Acetic acid bacteria are available to produce a variety of macromolecules (proteins, nucleic acids, and lipids) and metabolites (alcohols, sugar alcohols, esters, amino acids, and other aromatic compounds) throughout acetification.[13,82] Ammonium serves as a pivotal nitrogen source essential for various biosynthetic processes within AAB. These processes encompass the synthesis of amino acids, proteins, nucleotides, and volatile compounds. Amino acids, in particular, are generated through the utilization of nitrogen sources such as L-glutamine and L-glutamate, both of which are self-regulated by the cell to meet its specific requirements.[17,61] AAB possess a noteworthy capability for nitrogen recovery, allowing them to continuously convert nitrogen sources like proteins, nucleic acids, and apoptotic cells into ammonium and amino acids. This capacity enables AAB to replace any losses in cell materials that may occur during the submerged acetification process. In essence, AAB efficiently recycle nitrogen resources to sustain their growth and metabolic activities throughout vinegar production.[9,83,84] The deamination process, a well-established strategy in various bacterial groups, involves the conversion of L-glutamine into L-glutamate catalyzed by a glutaminase enzyme (such as YbaS). This enzymatic reaction results in the release of gaseous ammonia (NH_3). Consequently, this process contributes to an increase in the content of acid products, including protons (H^+) and ammonium ions (NH_4^+), toward the end of the acetification process. The deamination of L-glutamine to L-glutamate and the subsequent release of ammonia play a role in shaping the chemical composition of the final vinegar product.[85] Indeed, researchers have explored this strategy within vinegar microbiota, with a particular emphasis on acetolactate synthase (Als), a crucial enzyme involved in the synthesis of branched-chain amino acids (BCAA) from pyruvate. Als can play a significant role in providing ammonia (NH_3) and energy to counteract the increase in

TABLE 2 Main prevalent species, including acetic acid bacteria and lactic acid bacteria, in the microbiota of different types of vinegar elaborated according to the raw material used and the production method. The acidity range (%, w/v) between which each type of vinegar is usually elaborated is included.

	Submerged culture production				Traditional culture production (surface and solid-state)		
	Spirit vinegar	Wine vinegar	Cider vinegar	Fruit vinegar	Wine vinegar	Balsamic vinegar	Cereal vinegar
Microorganism	9%–15% (w/v)	4.5%–10% (w/v)	4%–9% (w/v)	4%–9% (w/v)	4%–7.5% (w/v)	4.5%–7.5% (w/v)	4%–7% (w/v)
Acetobacter[11,39,57,96]	–	*A. pasteurianus*	*A. pasteurianus*	*A. pasteurianus*	*A. pasteurianus*	*A. pasteurianus*	*A. pasteurianus*
	–	*A. aceti*	*A. pomorum*	*A. malorum*	*A. aceti*	*A. aceti*	*A. aceti*
	–	–	–	–	–	*A. malorum*	–
Komagataeibacter[8,11,15,25,29,77,79]	*K. europaeus*	*K. europaeus*	*K. europaeus*	*K. europaeus*	*K. europaeus*	*K. europaeus*	*K. europaeus*
	K. oboediens	*K. oboediens*	*K. intermedius*	*K. intermedius*	*K. intermedius*	*K. hansenii*	*K. medellinensis*
	K. intermedius	*K. intermedius*	*K. hansenii*	*K. rhaeticus*	*K. hansenii*	*K. xylinus*	*K. xylinus*
	–	*K. hansenii*	*K. xylinus*	*K. xylinus*	*K. rhaeticus*	–	–
	–	–	–	–	*K. xylinus*	–	–
Gluconacetobacter[80]	*Ga. entanii*	–	–	–	–	–	–
Lactic acid bacteria11[11,13,75]	–	–	*Lactobacillus*	*Lactobacillus*	–	–	*Lactobacillus*
	–	–	*Oenococcus*	*Lacticaseibacillus*	–	–	*Pediococcus*
	–	–	–	*Leuconostoc*	–	–	*Leuconostoc*
				Weissella			*Weissella*

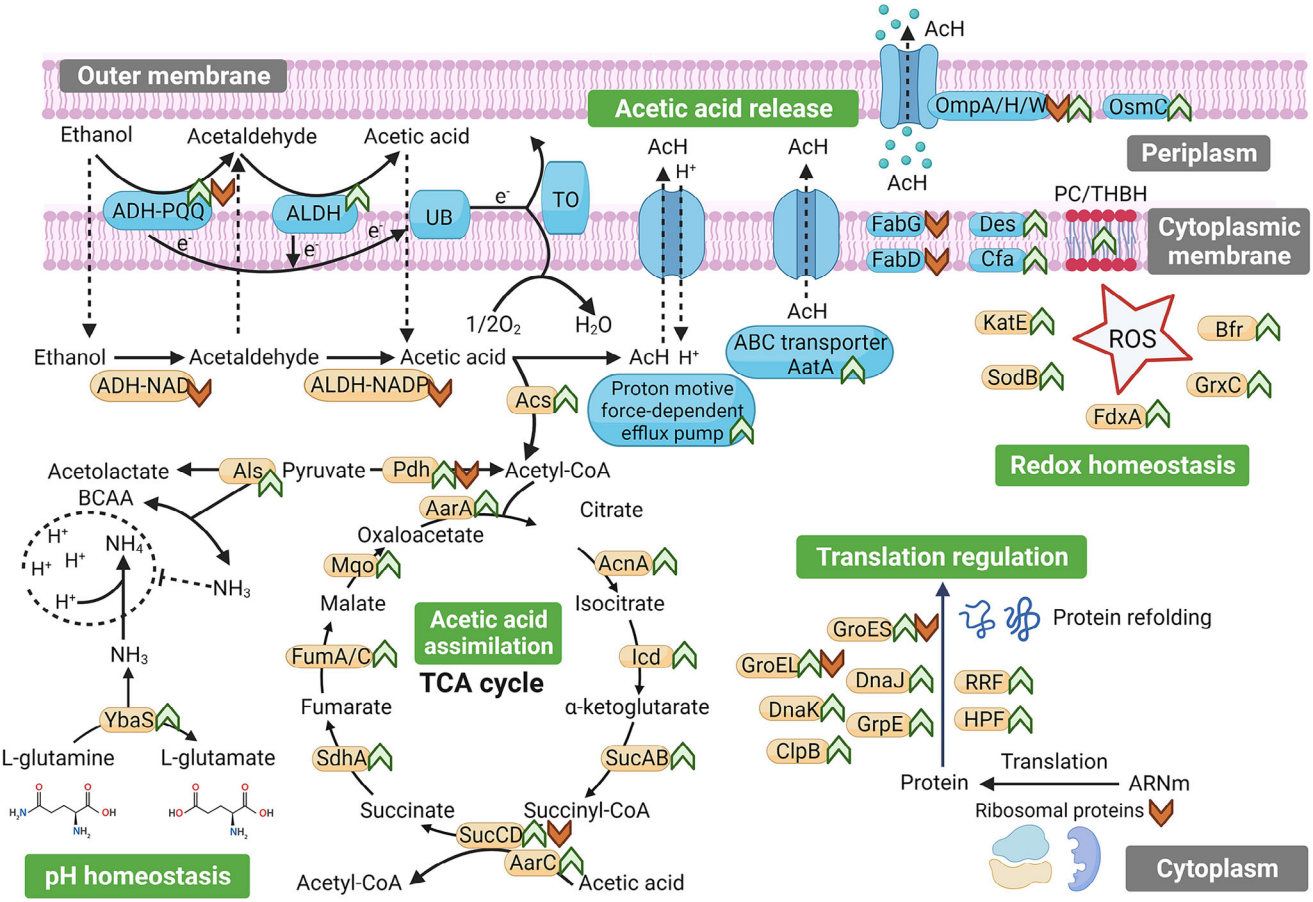

FIGURE 2 Main metabolic pathways performed by vinegar-producing microbiota under high acetic acid concentration conditions. The molecular strategies are included both at the cell membrane level (proteins in blue color): ADH-PQQ, alcohol dehydrogenase PQQ-dependent; ALDH, membrane-bound aldehyde dehydrogenase; UB, ubiquinone; TO, terminal oxidase; AatA, putative ABC transporter; FabD/G, acyl-carrier transacylase; Des, fatty acid dehydrogenase; Cfa, cyclopropane fatty acid synthase; Omp, outer membrane family protein; OsmC, osmotically inducible protein C; and at the cytoplasm level (proteins in orange color): ADH-NAD, alcohol dehydrogenase NAD-dependent; ALDH-NADP, aldehyde dehydrogenase NADP-dependent; Acs, acetyl-CoA synthase; AarA, citrate synthase; AcnA, aconitate hydratase; Icd, NAD^+ isocitrate dehydrogenase; SucAB, α-ketoglutarate dehydrogenase; SucCD, succinyl-CoA synthetase; AarC, succinyl-CoA transferase; SdhA, succinate dehydrogenase; FumA/C, fumarate hydratase; Mqo, malate dehydrogenase; Pdh, pyruvate dehydrogenase; Als, acetolactate synthase; YbaS, glutaminase S; GroESL, heat shock proteins; DnaKJ/GrpE/ClpB, molecular chaperonin proteins; RRF, ribosome recycling factor; HPF, hibernation promoting factor; KatE, catalase; SodB, superoxide dismutase; FdxA, ferredoxin; GrxC, glutaredoxin; Bfr, bacterioferritin. Next to each protein, it is shown if is normally upregulated (green arrow) or downregulated (red arrow) based on studies to date. AcH, acetic acid; BCAA, brain-chain amino acids; NH3, gaseous ammonia; NH4, ammonium; PC, phosphatidylcholine; THBH, tetrahydroxybacteriohopane; ROS, reactive oxygen species. Created with BioRender.com.

final acid products. By doing so, it helps maintain the balance of intracellular pH. The intricate metabolic pathways and enzymes involved in these processes are essential for the overall physiology and survival of the microbiota during vinegar production, as illustrated in Figure 2.[9,15,61]

During acetification, the biosynthesis of proteins represents one of the most prominent metabolic pathways within AAB. However, as acetic acid concentration increases in the later stages of acetification, certain proteins, including ribosomal proteins responsible for ensuring accurate translation processes, often experience a decrease in their activity.[86] This negative effect of increasing acidity on ribosome integrity and protein biosynthesis has been documented in various proteomic and transcriptomic studies.[18,47,55] Interestingly, under high-acidity conditions, the activity of proteins that regulate translation by recycling and inactivating ribosomes, such as ribosome recycling factor (RRF) and hibernation promoting factor (HPF), may actually increase. This adaptive response allows the microbiota to manage the challenges posed by elevated acidity during the later stages of acetification. Indeed, stress-related proteins, including heat shock proteins such as 60 GroES and 10 GroEL, along with molecular chaperones like DnaJ, DnaK, GrpE, and ClpB, have been recognized for their protective roles in preventing protein denaturation and facilitating refolding under stress conditions.[87] Studies conducted with *A. pasteurianus* have shed light on the up-regulation of these stress-related proteins when acidity levels increase. This suggests a potential regulatory mechanism for the formation and folding of proteins as a molecular strategy

to counteract the stress induced by elevated acetic acid levels. As illustrated in Figure 2, this molecular response appears to play a crucial role in helping AAB adapt and survive under challenging conditions in the later stages of acetification.[9,18,55]

During submerged vinegar fermentation, especially in semi-continuous systems widely used in the industry, the microbiota undergoes frequent and abrupt changes in the volume of the medium, substrate concentration, and product concentration. These fluctuations trigger constant biotransformations. Oxidation reactions, which occur under continuous aeration conditions, can lead to the generation of various toxic compounds and reactive oxygen species (ROS) within the cytoplasm of acetic acid bacteria (AAB) cells.[88] To cope with these oxidative stress conditions, AAB employ several proteins and enzymatic systems; these include catalase (KatE), superoxide dismutase (SodB), ferredoxin (FdxA), glutaredoxin (GrxC), bacterioferritin (Bfr), and numerous other oxidoreductases and cofactors such as $NADH/NAD^+$ and $NADPH/NADP^+$. Research has shown that these proteins are up-regulated under acetic acid stress in different AAB species, as depicted in Figure 2.[18,89] Maintaining redox homeostasis through these mechanisms is a valuable strategy and a critical metabolic pathway for controlling submerged vinegar fermentation effectively.

4.1.1 | The tricarboxylic acid cycle (TCA)

The tricarboxylic acid (TCA) cycle was among the earliest metabolic pathways in which enzymes were found to be associated with the assimilation of acetic acid within acetic acid bacteria (AAB).[90] In this process, cytoplasmic acetic acid can be completely oxidized to carbon dioxide (CO_2) and water (H_2O), yielding adenosine triphosphate (ATP) and simultaneously detoxifying the cell through a well-known overoxidation reaction.[91,92] The enzyme acetyl-CoA synthase (Acs) plays a critical role in this pathway. Acs catalyzes the conversion of acetate into acetyl-CoA, facilitating its entry into the TCA cycle. This metabolic adaptation is particularly relevant when the ethanol source in the medium becomes exhausted, promoting secondary growth through the utilization of acetic acid as an energy and carbon source.[93] *Acetobacter, Gluconacetobacter, and Komagataeibacter* species are capable of utilizing the tricarboxylic acid (TCA) cycle, which is a part of their metabolic repertoire. In contrast, *Gluconobacter* species lack some of the enzymes required for a functional TCA cycle, making it non-functional in these organisms.[26,94] Studies involving proteomic and genomic analyses have identified three specific genes (*aarA*, *aarB*, and *aarC*) that are influenced by acetic acid stress, and their deletion leads to a loss of acid resistance in *A. aceti* 1023.[53] Among these genes, *aarA* and *aarC* encode citrate synthase (AarA) and succinyl-CoA transferase (AarC), respectively. These genes represent some of the initial determinants associated with acetic acid resistance. Interestingly, AarC is produced by *Acetobacter* and *Komagataeibacter* species, replacing succinyl-CoA synthetase (SucCD) in *Acetobacter* spp., which belongs to the *A. pasteurianus* group. These genetic and metabolic adaptations help AAB species withstand the challenges posed by acetic acid stress.[95] Aconitate hydratase (AcnA), another enzyme involved in the tricarboxylic acid (TCA) cycle, has been shown to be up-regulated when *A. aceti* is grown in a medium containing 1% ethanol (EtOH). Overexpression of acnA has been demonstrated to increase the acetic acid resistance of the strain.[54] Furthermore, proteomic and metaproteomic analyses of *A. pasteurianus* in a 4% (w/v) submerged vinegar production medium and *Komagataeibacter* species in a medium with over 10% (w/v) ethanol revealed the up-regulation of various enzymes associated with the TCA cycle under high-acid conditions. These enzymes include citrate synthase (AarA), aconitate hydratase (AcnA), isocitrate dehydrogenase NAD^+ (Icd), succinate dehydrogenase (SdhA), fumarate hydratase (FumA/C), succinyl-CoA transferase (AarC), and several others. These up-regulated enzymes are part of the microbial response to cope with the challenges posed by high-acid conditions during vinegar production.[15,55] Indeed, the tricarboxylic acid (TCA) cycle plays a crucial role as a metabolic strategy for acetic acid bacteria (AAB) during submerged acetification, where they must adapt to continuous changes in ethanol concentration, acetic acid concentration, and cell concentrations. The TCA cycle is instrumental in assimilating cytoplasmic acetic acid derived from ethanol, providing both energy and essential biosynthetic precursors for the microbial cells, as depicted in Figure 2. By utilizing the TCA cycle, AAB can efficiently convert acetic acid into metabolic intermediates that can be used for various cellular processes. This metabolic adaptation contributes to the inherent resistance of vinegar microbiota, allowing them to not only survive but thrive in the challenging and aggressive conditions of their natural environment within bioreactors during vinegar production. The TCA cycle is a key component of their metabolic toolkit for maintaining growth and productivity in these conditions.[9,14,15,81]

4.2 | Acetic acid resistance mechanisms on the cell membrane

The concentration of acetic acid in the medium can induce modifications in the cell membrane morphology of vinegar microbiota. *Acetobacter* and *Komagataeibacter* are two common acetic acid-producing species in the vinegar industry, with the latter exhibiting higher acid resistance compared to the former.[81] In the case of *Acetobacter* spp., they can be classified based on their cell surface morphology into two types: R (rough cell surface) and S (smooth cell surface). The R strains are associated with the formation of a pellicle polysaccharide layer.[81] Notably, *A. pasteurianus* R strains have demonstrated a higher capability for the production and tolerance of acetic acid compared to S strains. The intracellular acetic acid content in S strains is significantly higher, being 3 or 4 times that of R strains. This suggests that S strains exhibit a higher diffusion of acetate molecules into the cell due to the absence of a pellicle polysaccharide layer, which functions as a barrier.[96] In *Komagataeibacter* spp., there is a reported absence of capsular polysaccharides (CPS) layers during industrial vinegar production, which is in contrast to *Acetobacter* spp. The absence of the CPS layer in *Komagataeibacter* allows ethanol and acetic acid to freely dif-

fuse through the outer membrane during acetification. This absence of a CPS layer potentially enhances the exchange of metabolites between the cellular interior and the medium, thereby promoting industrial vinegar production.[15] While CPS may not directly contribute to the acetic acid resistance of *Komagataeibacter*, they likely play a role in improving yield during vinegar production, as illustrated in Figure 2. These structural and functional adaptations in response to acetic acid concentrations highlight the remarkable versatility and adaptability of vinegar microbiota in optimizing their performance under varying environmental conditions.

The composition of the cell membrane in *Komagataeibacter* strains, particularly *K. europaeus*, exhibits a higher content of phosphatidylcholine (PC) compared to *Acetobacter* strains, making PC the predominant phospholipid in the cell membrane. This phenomenon is particularly pronounced in the presence of acetic acid in the medium, as depicted in Figure 2. Additionally, the content of non-polar glycolipids may increase under these conditions, potentially strengthening the hydrophobic layer of the cell membrane.[79] Sphingolipids content can also increase, particularly through the synthesis of dihydroceramide. This increase in sphingolipids has been directly linked to acetic acid tolerance and the stability of the PQQ-ADH enzyme in *Acetobacter malorum* during vinegar elaboration.[97] Certain *Komagataeibacter* species may exhibit higher levels of hopanoids, specifically tetrahydroxybacteriohopane (THBH), which contributes to the stabilization of the cellular membrane, particularly at high ethanol concentrations. This stabilization has also been linked to acetic acid resistance in acetic acid bacteria (AAB).[91] Modifications in the composition of fatty acids in the membrane have also been observed, often involving a reduction in the total lipid content due to the attenuation of the fatty acid biosynthesis pathway. For instance, the downregulation of two effector proteins, FabD and FabG, which are involved in the biosynthesis and elongation of fatty acids, occurs under acetic acid stress in *A. pasteurianus*.[18] However, there can be variations in the strategies used among different AAB species to adapt their membrane composition to the conditions imposed by the medium. For example, in a *K. hansenii* strain under acetic acid stress, two genes (*des* and *cfa*) involved in increasing the proportion and chain length of unsaturated fatty acids were activated. These variations reflect the diversity of strategies employed by AAB to adjust their membrane composition to cope with changing environmental conditions, as illustrated in Figure 2.[16]

Proteins and enzymatic complexes situated in the cell membrane play a significant role in the molecular strategy of the vinegar-producing microbiota. While the incomplete oxidation reaction conducted by the membrane-bound alcohol dehydrogenase with pyrroloquinoline quinone (ADH-PQQ) and aldehyde dehydrogenase (ALDH) to generate acetic acid from ethanol is well-established, it's important to understand the activity and function of these enzymes in the process. ADH-PQQ enzymes are responsible for oxidizing ethanol into acetaldehyde. Throughout acetification, these enzymes are typically found in high quantities. However, their behavior can become less stable due to factors such as a shift in the carbon source from glucose to ethanol or the growth phase in some acetic acid bacteria (AAB) strains.[55,98] Despite this variability, ADH-PQQ remains highly relevant in this transformation, with its activity usually being more pronounced than that of membrane-bound ALDH. ALDH is responsible for the subsequent conversion of acetaldehyde to acetic acid. Overall, these enzymatic reactions within the cell membrane are crucial for the conversion of ethanol to acetic acid, which is the primary metabolic process underlying vinegar production. Understanding the dynamics and regulation of these enzymes is essential for optimizing vinegar fermentation processes.[47] The cytoplasmic enzymes alcohol dehydrogenase with NAD (ADH-NAD) and aldehyde dehydrogenase with NADP (ALDH-NADP) play a distinct role in the conversion of cytoplasmic ethanol. During acetification, their activities on the cell membrane are entirely inhibited.[8,81] The release of acetic acid from the cytoplasm to the periplasm is facilitated by systems of efflux pumps that are proton motive force-dependent and ATP-binding cassette (ABC) transporters. A putative ABC transporter in *A. aceti*, named AatA, has been associated with acid resistance and acts as an efflux pump for the release of acetic acid. This efflux mechanism helps in maintaining a lower concentration of acetic acid within the bacterial cell, contributing to the ability of the bacteria to survive and thrive in acetic acid-rich environments during vinegar production.[54,99] Comparative genomic analysis has shown that species of *Komagataeibacter* contain a greater number of genes encoding putative ABC transporter proteins compared to *Acetobacter* species.[100] This observation suggests a correlation between the presence of ABC transporters and acetic acid resistance.[81] ABC transporters are likely directly involved in helping the cells cope with the challenges posed by acetic acid-rich environments. The outer membrane of AAB also plays a role in cell membrane function. Outer membrane proteins (OMPs) act as permeable porins for small solutes and help maintain the structural stability of the outer membrane.[101] Some OMPs, such as OmpA, OmpH, OmpW, and OsmC, have been implicated in balancing nutrient uptake and resistance to the toxicity of molecular stressors, particularly in response to high acidity in *A. pasteurianus* and *K. europaeus*. However, there are notable differences in the behavior of these proteins and their corresponding coding genes between different AAB genera during acetification. These variations suggest diverse strategies that contribute to acetic acid resistance and highlight the complexity of the molecular mechanisms involved.[9,18,47,55] These molecular strategies at the cell membrane level are detailed in Figure 2.

5 | *KOMAGATAEIBACTER EUROPAEUS*: A CRUCIAL SPECIES FOR INDUSTRIAL VINEGAR PRODUCTION

Komagataeibacter europaeus, firstly observed by Sievers, Sellmer, & Teuber in 1992[31] (formerly named *Acetobacter europaeus*), was subsequently isolated and characterized from high-acid vinegar fermentations in central Europe. This species has garnered attention as one of the most suitable AAB for industrial vinegar production due to its unique growth characteristics. These characteristics include a high capability to oxidize ethanol and produce acetic acid, which is beneficial for both its requirement and tolerance. *K. europaeus* is capable of thriving in environments with a pH as low as 2.5 and requires a contin-

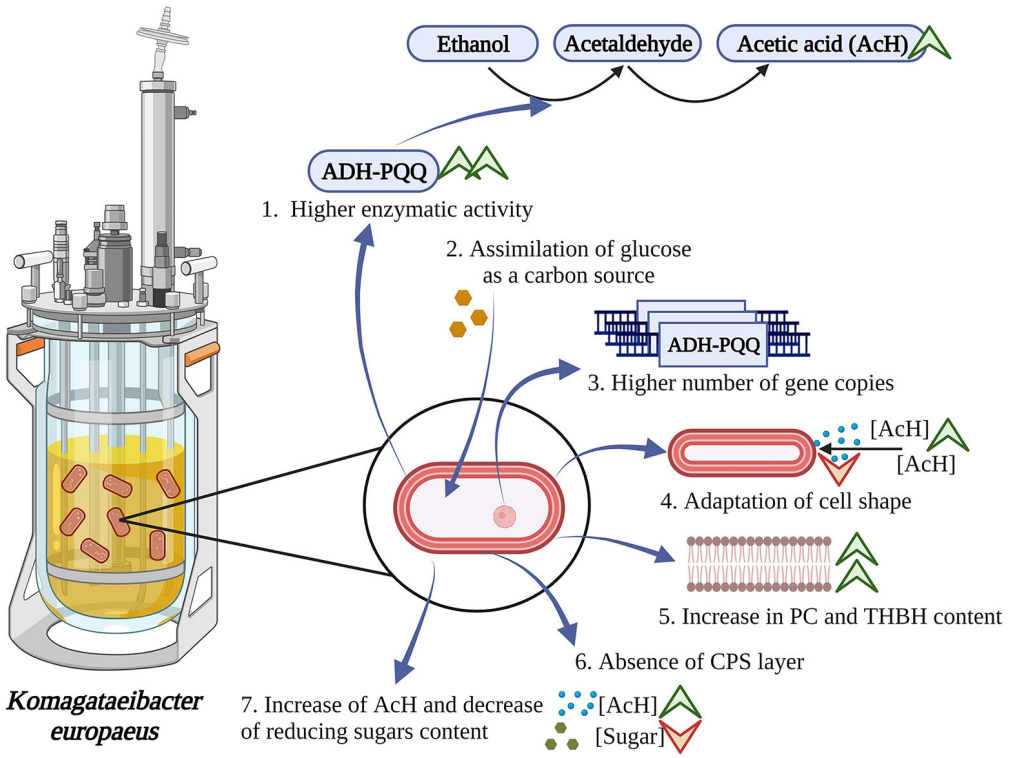

FIGURE 3 Metabolic strategies used by *Komagataeibacter europaeus* strains to impose themselves and survive on the rest of the vinegar microbiota throughout the submerged acetification process. AcH, acetic acid; CPS, capsular polysaccharides; PC, phosphatidylcholine; ADH-PQQ, alcohol dehydrogenase pyrroloquinoline quinone dependent; THBH, tetrahydroxybacteriohopane. Created with BioRender.com.

uous supply of oxygen for its growth.[8,31,76] These metabolic features make *K. europaeus* well-suited for growth in various types of vinegars produced via submerged culture, including wine vinegar (with ethanol concentrations ranging from 4.5% to 10% w/v), spirit vinegar (with ethanol concentrations ranging from 10% to 20% w/v), and even low-acidity vinegars such as traditional balsamic vinegar and cereal vinegar (with ethanol concentrations ranging from 4% to 7% w/v). Its adaptability to a wide range of vinegar types makes it a valuable asset in vinegar production processes.[25,26,102]

K. europaeus is often dominant in industrial vinegar production, and ongoing research is focused on understanding the various metabolic strategies employed by different strains of this species to achieve dominance. Recent advances and innovative strategies used by *K. europaeus* are outlined in Figure 3. *K. europaeus*, like other *Komagataeibacter* species, utilizes coenzyme Q10 in its respiratory chain. The key enzyme responsible for converting ethanol into acetic acid in this species is ADH-PQQ (alcohol dehydrogenase with pyrroloquinoline quinone). Notably, the enzymatic activity of ADH-PQQ in *K. europaeus* cells under high acidity conditions is approximately twice that of *A. pasteurianus*. This higher ADH activity provides a larger energy pool for membrane-associated processes, including acetate/acetic acid export systems.[8] Comparative analysis of published AAB genomes has revealed differences in the number of gene copies of ADH-PQQ. The *Komagataeibacter* genus, including *K. europaeus*, contains the highest number of encoding gene copies. For example, *K. europaeus* 5P3 possesses six copies of the gene, while it is absent in strains like *K. hansenii* ATCC 23769 and *K. medellinensis* NBRC 3288. These variations in the number of ADH-PQQ genes may play a crucial role in allowing *K. europaeus* to dominate high-acid acetification processes.[81] Furthermore, *K. europaeus* has the potential to assimilate other carbon sources besides ethanol. Recent studies have proposed a molecular strategy in which this species, when predominant in a microbiota involved in submerged vinegar production using raw materials with high sugar content, may utilize glucose from the medium before ethanol. This strategy could provide *K. europaeus* with a competitive advantage over other species that preferentially consume glucose (see Figure 3).[9]

The modification of cellular structure and membrane composition plays a critical role in the molecular strategies employed by *K. europaeus*, as illustrated in Figure 3. *K. europaeus* exhibits adaptability in terms of its cellular morphology. In the absence of acetic acid, it typically starts as short rod-shaped cells but can undergo morphological changes to become longer and thinner rods as the acidity level in the environment increases. This change in morphology reduces the effective area available for passive diffusion and storage of acetic acid within the cells. As a result, *K. europaeus* can tolerate higher levels of acetic acid activity.[79,81] In terms of membrane composition, *K. europaeus* has been associated with an increase in lipid content in the cell membrane, including phosphatidylcholine (PC) and tetrahydroxybacteriohopane (THBH). Additionally, it lacks capsular polysaccharides (CPS), which are present in some other acetic acid bacteria. These differences in membrane composition serve as distinctive strategies that

contribute to *K. europaeus*'s ability to dominate submerged fermentation processes.[79,91] Recently, an O-antigen polysaccharide fraction known as Ke-PS was isolated from *K. europaeus* NBRC 3261, and it may be involved in acetic acid resistance mechanisms.[103] Furthermore, when examining the population dynamics during submerged vinegar production using mixed starting cultures of acetic acid bacteria (AAB), metagenomic tools have revealed that although *A. pasteurianus* is initially predominant, particularly at high ethanol concentrations (ranging from 5% to 7% v/v), *K. europaeus* consistently prevails as fermentation progresses.[81] This dominance shift highlights the competitive advantage of *K. europaeus* in acetic acid-rich environments.[25]

In the solid-state fermentation of Chinese cereal vinegar, it has been observed that non-abundant microbiota communities can have crucial roles in network stability. A study conducted by Peng et al. 2021[69] identified *K. europaeus* as the most co-occurring non-abundant species with a significant role in the function and resilience of the microbial community. When K. europaeus JNP1 was bioaugmented, it was found to modulate the composition of the microbiota and enhance bioprocess efficiency. This was achieved by increasing acetic acid content and reducing the level of reducing sugars, as depicted in Figure 3. *K. europaeus* exhibited the capability to confer stability to both predominant and minor abundant members of the microbiota, ultimately enhancing the final vinegar properties.

In summary, gaining a better understanding of the molecular mechanisms employed by *K. europaeus* to adapt to its environment and dominate the microbiota is essential for improving fermentation conditions. The characterization of suitable strains of *K. europaeus* holds the potential to enhance starter cultures, resulting in final products with improved organoleptic properties and higher quality.

6 | CONCLUSIONS AND FUTURE TRENDS

In this review, a comprehensive compilation of the current omic and meta-omic technologies and their multiple applications for the identification of vinegar microbiota members, as well as key metabolic strategies used, has been conducted. Indeed, with the ongoing advancements in research and the changing landscape of the agro-alimentary market, gaining this knowledge is undeniably vital for enhancing the acetification process and ensuring the quality of the final products. As we continue to uncover more about the complex microbial communities involved, it opens up opportunities for optimizing production methods, enhancing product consistency, and even developing innovative vinegar and bio-product variations that cater to evolving consumer preferences. This intersection of science and industry is instrumental in meeting the demands of a dynamic market while maintaining the tradition of vinegar production. In this sense, omic technologies are becoming more attractive interest of researchers because they are allowed to approach these issues with high throughput and without compromising the integrity of the microbiota and properties of the fermented product. In the case of vinegar, they are allowing to solve many of the traditional hurdles for isolation and characterization of microorganisms inhabiting these aggressive media. However, there are still several limitations of the current omic analysis, such as the limitation to detect low-abundant species, high level of technical and biological noise, and few biological replicates normally due to the high cost. Moreover, powerful bioinformatic methods for the integration, visualization, and validation of meta-omic data need to be developed. Therefore, the progress in sequencing and mass spectrometry technologies may allow the development and improvement of the current omic sciences including (meta)genomics, (meta)transcriptomics, (meta)proteomics and metabolomics. Studying vinegar microbes with advanced tools can help create new, high-quality vinegars with better taste and health benefits. It can also improve starter cultures for vinegar production by selecting key species or strains; progress in obtaining isolates from vinegar, their phenotypic characterization, and biotechnological enhancement; and finding of marker genes, proteins, and metabolites throughout the process. Ultimately, this review aims to provide useful guidance for the industry of vinegar and other related bioproducts targeted for the improvement of the elaboration procedures and operating conditions in the coming years.

AUTHOR CONTRIBUTIONS

J. J. Román-Camacho: Methodology; validation; investigation; data curation; writing—original draft; visualization. **I. García-García**: Conceptualization; methodology; writing-review and funding acquisition. **I. M. Santos-Dueñas**: Data curation; original draft preparation and editing. **T. García-Martínez**: Data curation; original draft preparation and editing. **J. C. Mauricio**: Conceptualization; methodology; writing-review and funding acquisition. **Teresa García-Martínez**: Data curation (equal); methodology (equal); supervision (equal); writing—original draft (equal); writing—review and editing (equal). **Isidoro García-García**: Conceptualization (equal); funding acquisition (equal); methodology (equal); writing—review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review. All the authors declare there are no competing interests to declare.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study can be made available by the corresponding author upon request. No data was used for the research described in the article.

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REFERENCES

1. Ali, Z., Wang, Z., Amir, R. M., Younas, S., Wali, A., Adowa, N., & Ayim, I. (2016). Potential uses of vinegar as a medicine and related in vivo mechanisms. *International Journal for Vitamin and Nutrition Research*, *86*(3–4), 140–151. https://doi.org/10.1024/0300-9831/a000440
2. Budak, N. H., Aykin, E., Seydim, A. C., Greene, A. K., & Güzel-Seydim, Z. B. (2014). Functional properties of vinegar. *Journal of Food Science*, *79*(5), R757–R764. https://doi.org/10.1111/1750-3841.12434
3. Chou, C. H., Liu, C. W., Yang, D. J., Wu, Y. H. S., & Chen, Y. C. (2015). Amino acid, mineral, and polyphenolic profiles of black vinegar, and its lipid lowering and antioxidant effects in vivo. *Food Chemistry*, *168*, 63–69. https://doi.org/10.1016/j.foodchem.2014.07.035
4. Mas, A., Torija, M. J., García-Parrilla, M. D. C., & Troncoso, A. M. (2014). Acetic acid bacteria and the production and quality of wine vinegar. *The Scientific World Journal*, *2014*, 1–6. https://doi.org/10.1155/2014/394671
5. Román-Camacho, J. J., Santos-Dueñas, I. M., García-García, I., Moreno-García, J., García-Martínez, T., & Mauricio, J. C. (2020). Metaproteomics of microbiota involved in submerged culture production of alcohol wine vinegar: A first approach. *International Journal of Food Microbiology*, *333*, 108797. https://doi.org/10.1016/j.ijfoodmicro.2020.108797
6. Shi, H., Zhou, X., Yao, Y., Qu, A., Ding, K., Zhao, G., & Liu, S. Q. (2022). Insights into the microbiota and driving forces to control the quality of vinegar. *LWT*, *157*, 113085. https://doi.org/10.1016/j.lwt.2022.113085
7. García-García, I., Santos-Dueñas, I. M., Jiménez-Ot, C., Jiménez-Hornero, J. E., & Bonilla-Venceslada, J. L. (2009). Vinegar Engineering. In L. Solieri, & P. Giudici (Eds.), *Vinegars of the World* (pp. 97–120). Springer Milan. https://doi.org/10.1007/978-88-470-0866-3
8. Gullo, M., Verzelloni, E., & Canonico, M. (2014). Aerobic submerged fermentation by acetic acid bacteria for vinegar production: Process and biotechnological aspects. *Process Biochemistry*, *49*(10), 1571–1579. https://doi.org/10.1016/j.procbio.2014.07.003
9. Román-Camacho, J. J., García-García, I., Santos-Dueñas, I. M., Ehrenreich, A., Liebl, W., García-Martínez, T., & Mauricio, J. C. (2022). Combining omics tools for the characterization of the microbiota of diverse vinegars obtained by submerged culture: 16S rRNA amplicon sequencing and MALDI-TOF MS. *Frontiers in Microbiology*, *13*, 1055010. https://doi.org/10.3389/fmicb.2022.1055010
10. Román-Camacho, J. J., Mauricio, J. C., Santos-Dueñas, I. M., García-Martínez, T., & García-García, I. (2022). Unraveling the role of acetic acid bacteria comparing two acetification profiles from natural raw materials: A quantitative approach in *Komagataeibacter europaeus*. *Frontiers in Microbiology*, *13*, 840119. https://doi.org/10.3389/fmicb.2022.840119
11. Trček, J., Mahnič, A., & Rupnik, M. (2016). Diversity of the microbiota involved in wine and organic apple cider submerged vinegar production as revealed by DHPLC analysis and next-generation sequencing. *International Journal of Food Microbiology*, *223*, 57–62. https://doi.org/10.1016/j.ijfoodmicro.2016.02.007
12. Wu, L.-H., Lu, Z.-M., Zhang, X.-J., Wang, Z.-M., Yu, Y.-J., Shi, J.-S., & Xu, Z.-H. (2017). Metagenomics reveals flavour metabolic network of cereal vinegar microbiota. *Food Microbiology*, *62*, 23–31. https://doi.org/10.1016/j.fm.2016.09.010
13. Zhu, Y., Zhang, F., Zhang, C., Yang, L., Fan, G., Xu, Y., Sun, B., & Li, X. (2018). Dynamic microbial succession of Shanxi aged vinegar and its correlation with flavor metabolites during different stages of acetic acid fermentation. *Scientific Reports*, *8*(1), 8612. https://doi.org/10.1038/s41598-018-26787-6
14. Adler, P., Frey, L. J., Berger, A., Bolten, C. J., Hansen, C. E., & Wittmann, C. (2014). The key to acetate: Metabolic fluxes of acetic acid bacteria under cocoa pulp Fermentation-simulating conditions. *Applied and Environmental Microbiology*, *80*(15), 4702–4716. https://doi.org/10.1128/AEM.01048-14
15. Andrés-Barrao, C., Saad, M. M., Cabello Ferrete, E., Bravo, D., Chappuis, M.-L., Ortega Pérez, R., Junier, P., Perret, X., & Barja, F. (2016). Metaproteomics and ultrastructure characterization of *Komagataeibacter* spp. Involved in high-acid spirit vinegar production. *Food Microbiology*, *55*, 112–122. https://doi.org/10.1016/j.fm.2015.10.012
16. Li, Y., Yan, P., Lei, Q., Li, B., Sun, Y., Li, S., Lei, H., & Xie, N. (2019). Metabolic adaptability shifts of cell membrane fatty acids of *Komagataeibacter hansenii* HDM1-3 improve acid stress resistance and survival in acidic environments. *Journal of Industrial Microbiology and Biotechnology*, *46*(11), 1491–1503. https://doi.org/10.1007/s10295-019-02225-y
17. Sankuan, X., Cuimei, Z., Bingqian, F., Yu, Z., Menglei, X., Linna, T., Jia, S., Xinyi, Z., & Min, W. (2021). Metabolic network of ammonium in cereal vinegar solid-state fermentation and its response to acid stress. *Food Microbiology*, *95*, 103684. https://doi.org/10.1016/j.fm.2020.103684
18. Xia, K., Zang, N., Zhang, J., Zhang, H., Li, Y., Liu, Y., Feng, W., & Liang, X. (2016). New insights into the mechanisms of acetic acid resistance in Acetobacter pasteurianus using iTRAQ-dependent quantitative proteomic analysis. *International Journal of Food Microbiology*, *238*, 241–251. https://doi.org/10.1016/j.ijfoodmicro.2016.09.016
19. De Roos, J., & De Vuyst, L. (2018). Acetic acid bacteria in fermented foods and beverages. *Current Opinion in Biotechnology*, *49*, 115–119. https://doi.org/10.1016/j.copbio.2017.08.007
20. Rizo, J., Guillén, D., Farrés, A., Díaz-Ruiz, G., Sánchez, S., Wacher, C., & Rodríguez-Sanoja, R. (2018). Omics in traditional vegetable fermented foods and beverages. *Critical Reviews in Food Science and Nutrition*, *60*(5), 791–809. https://doi.org/10.1080/10408398.2018.1551189
21. Pinu, F., De Carvalho-Silva, S., Trovatti Uetanabaro, A., & Villas-Boas, S. (2016). Vinegar metabolomics: An explorative study of commercial balsamic vinegars using gas chromatography-mass spectrometry. *Metabolites*, *6*(3), 22. https://doi.org/10.3390/metabo6030022
22. Yang, H., He, Y., Liao, J., Li, X., Zhang, J., Liebl, W., & Chen, F. (2022). RNA-Seq transcriptomic analysis reveals gene expression profiles of acetic acid bacteria under high-acidity submerged industrial fermentation process. *Frontiers in Microbiology*, *13*, 956729. https://doi.org/10.3389/fmicb.2022.956729
23. Kuo, E. Y., Yang, R., Chin, Y. Y., Chien, Y., Chen, Y. C., Wei, C., Kao, L., Chang, Y., Li, Y., Chen, T., & Lee, T. (2022). Multiomics approaches and genetic engineering of metabolism for improved biorefinery and wastewater treatment in microalgae. *Biotechnology Journal*, *17*(8), 2100603. https://doi.org/10.1002/biot.202100603
24. Chen, G., Chen, C., & Lei, Z. (2017). Meta-omics insights in the microbial community profiling and functional characterization of fermented foods. *Trends in Food Science & Technology*, *65*, 23–31. https://doi.org/10.1016/j.tifs.2017.05.002
25. Fernández-Pérez, R., Torres, C., Sanz, S., & Ruiz-Larrea, F. (2010). Strain typing of acetic acid bacteria responsible for vinegar production by the submerged elaboration method. *Food Microbiology*, *27*(8), 973–978. https://doi.org/10.1016/j.fm.2010.05.020
26. Mamlouk, D., & Gullo, M. (2013). Acetic acid bacteria: physiology and carbon sources oxidation. *Indian Journal of Microbiology*, *53*(4), 377–384. https://doi.org/10.1007/s12088-013-0414-z

27. García-García, I., Jiménez-Hornero, J. E., Santos-Dueñas, I. M., González-Granados, Z., & Cañete-Rodríguez, A. M. (2019). Modeling and optimization of acetic acid fermentation. In A. Bekatorou (Ed.), *Advances in vinegar production* (1st ed., pp. 299–325). CRC Press. https://doi.org/10.1201/9781351208475-15
28. Cleenwerck, I., & De Vos, P. (2008). Polyphasic taxonomy of acetic acid bacteria: An overview of the currently applied methodology. *International Journal of Food Microbiology*, *125*(1), 2–14. https://doi.org/10.1016/j.ijfoodmicro.2007.04.017
29. Boesch, C., Trček, J., Sievers, M., & Teuber, M. (1998). Acetobacter intermedius, sp. Nov. *Systematic and Applied Microbiology*, *21*(2), 220–229. https://doi.org/10.1016/S0723-2020(98)80026-X
30. Ruiz, A., Poblet, M., Mas, A., & Guillamón, J. M. (2000). Identification of acetic acid bacteria by RFLP of PCR-amplified 16S rDNA and 16S-23S rDNA intergenic spacer. *International Journal of Systematic and Evolutionary Microbiology*, *50*(6), 1981–1987. https://doi.org/10.1099/00207713-50-6-1981
31. Sievers, M., Sellmer, S., & Teuber, M. (1992). Acetobacter europaeus sp. Nov., a main component of industrial vinegar fermenters in Central Europe. *Systematic and Applied Microbiology*, *15*(3), 386–392. https://doi.org/10.1016/S0723-2020(11)80212-2
32. González, Á., Guillamón, J. M., Mas, A., & Poblet, M. (2006). Application of molecular methods for routine identification of acetic acid bacteria. *International Journal of Food Microbiology*, *108*(1), 141–146. https://doi.org/10.1016/j.ijfoodmicro.2005.10.025
33. Dutta, D., & Gachhui, R. (2006). Novel nitrogen-fixing Acetobacter nitrogenifigens sp. Nov., isolated from Kombucha tea. *International Journal of Systematic and Evolutionary Microbiology*, *56*(8), 1899–1903. https://doi.org/10.1099/ijs.0.64101-0
34. Trček, J. (2005). Quick identification of acetic acid bacteria based on nucleotide sequences of the 16S–23S rDNA internal transcribed spacer region and of the PQQ-dependent alcohol dehydrogenase gene. *Systematic and Applied Microbiology*, *28*(8), 735–745. https://doi.org/10.1016/j.syapm.2005.05.001
35. De Vuyst, L., Camu, N., De Winter, T., Vandemeulebroecke, K., Van De Perre, V., Vancanneyt, M., De Vos, P., & Cleenwerck, I. (2008). Validation of the (GTG)5-rep-PCR fingerprinting technique for rapid classification and identification of acetic acid bacteria, with a focus on isolates from Ghanaian fermented cocoa beans. *International Journal of Food Microbiology*, *125*(1), 79–90. https://doi.org/10.1016/j.ijfoodmicro.2007.02.030
36. González, Á., Hierro, N., Poblet, M., Mas, A., & Guillamón, J. M. (2005). Application of molecular methods to demonstrate species and strain evolution of acetic acid bacteria population during wine production. *International Journal of Food Microbiology*, *102*(3), 295–304. https://doi.org/10.1016/j.ijfoodmicro.2004.11.020
37. González, Á., Hierro, N., Poblet, M., Mas, A., & Guillamón, J. M. (2006). Enumeration and detection of acetic acid bacteria by real-time PCR and nested PCR. *FEMS Microbiology Letters*, *267*(1), 129–129. https://doi.org/10.1111/j.1574-6968.2006.00586.x
38. Vegas, C., Mateo, E., González, Á., Jara, C., Guillamón, J. M., Poblet, M., Torija, M. J., & Mas, A. (2010). Population dynamics of acetic acid bacteria during traditional wine vinegar production. *International Journal of Food Microbiology*, *138*(1–2), 130–136. https://doi.org/10.1016/j.ijfoodmicro.2010.01.006
39. De Vero, L., & Giudici, P. (2008). Genus-specific profile of acetic acid bacteria by 16S rDNA PCR-DGGE. *International Journal of Food Microbiology*, *125*(1), 96–101. https://doi.org/10.1016/j.ijfoodmicro.2007.02.029
40. Escobar-Zepeda, A., Vera-Ponce De León, A., & Sanchez-Flores, A. (2015). The road to metagenomics: From microbiology to DNA sequencing technologies and bioinformatics. *Frontiers in Genetics*, *6*, https://doi.org/10.3389/fgene.2015.00348
41. Peng, Q., Yang, Y., Guo, Y., & Han, Y. (2015). Analysis of bacterial diversity during acetic acid fermentation of Tianjin Duliu aged vinegar by 454 pyrosequencing. *Current Microbiology*, *71*(2), 195–203. https://doi.org/10.1007/s00284-015-0823-9
42. Valera, M. J., Torija, M. J., Mas, A., & Mateo, E. (2015). Acetic acid bacteria from biofilm of strawberry vinegar visualized by microscopy and detected by complementing culture-dependent and culture-independent techniques. *Food Microbiology*, *46*, 452–462. https://doi.org/10.1016/j.fm.2014.09.006
43. Verce, M., De Vuyst, L., & Weckx, S. (2019). Shotgun metagenomics of a water kefir fermentation ecosystem reveals a novel Oenococcus species. *Frontiers in Microbiology*, *10*, 479. https://doi.org/10.3389/fmicb.2019.00479
44. Sakurai, K., Arai, H., Ishii, M., & Igarashi, Y. (2011). Transcriptome response to different carbon sources in Acetobacter aceti. *Microbiology*, *157*(3), 899–910. https://doi.org/10.1099/mic.0.045906-0
45. Okamoto-Kainuma, A., & Ishikawa, M. (2016). Physiology of Acetobacter spp.: Involvement of molecular chaperones during acetic acid fermentation. In K. Matsushita, H. Toyama, N. Tonouchi, & A. Okamoto-Kainuma (Eds.), *Acetic Acid Bacteria* (pp. 179–199). Springer Japan. https://doi.org/10.1007/978-4-431-55933-7_8
46. Yang, H., Yu, Y., Fu, C., & Chen, F. (2019). Bacterial acid resistance toward organic weak acid revealed by RNA-Seq transcriptomic analysis in Acetobacter pasteurianus. *Frontiers in Microbiology*, *10*, 1616. https://doi.org/10.3389/fmicb.2019.01616
47. Wang, L., Hong, H., Zhang, C., Huang, Z., & Guo, H. (2021). Transcriptome analysis of *Komagataeibacter europaeus* CGMCC 20445 responses to different acidity levels during acetic acid fermentation. *Polish Journal of Microbiology*, *70*(3), 305–313. https://doi.org/10.33073/pjm-2021-027
48. Wu, Y., Xia, M., Zhao, N., Tu, L., Xue, D., Zhang, X., Zhao, C., Cheng, Y., Zheng, Y., & Wang, M. (2021). Metabolic profile of main organic acids and its regulatory mechanism in solid-state fermentation of Chinese cereal vinegar. *Food Research International*, *145*, 110400. https://doi.org/10.1016/j.foodres.2021.110400
49. Huang, T., Lu, Z.-M., Peng, M.-Y., Chai, L.-J., Zhang, X.-J., Shi, J.-S., Li, Q., & Xu, Z.-H. (2022). Constructing a defined starter for multispecies vinegar fermentation via evaluation of the vitality and dominance of functional microbes in an autochthonous starter. *Applied and Environmental Microbiology*, *88*(3), e02175-21. https://doi.org/10.1128/aem.02175-21
50. Elviri, L., & Mattarozzi, M. (2012). Food proteomics. In *Chemical analysis of food: Techniques and applications* (pp. 519–537). Elsevier. https://doi.org/10.1016/B978-0-12-384862-8.00016-9
51. Heyer, R., Schallert, K., Zoun, R., Becher, B., Saake, G., & Benndorf, D. (2017). Challenges and perspectives of metaproteomic data analysis. *Journal of Biotechnology*, *261*, 24–36. https://doi.org/10.1016/j.jbiotec.2017.06.1201
52. Lasko, D. R., Schwerdel, C., Bailey, J. E., & Sauer, U. (1997). Acetate-specific stress response in acetate-resistant bacteria: An analysis of protein patterns. *Biotechnology Progress*, *13*(5), 519–523. https://doi.org/10.1021/bp970075f
53. Nakano, S., Fukaya, M., & Horinouchi, S. (2004). Enhanced expression of aconitase raises acetic acid resistance in *Acetobacter aceti*. *FEMS Microbiology Letters*, *235*(2), 315–322. https://doi.org/10.1111/j.1574-6968.2004.tb09605.x
54. Nakano, S., Fukaya, M., & Horinouchi, S. (2006). Putative ABC transporter responsible for acetic acid resistance in *Acetobacter aceti*. *Applied and Environmental Microbiology*, *72*(1), 497–505. https://doi.org/10.1128/AEM.72.1.497-505.2006
55. Andrés-Barrao, C., Saad, M. M., Chappuis, M.-L., Boffa, M., Perret, X., Ortega Pérez, R., & Barja, F. (2012). Proteome analysis of Acetobacter pasteurianus during acetic acid fermentation. *Journal of Proteomics*, *75*(6), 1701–1717. https://doi.org/10.1016/j.jprot.2011.11.027
56. Trček, J., & Barja, F. (2015). Updates on quick identification of acetic acid bacteria with a focus on the 16S–23S rRNA gene internal transcribed spacer and the analysis of cell proteins by MALDI-TOF

mass spectrometry. *International Journal of Food Microbiology*, *196*, 137–144. https://doi.org/10.1016/j.ijfoodmicro.2014.12.003
57. Andrés-Barrao, C., Benagli, C., Chappuis, M., Ortega Pérez, R., Tonolla, M., & Barja, F. (2013). Rapid identification of acetic acid bacteria using MALDI-TOF mass spectrometry fingerprinting. *Systematic and Applied Microbiology*, *36*(2), 75–81. https://doi.org/10.1016/j.syapm.2012.09.002
58. Wieme, A. D., Spitaels, F., Aerts, M., De Bruyne, K., Van Landschoot, A., & Vandamme, P. (2014). Effects of growth medium on matrix-assisted laser desorption–ionization time of flight mass spectra: A case study of acetic acid bacteria. *Applied and Environmental Microbiology*, *80*(4), 1528–1538. https://doi.org/10.1128/AEM.03708-13
59. Malachová, A., Stránská, M., Václavíková, M., Elliott, C. T., Black, C., Meneely, J., Hajšlová, J., Ezekiel, C. N., Schuhmacher, R., & Krska, R. (2018). Advanced LC–MS-based methods to study the co-occurrence and metabolization of multiple mycotoxins in cereals and cereal-based food. *Analytical and Bioanalytical Chemistry*, *410*(3), 801–825. https://doi.org/10.1007/s00216-017-0750-7
60. Zhang, P., Zhang, P., Xie, M., An, F., Qiu, B., & Wu, R. (2018). Metaproteomics of microbiota in naturally fermented soybean paste, *Da-jiang*. *Journal of Food Science*, *83*(5), 1342–1349. https://doi.org/10.1111/1750-3841.14146
61. Yin, H., Zhang, R., Xia, M., Bai, X., Mou, J., Zheng, Y., & Wang, M. (2017). Effect of aspartic acid and glutamate on metabolism and acid stress resistance of *Acetobacter pasteurianus*. *Microbial Cell Factories*, *16*(1), 109. https://doi.org/10.1186/s12934-017-0717-6
62. Patti, G. J., Yanes, O., & Siuzdak, G. (2012). Metabolomics: The apogee of the omics trilogy. *Nature Reviews Molecular Cell Biology*, *13*(4), 263–269. https://doi.org/10.1038/nrm3314
63. Sanarico, D., Motta, S., Bertolini, L., & Antonelli, A. (2003). HPLC determination of organic acids in traditional balsamic vinegar of reggio emilia. *Journal of Liquid Chromatography & Related Technologies*, *26*(13), 2177–2187. https://doi.org/10.1081/JLC-120022402
64. Lee, S., Kim, S.-J., Bang, E., & Na, Y.-C. (2019). Chiral separation of intact amino acids by capillary electrophoresis-mass spectrometry employing a partial filling technique with a crown ether carboxylic acid. *Journal of Chromatography A*, *1586*, 128–138. https://doi.org/10.1016/j.chroma.2018.12.001
65. Caligiani, A., Acquotti, D., Palla, G., & Bocchi, V. (2007). Identification and quantification of the main organic components of vinegars by high resolution 1H NMR spectroscopy. *Analytica Chimica Acta*, *585*(1), 110–119. https://doi.org/10.1016/j.aca.2006.12.016
66. Smart, K. F., Aggio, R. B. M., Van Houtte, J. R., & Villas-Bôas, S. G. (2010). Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography–mass spectrometry. *Nature Protocols*, *5*(10), 1709–1729. https://doi.org/10.1038/nprot.2010.108
67. Durán-Guerrero, E., Marín, R. N., Mejías, R. C., & Barroso, C. G. (2006). Optimisation of stir bar sorptive extraction applied to the determination of volatile compounds in vinegars. *Journal of Chromatography A*, *1104*(1–2), 47–53. https://doi.org/10.1016/j.chroma.2005.12.006
68. Durán-Guerrero, E., Natera-Marín, R., Castro-Mejías, R., & García-Barroso, C. (2007). Stir bar sorptive extraction of volatile compounds in vinegar: Validation study and comparison with solid phase microextraction. *Journal of Chromatography A*, *1167*(1), 18–26. https://doi.org/10.1016/j.chroma.2007.08.039
69. Peng, M.-Y., Zhang, X.-J., Huang, T., Zhong, X.-Z., Chai, L.-J., Lu, Z.-M., Shi, J.-S., & Xu, Z.-H. (2021). Komagataeibacter europaeus improves community stability and function in solid-state cereal vinegar fermentation ecosystem: Non-abundant species plays important role. *Food Research International*, *150*, 110815. https://doi.org/10.1016/j.foodres.2021.110815
70. Peters, B., Mientus, M., Kostner, D., Daniel, R., Liebl, W., & Ehrenreich, A. (2017). Expression of membrane-bound dehydrogenases from a mother of vinegar metagenome in Gluconobacter oxydans. *Applied Microbiology and Biotechnology*, *101*(21), 7901–7912. https://doi.org/10.1007/s00253-017-8479-y
71. Zhang, Z., Ma, H., Yang, Y., Dai, L., & Chen, K. (2015). Protein profile of acetobacter pasteurianus HSZ3-21. *Current Microbiology*, *70*(5), 724–729. https://doi.org/10.1007/s00284-015-0777-y
72. Zheng, Y., Zhang, R., Yin, H., Bai, X., Chang, Y., Xia, M., & Wang, M. (2017). Acetobacter pasteurianus metabolic change induced by initial acetic acid to adapt to acetic acid fermentation conditions. *Applied Microbiology and Biotechnology*, *101*(18), 7007–7016. https://doi.org/10.1007/s00253-017-8453-8
73. Jiang, Y., Lv, X., Zhang, C., Zheng, Y., Zheng, B., Duan, X., & Tian, Y. (2019). Microbial dynamics and flavor formation during the traditional brewing of Monascus vinegar. *Food Research International*, *125*, 108531. https://doi.org/10.1016/j.foodres.2019.108531
74. Zhang, X., Wang, P., Xu, D., Wang, W., & Zhao, Y. (2019). Aroma patterns of Beijing rice vinegar and their potential biomarker for traditional Chinese cereal vinegars. *Food Research International*, *119*, 398–410. https://doi.org/10.1016/j.foodres.2019.02.008
75. Sengun, I. Y., Kilic, G., Charoenyingcharoen, P., Yukphan, P., & Yamada, Y. (2022). Investigation of the microbiota associated with traditionally produced fruit vinegars with focus on acetic acid bacteria and lactic acid bacteria. *Food Bioscience*, *47*, 101636. https://doi.org/10.1016/j.fbio.2022.101636
76. Yamada, Y., Yukphan, P., Lan Vu, H. T., Muramatsu, Y., Ochaikul, D., Tanasupawat, S., & Nakagawa, Y. (2012). Description of Komagataeibacter gen. Nov., with proposals of new combinations (Acetobacteraceae). *The Journal of General and Applied Microbiology*, *58*(5), 397–404. https://doi.org/10.2323/jgam.58.397
77. Fu, L., Chen, S., Yi, J., & Hou, Z. (2014). Effects of different fermentation methods on bacterial cellulose and acid production by Gluconacetobacter xylinus in Cantonese-style rice vinegar. *Food Science and Technology International*, *20*(5), 321–331. https://doi.org/10.1177/1082013213486663
78. Román-Camacho, J. J., Mauricio, J. C., Santos-Dueñas, I. M., García-Martínez, T., & García-García, I. (2021). Functional metaproteomic analysis of alcohol vinegar microbiota during an acetification process: A quantitative proteomic approach. *Food Microbiology*, *98*, 103799. https://doi.org/10.1016/j.fm.2021.103799
79. Trček, J., Jernejc, K., & Matsushita, K. (2007). The highly tolerant acetic acid bacterium Gluconacetobacter europaeus adapts to the presence of acetic acid by changes in lipid composition, morphological properties and PQQ-dependent ADH expression. *Extremophiles*, *11*(4), 627–635. https://doi.org/10.1007/s00792-007-0077-y
80. Schüller, G., Hertel, C., & Hammes, W. P. (2000). Gluconacetobacter entanii sp. Nov., isolated from submerged high-acid industrial vinegar fermentations. *International Journal of Systematic and Evolutionary Microbiology*, *50*(6), 2013–2020. https://doi.org/10.1099/00207713-50-6-2013
81. Qiu, X., Zhang, Y., & Hong, H. (2021). Classification of acetic acid bacteria and their acid resistant mechanism. *AMB Express*, *11*(1), 29. https://doi.org/10.1186/s13568-021-01189-6
82. Li, S., Li, P., Liu, X., Luo, L., & Lin, W. (2016). Bacterial dynamics and metabolite changes in solid-state acetic acid fermentation of Shanxi aged vinegar. *Applied Microbiology and Biotechnology*, *100*(10), 4395–4411. https://doi.org/10.1007/s00253-016-7284-3
83. Álvarez-Cáliz, C., Santos-Dueñas, I. M., Cañete-Rodríguez, A. M., García-Martínez, T., Mauricio, J. C., & García-García, I. (2012). Free amino acids, urea and ammonium ion contents for submerged wine vinegar production: Influence of loading rate and air-flow rate. *Acetic Acid Bacteria*, *1*(1), 1. https://doi.org/10.4081/aab.2012.e1
84. Kuypers, M. M. M., Marchant, H. K., & Kartal, B. (2018). The microbial nitrogen-cycling network. *Nature Reviews Microbiology*, *16*(5), 263–276. https://doi.org/10.1038/nrmicro.2018.9
85. Trček, J. (2015). Plasmid analysis of high acetic acid-resistant bacterial strains by two-dimensional agarose gel electrophoresis and

insights into the phenotype of plasmid pJK2-1. *Annals of Microbiology*, *65*(3), 1287–1292. https://doi.org/10.1007/s13213-014-0966-0
86. Rubio Gomez, M. A., & Ibba, M. (2020). Aminoacyl-tRNA synthetases. *RNA*, *26*(8), 910–936. https://doi.org/10.1261/rna.071720.119
87. Matzov, D., Bashan, A., Yap, M. F., & Yonath, A. (2019). Stress response as implemented by hibernating ribosomes: A structural overview. *The FEBS Journal*, *286*(18), 3558–3565. https://doi.org/10.1111/febs.14968
88. Okamoto-Kainuma, A., Ehata, Y., Ikeda, M., Osono, T., Ishikawa, M., Kaga, T., & Koizumi, Y. (2008). Hydrogen peroxide resistance of *Acetobacter pasteurianus* NBRC3283 and its relationship to acetic acid fermentation. *Bioscience, Biotechnology, and Biochemistry*, *72*(10), 2526–2534. https://doi.org/10.1271/bbb.80136
89. Sriherfyna, F. H., Matsutani, M., Hirano, K., Koike, H., Kataoka, N., Yamashita, T., Nakamaru-Ogiso, E., Matsushita, K., & Yakushi, T. (2021). The auxiliary NADH dehydrogenase plays a crucial role in redox homeostasis of nicotinamide cofactors in the absence of the periplasmic oxidation system in Gluconobacter oxydans NBRC3293. *Applied and Environmental Microbiology*, *87*(2), e02155-20. https://doi.org/10.1128/AEM.02155-20
90. Nakano, S., & Fukaya, M. (2008). Analysis of proteins responsive to acetic acid in Acetobacter: Molecular mechanisms conferring acetic acid resistance in acetic acid bacteria. *International Journal of Food Microbiology*, *125*(1), 54–59. https://doi.org/10.1016/j.ijfoodmicro.2007.05.015
91. Matsushita, K., Toyama, H., Tonouchi, N., & Okamoto-Kainuma, A. (Eds.). (2016). *Acetic acid bacteria*. Springer Japan. https://doi.org/10.1007/978-4-431-55933-7
92. Arslan, K., Schoch, T., Höfele, F., Herrschaft, S., Oberlies, C., Bengelsdorf, F., Veiga, M. C., Dürre, P., & Kennes, C. (2022). Engineering Acetobacterium woodii for the production of isopropanol and acetone from carbon dioxide and hydrogen. *Biotechnology Journal*, *17*(5), 2100515. https://doi.org/10.1002/biot.202100515
93. Ramírez-Bahena, M. H., Tejedor, C., Martín, I., Velázquez, E., & Peix, A. (2013). Endobacter medicaginis gen. Nov., sp. Nov., isolated from alfalfa nodules in an acidic soil. *International Journal of Systematic and Evolutionary Microbiology*, *63*(Pt_5), 1760–1765. https://doi.org/10.1099/ijs.0.041368-0
94. Kiefler, I., Bringer, S., & Bott, M. (2017). Metabolic engineering of Gluconobacter oxydans 621H for increased biomass yield. *Applied Microbiology and Biotechnology*, *101*(13), 5453–5467. https://doi.org/10.1007/s00253-017-8308-3
95. Mullins, E. A., & Kappock, T. J. (2012). Crystal structures of acetobacter aceti succinyl-coenzyme A (CoA):Acetate CoA-transferase reveal specificity determinants and illustrate the mechanism used by Class I CoA-Transferases. *Biochemistry*, *51*(42), 8422–8434. https://doi.org/10.1021/bi300957f
96. Kanchanarach, W., Theeragool, G., Inoue, T., Yakushi, T., Adachi, O., & Matsushita, K. (2010). Acetic acid fermentation of acetobacter pasteurianus: Relationship between acetic acid resistance and pellicle polysaccharide formation. *Bioscience, Biotechnology, and Biochemistry*, *74*(8), 1591–1597. https://doi.org/10.1271/bbb.100183
97. Ogawa, S., Tachimoto, H., & Kaga, T. (2010). Elevation of ceramide in Acetobacter malorum S24 by low pH stress and high temperature stress. *Journal of Bioscience and Bioengineering*, *109*(1), 32–36. https://doi.org/10.1016/j.jbiosc.2009.07.007
98. Quintero, Y., Poblet, M., Guillamón, J. M., & Mas, A. (2009). Quantification of the expression of reference and alcohol dehydrogenase genes of some acetic acid bacteria in different growth conditions. *Journal of Applied Microbiology*, *106*(2), 666–674. https://doi.org/10.1111/j.1365-2672.2008.04046.x
99. Matsushita, K., Inoue, T., Adachi, O., & Toyama, H. (2005). Acetobacter aceti possesses a proton motive force-dependent efflux system for acetic acid. *Journal of Bacteriology*, *187*(13), 4346–4352. https://doi.org/10.1128/JB.187.13.4346-4352.2005
100. Wang, B., Shao, Y., Chen, T., Chen, W., & Chen, F. (2015). Global insights into acetic acid resistance mechanisms and genetic stability of Acetobacter pasteurianus strains by comparative genomics. *Scientific Reports*, *5*(1), 18330. https://doi.org/10.1038/srep18330
101. Confer, A. W., & Ayalew, S. (2013). The OmpA family of proteins: Roles in bacterial pathogenesis and immunity. *Veterinary Microbiology*, *163*(3–4), 207–222. https://doi.org/10.1016/j.vetmic.2012.08.019
102. Gullo, M., & Giudici, P. (2008). Acetic acid bacteria in traditional balsamic vinegar: Phenotypic traits relevant for starter cultures selection. *International Journal of Food Microbiology*, *125*(1), 46–53. https://doi.org/10.1016/j.ijfoodmicro.2007.11.076
103. Devanthéry, T., Nakamura, K., Hashiguchi, S., & Hashimoto, M. (2020). Structure of a heptose-containing polysaccharide derived from Komagataeibacter europaeus NBRC 3261. *Carbohydrate Research*, *492*, 107989. https://doi.org/10.1016/j.carres.2020.107989
104. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2
105. 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., & Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9
106. Parte, A. C., Sardà Carbasse, J., Meier-Kolthoff, J. P., Reimer, L. C., & Göker, M. (2020). List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology*, *70*(11), 5607–5612. https://doi.org/10.1099/ijsem.0.004332
107. UniProt Consortium, T. (2018). UniProt: The universal protein knowledgebase. *Nucleic Acids Research*, *46*(5), 2699–2699. https://doi.org/10.1093/nar/gky092
108. Tyanova, S., Temu, T., & Cox, J. (2016). The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. *Nature Protocols*, *11*(12), 2301–2319. https://doi.org/10.1038/nprot.2016.136
109. Tyanova, S., Temu, T., Sinitcyn, P., Carlson, A., Hein, M. Y., Geiger, T., Mann, M., & Cox, J. (2016). The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nature Methods*, *13*(9), 731–740. https://doi.org/10.1038/nmeth.3901
110. Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: Protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, *51*(D1), D638–D646. https://doi.org/10.1093/nar/gkac1000
111. Kanehisa, M. (2004). The KEGG resource for deciphering the genome. *Nucleic Acids Research*, *32*(90001), 277D–280. https://doi.org/10.1093/nar/gkh063
112. Caspi, R., Altman, T., Dale, J. M., Dreher, K., Fulcher, C. A., Gilham, F., Kaipa, P., Karthikeyan, A. S., Kothari, A., Krummenacker, M., Latendresse, M., Mueller, L. A., Paley, S., Popescu, L., Pujar, A., Shearer, A. G., Zhang, P., & Karp, P. D. (2010). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Research*, *38*(1), D473–D479. https://doi.org/10.1093/nar/gkp875
113. Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., Wishart, D. S., & Xia, J. (2018). MetaboAnalyst 4.0: Towards more transparent and integrative metabolomics analysis. *Nucleic Acids Research*, *46*(W1), W486–W494. https://doi.org/10.1093/nar/gky310

114. Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2012). GenBank. *Nucleic Acids Research*, *41*(D1), D36–D42. https://doi.org/10.1093/nar/gks1195
115. Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P., Haw, R., Jassal, B., Korninger, F., May, B., Milacic, M., Roca, C. D., Rothfels, K., Sevilla, C., Shamovsky, V., Shorser, S., Varusai, T., Viteri, G., Weiser, J., & D'Eustachio, P. (2018). The reactome pathway knowledgebase. *Nucleic Acids Research*, *46*(D1), D649–D655. https://doi.org/10.1093/nar/gkx1132
116. Crampin, E. J., Cooling, M. T., & Hunter, P. (2008). Modelling biological modularity with CellML. *IET Systems Biology*, *2*(2), 73–79. https://doi.org/10.1049/iet-syb:20070020
117. King, Z. A., Dräger, A., Ebrahim, A., Sonnenschein, N., Lewis, N. E., & Palsson, B. O. (2015). Escher: A web application for building, sharing, and embedding data-rich visualizations of biological pathways. *PLOS Computational Biology*, *11*(8), e1004321. https://doi.org/10.1371/journal.pcbi.1004321

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