Trends in Food Science & Technology 65 (2017) 23–31

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: http://www.journals.elsevier.com/trends-in-food-science-and-technology

Meta-omics insights in the microbial community profiling and functional characterization of fermented foods

Gu Chen*, Congcong Chen, Zhonghua Lei

School of Food Science and Engineering, South China University of Technology, Guangzhou, 510640, China

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form
2 March 2017
Accepted 2 May 2017
Available online 8 May 2017

Keywords:
Fermented food microbial community
Amplicon analysis
Metagenomics
Metatranscriptomics
Metaproteomics
Metabolomics

ABSTRACT

Background: Food fermentation is an important pillar of societal traditions, but also crucial in terms of economy for numerous regional products, as well as rich microbiological resources awaiting exploration. During the past few years, tremendous efforts have been made to provide important and deeper insights into the underlying molecular mechanisms of fermented foods through amplicon analysis, metagenomic and metatranscriptomic sequencing, as well as metaproteomic investigation, and integrated with metabolomics analysis.

Scope and approach: In this review the meta-omic tools applied in fermented foods are summarized. Then the recent meta-omic studies in cheese, vinegar, fermented alcoholic beverage, fermented vegetables, fermented tea, and soybean products are reviewed and emphasized.

Key findings and conclusions: Mainly based on high-throughput DNA sequencing, amplicon analysis, metagenomics and metatranscriptomics, together with metaproteomics and high-resolution metabolomics have allowed exploration of microbial community dynamics and functional characterization in traditional fermented foods in astonishing scale and speed during the past few years. The recent applications of meta-omics in cheese, vinegar, fermented alcoholic beverage, fermented vegetables, fermented tea, and soybean products explore the underlying mechanism of food fermentation and reveal fermented foods as rich sources of valuable genes and bioactive substances. Though there is limitation, promising clues exist for future investigation. Further integration of multiple meta-omic tools, together with powerful statistical analysis methods will assuredly lead to more detailed functional characterization and more efficient and sustainable production practices in fermented foods worldwide.

1. Introduction

Food fermentation is traditionally created and reserved worldwide in different regions, which represents valuable cultural heritage and provides economy regional products and rich microbiological resources awaiting our exploration (Bokulich, Lewis, Boundy-Mills, & Mills, 2016; Gibbons & Rinker, 2015; Kergourlay, Taminiau, Daube, & Champomier Verges, 2015; Tamang, Watanabe, & Holzapfel, 2016). Other than their special quality, flavor and texture, more and more data confirm the health benefit of fermented foods, such as providing protection against *Helicobacter pylori* associated peptic ulcer and gastric cancer (Nair, Chouhan, Sen Gupta, & Chattopadhyay, 2016). In the past two decades, next-generation sequencing and advanced mass analyzers, as well as other new tools greatly enhanced the throughput and sensitivity of fermented foods study. Mainly based on high-throughput sequencing, culture-independent taxonomic methods were frequently applied and contributed a lot to fermented food microbial community analysis, for example, studies on cheese (Wolfe, Button, Santarelli, & Dutton, 2014), wine (Stefanini et al., 2016), fermented cocoa bean (Illeghems, De Vuyst, Papalexandratou, & Weckx, 2012) and tea (Zhao et al., 2015b). Several reviews summarized recent progress in food fermentation from different aspects. Kergourlay et al. reviewed the dynamics of microbial communities in food mainly based on amplicon analysis (Kergourlay et al., 2015). Bokulich et al. discussed the application of high-throughput sequencing for revealing the microbial landscapes within fermented foods and the food-processing built environment (Bokulich et al., 2016). Gibbons and Rinker reviewed the genomics of microbial domestication in the fermented food environment to gain microorganisms strains with enhanced fermentative capacities (Gibbons & Rinker, 2015). A rather comprehensive report

* Corresponding author.
E-mail address: chengu@scut.edu.cn (G. Chen).

http://dx.doi.org/10.1016/j.tifs.2017.05.002
0924-2244/

reviewed the microbial diversity of nine major groups of fermented foods (Tamang et al., 2016). However, in the past few years, tremendous efforts have been made to provide important and deeper insights into the underlying molecular mechanisms of fermented foods through metagenomic and metatranscriptomic sequencing, as well as the metaproteomic investigation, and integrating with metabolomics analysis. Thus, we review the updated omic and meta-omic studies of fermented foods in the past decade, mainly focus on the microbial succession contribution and exploration of functional genes and enzymes, and discuss the existing limitations, as well as touch the promising clues for future investigation in food fermentation.

2. Tools

2.1. High-throughput DNA sequencing

Mainly based on **high-throughput DNA sequencing**, amplicon analysis, metagenomic sequencing, and metatranscriptomic sequencing are applied in ecosystematic studies of microbial communities, including fermented foods (Bokulich et al., 2016; Franzosa et al., 2015).

Amplicon analysis amplifies conserved marker DNA region, such as the commonly used 16S rRNA gene for bacteria and ITS region for fungi, from mixed genomic DNA with universal PCR primers. It sequences PCR products directly, or displays them through DGGE (denaturing gradient gel electrophoresis), or quantifies through quantitative-PCR. The taxonomic compositions of whole microbial communities are identified through aligning marker DNA sequences against reference database. Amplicon analysis has been prevalently utilized to describe the microbial succession in global fermented foods (Tamang et al., 2016). Among fermentation without defined starter culture, or involving adventitious microbiota, or with multiple stages of complex microbiota, amplicon analysis helps to identify the normal and weird microbial successions, which is responsible for product quality and consistency. Other than amplifying DNA, amplicon analysis can amplify cDNA after reverse-transcription of mixed RNA, which can profile active microbial populations (Alessandria et al., 2016). Different from rRNA gene region, specific genes can be used as marker regions. For example, dextransucrase and surface layer protein D (SlpD) genes were used in competitive quantitative-PCR to differentiate different lactic acid bacteria (LAB) species, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, and *Lactobacillus brevis* in kimchi (Ahn et al., 2015). The relatively higher throughput and lower cost of amplicon analysis make it suitable for large-scale studies to display microbial community dynamics across time and space. However, amplicon analysis suffers from several disadvantages, such as the low resolution of taxonomy assignment at species level, PCR amplification bias, and unable to detect organisms without marker genes (Franzosa et al., 2015).

Metagenomic sequencing overcomes these problems. Different from genomics reveals the DNA sequences of an individual organism, metagenomics directly sequences the mixed genomic DNA after fragmentation and library construction. Thus, intact marker genes can be reconstructed and assigned to accurate taxonomy at species level without over or under representation. Compared with rRNA gene sequencing, metagenomic sequencing identified novel species, especially low abundance species in kefir grains, indicating its higher resolution and better abundance accuracy in microbial community analysis (Nalbantoglu et al., 2014). Metagenomics revealed virus as part of the microbial communities during several food fermentation (Jung et al., 2011; Park et al., 2011), which would not otherwise be detected through amplicon analysis. After annotation and quantification, metagenomics can also unravel functional gene cluster and pathways that might contribute to the characteristic flavor or structure of fermented foods, such as in cheese (Wolfe et al., 2014). Comparative metagenomics can discover differentially abundant genes between metagenomes, and provide information about differences in community structure, diversity and biological function. However, metagenomics cannot describe actively transcribing community.

Targeting on the actively expressed genes, **metatranscriptomics** sequences the cDNA after reverse-transcription of community RNA. It reveals the active population and actively expressed genes under specific time and space, which can be associated with ongoing metabolomic changes as well as the flavor and taste formation in fermented foods.

2.2. Metaproteomics

Metaproteomics is the large-scale characterization of the entire protein complement of environmental microbiota at a given point in time (Wilmes & Bond, 2004). Relying on proper protein extraction, fractionation procedures, and advanced tandem mass spectrometry, metaproteomics resolves proteins, the major functional components in microbial populations and directly links genotype to phenotype in situ. In its first decade, metaproteomics has yielded many important insights into microbial ecosystem function in various environmental niches, from activated sludge, soil to human gut microbiota (reviewed in (Wilmes, Heintz-Buschart, & Bond, 2015)). Interpreting the proteomic data can be relatively straightforward if the organism genome sequence or draft sequence is available; but it can be challenge if the organism is distantly related to well characterized organisms or in the analysis of mixtures of organisms (Armengaud, 2016), which is usually the case in metaproteomics of fermented foods. Despite of these difficulties, there are a few proteomic and metaproteomic reports in fermented foods, such as in lactic acid bacteria (Mangiapane et al., 2015; Siragusa et al., 2014), acetic acid bacteria *Acetobacter pasteurianus* during high acidity rice vinegar fermentation (Wang et al., 2015b), solid-state fermentation of Pu-erh tea (Zhao et al., 2015b), and Chinese liquor (Zheng et al., 2015). Metaproteomics remains a promising yet largely underexplored field in fermented foods.

2.3. Metabolomics

Metabolomics is the simultaneous and high-throughput determination and quantification of small molecule metabolites generated through metabolism. Both targeted or untargeted metabolomic analyses dependent on metabolite separation, detection, identification and quantification as well as multivariate data analysis (Mozzi, Ortiz, Bleckwedel, De Vuyst, & Pescuma, 2013). The most popular separation techniques are liquid chromatography (LC), including high-performance LC (HPLC) or ultra-performance LC (UPLC), gas chromatography (GC) and capillary electrophoresis (CE). While mass spectrometry (MS), nuclear magnetic resonance (NMR), and near infrared spectrometry (NIR) are the most used detection techniques. Metabolomics have been successfully applied to display the metabolite profiles in various fermented foods to assess food quality, traceability, authenticity, and safety (Johanningsmeier, Harris, & Klevorn, 2016; Lee, Jung, & Jeon, 2015; Mozzi et al., 2013; Oh, Jang, Woo, Kim, & Lee, 2016). When integrated with other meta-omic approaches, metabolomics provides important insights to unravel the underlying mechanisms of fermented foods production (Jung et al., 2013; Wu, Chen, & Xu, 2015a).