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Investigating bacterial and yeast diversity of spontaneous fermentation beer and cider using Hi-C metagenomics

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Motivation and Aim: Microbiome of fermented foods and beverages is a contributor to their organoleptic properties and health-related effects. Health claims must be based on scientific evidence supported by robust and detailed omics-based analysis of food products. The classic method for investigating taxonomic and functional profile is high-throughput shotgun sequencing, but metagenome-assembled genomes (MAGs) yielded from pure WGS data are often fragmentary and consist of a set of unordered scaffolds. Augmentation of metagenomics with chromosome conformation capture methods (like Hi-C/3-C) provides more accurate profiles of microbiome structure and functions. Combined investigation of food community structure along with the functional potential of each of its members will allow better understanding of microbial ecology and contribute to the improved quality control and innovative formulation development of beneficial products including low-alcohol/alcohol-free beverages. Apart from improving genome assembly, usage of Hi-C methods and its modifications allow revealing the chromatin structure and genome structural organization (i.e. number of chromosomes, folding properties, localisation in nucleus etc.).
Methods and Algorithms: We present a Hi-C metagenomics-based pipeline for exploring food microbial communities – with a particular focus on beverages yeast identification and comparative genomics. This pipeline includes Hi-C metagenomic library preparation and sequencing; reads preprocessing, Hi-C-aided reconstruction of metagenome-assembled-genomes (MAGs) from sequencing data and taxonomic profiling, and yeast comparative genomic analysis as a separate module. Yeast comparative genomics module includes the following steps: *de novo* gene annotation, comparative genomics with published reference genomes, phylogenetic analysis, average nucleotide identity analysis and functional enrichment analysis. In our pilot study, we applied Hi-C and common WGS metagenomic library preparation and sequencing for each of the 12 spontaneous fermentation beverages based on a larger collection analyzed using high-throughput 16S rRNA and ITS sequencing (https://biota.knomics.ru/beer-cider-2020) [1, 2]. We recovered multiple microbial genomes mainly represented by the prokaryotes from the *Acetobacteraceae*, *Enterobacteriaceae* and *Lactobacillaceae* families. Among the yeasts, the major species detected were *Brettanomyces*/*Dekkera bruxellensis* (a non-conventional yeast considered a spoilage organism in products like wine but an essential component in beers like lambics and American wild ales) and *Saccharomyces cerevisiae* (well-known yeast

prevalent in foods and beverages). We focused on recovered MAGs (bacteria and yeasts) as a showcase for validating the developed pipeline.

Results: Up to 6 bacterial Hi-C MAGs per sample was recovered; yeast MAGs were found in 5 samples. Recovered MAGs tended to have high completion (≥ 80 %) and low contamination (≤ 5 %). Features of phylogeny and gene content of the identified *Lactobacilli* genomes reflected their ability to survive in sour beers (like presence of hop resistance genes). For one *Lacticaseibacillus casei* MAG recovered from a mixed-fermentation ale sample, we found enrichment of genes possibly reflecting its high probiotic potential. As a showcase of Hi-C metagenomic aid in linking plasmids to bacterial hosts, we explored *Pediococcus damnosus* plasmidome. By utilizing Hi-C reads to improve genome assembly of *B.bruxellensis* MAGs, we achieved significant improvement in comparison with WGS-only genome assembly (up to 95 % completion and 4 % contamination). The obtained chromosome contact maps demonstrated chromosome conformation structure similar to *S. cerevisiae* Rabl-like conformation. Yeast comparative genomic analysis clearly demonstrated clustering by niche, and functional enrichment analysis showed prevalence of specific metabolic modules which are likely responsible for adaptation to niche conditions such as SO_2 tolerance (applied in winemaking as a preservative agent) or reoxidation of pyruvate (thus outperforming *S. cerevisiae* in beer production).

Conclusion: Hi-C metagenomics is a promising technique for in-depth microbiome analysis of spontaneously fermented foods and beverages. It allows improved reconstruction of bacterial and yeast genomes compared to conventional metagenomics, thus facilitating downstream analysis steps like comparative genomics. In the future, the approach might aid in developing advanced food quality control as well as in discovery of novel strains with high biotechnological potential.

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