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Revolutionizing Beer Quality Control: Rapid Microbial Identification Via Nanopore Sequencing

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Abstract:

- ***Background:***

Microbial diversity is what decides the flavor, aroma, and quality of beer. These microbes include bacteria and yeast. These microorganisms can cause spoilage when their population grows unchecked and fermentation when in check. Current traditional techniques to identify the microbial flora have poor resolution and scale up very badly. High-resolution sequencing technologies, such as nanopore sequencing, offer a great look into microbial communities, making their role in the brewing process considerably clearer. Bioinformatics platforms, such as Galaxy.org, offer user-friendly tools for processing and analyzing complex sequencing data, providing advanced microbial characterization capability to researchers and industry professionals

- ***Objectives:***

This study aimed to isolate and characterize microbial communities in beer using advanced nanopore sequencing technologies combined with the Galaxy.org platform for bioinformatics analysis. Specifically, the objectives were to:

- **Extract DNA from beer samples and generate long-read sequencing data through the Oxford Nanopore MinION platform.**
- **Use Galaxy.org to process and analyze data: base calling, quality control, taxonomic classification, and visualization.**
- **Identify key microbes, such as bacteria and yeast, emphasizing species linked to fermentation and spoilage.**
- **Demonstrate nanopore sequencing and Galaxy.org as a powerful, user-friendly tool for the identification of microorganisms in brewing, helping quality control and optimization of processes.**
- **Explain how the methodology has wider applications in food and beverage microbiology**

Keywords: *Nanopore Sequencing, Galaxy.Org, Beer Microbiology, Microbial Identification, Brewing, Bioinformatics.*

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I. INTRODUCTION

Beer's flavor, aroma, and quality are largely affected by microbial communities during fermentation and storage. Desirable yeast and bacteria contribute to important biochemical reactions, whereas unwanted microbes cause spoilage, off-flavors, and instability. Traditional approaches, such as culture-based methods, typically cannot detect low-abundance or non-culturable species, and high-resolution methods are needed to improve our understanding of the complex communities. Nanopore sequencing, through Oxford Nanopore Technologies, is one of the cutting-edge technologies for providing real-time long-read sequencing in detailed insight to complex microbial communities. Coupled with Galaxy.org, an easy-to-use and open-source bioinformatics platform, it simplifies data analysis—quality control through taxonomic classification and visualization—even for non-experts.

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This study uses nanopore sequencing and Galaxy.org to characterize beer microbiomes, identifying key microorganisms linked to fermentation and spoilage. It demonstrates the utility of this approach for improving quality control and process optimization in brewing by analyzing taxonomic composition. The findings also highlight its broader applicability in food and beverage microbiology, offering a powerful tool for enhancing product quality and stability.

II. WORKFLOW

A. Data Acquisition

To begin with, the raw sequencing data in the form of FASTQ files should be imported into the Galaxy workspace. This can be done by uploading from a local system or by fetching data directly from external repositories such as Zenodo. Once the data has been uploaded, the dataset appears in the Galaxy history, and the file contents can also be verified through the preview built-in function.

B. Quality Assessment

The downstream analyses can only be done appropriately with proper quality evaluation of the data. For this, base quality scores, sequence length distribution, and GC content are checked using FASTQC. A detailed HTML report will be provided for the given sequencing reads so that decisions in the processing step can be taken on the basis of quality metric.

- **Objective**: Quality assessment of the raw sequencing data.

Fig 1: Sequence Quality Drops at Start and End

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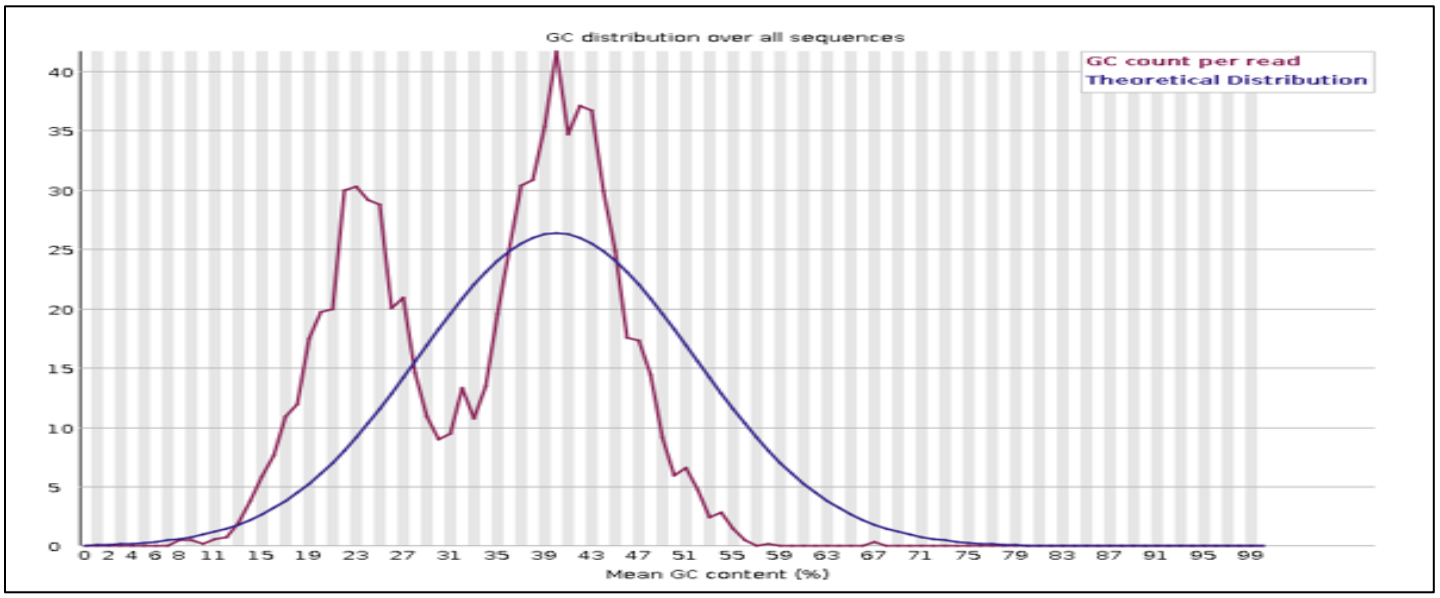

Fig 2: GC Content Distribution Shows Deviation from Theoretical Mode

C. Data Refining

➢ *Quality Improvement in Sequencing Includes Adapter Removal and Filtering out Low-Quality Reads.*

- *Adapter Removal (Porechop)*

✓ Deletes the sequencing adapters and chimeric sequences
✓ The actual biological sequences are retained for downstream analysis

- *Quality Filtering (FASTP)*

✓ Removes low quality reads by applying phred score cut-offs.
✓ Adapter trimming is disabled to avoid any accidental sequence modifications.
✓ The sequences with a phred score of less than 10 are filtered out and the quality of data is improved.

- *Reports*

✓ Sequence retention summary and Qualities improve.
✓ Guarantees the processed dataset meets the required standards for downstream analysis.

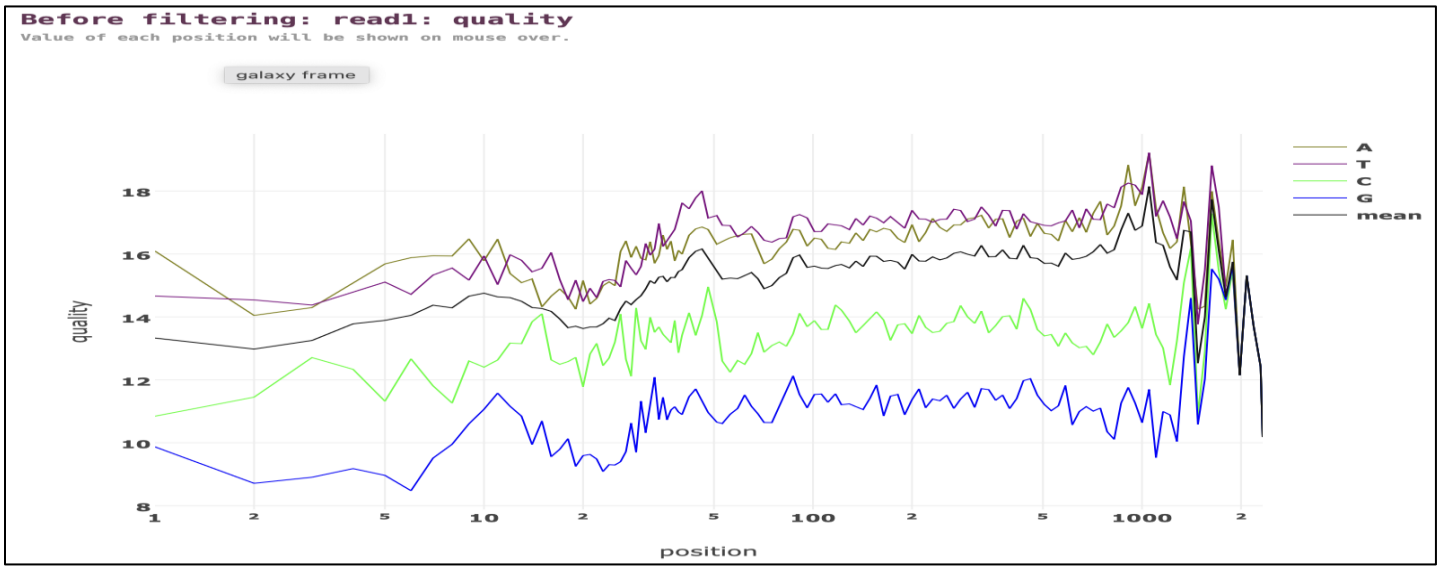

Fig 3: Before Filtering Quality

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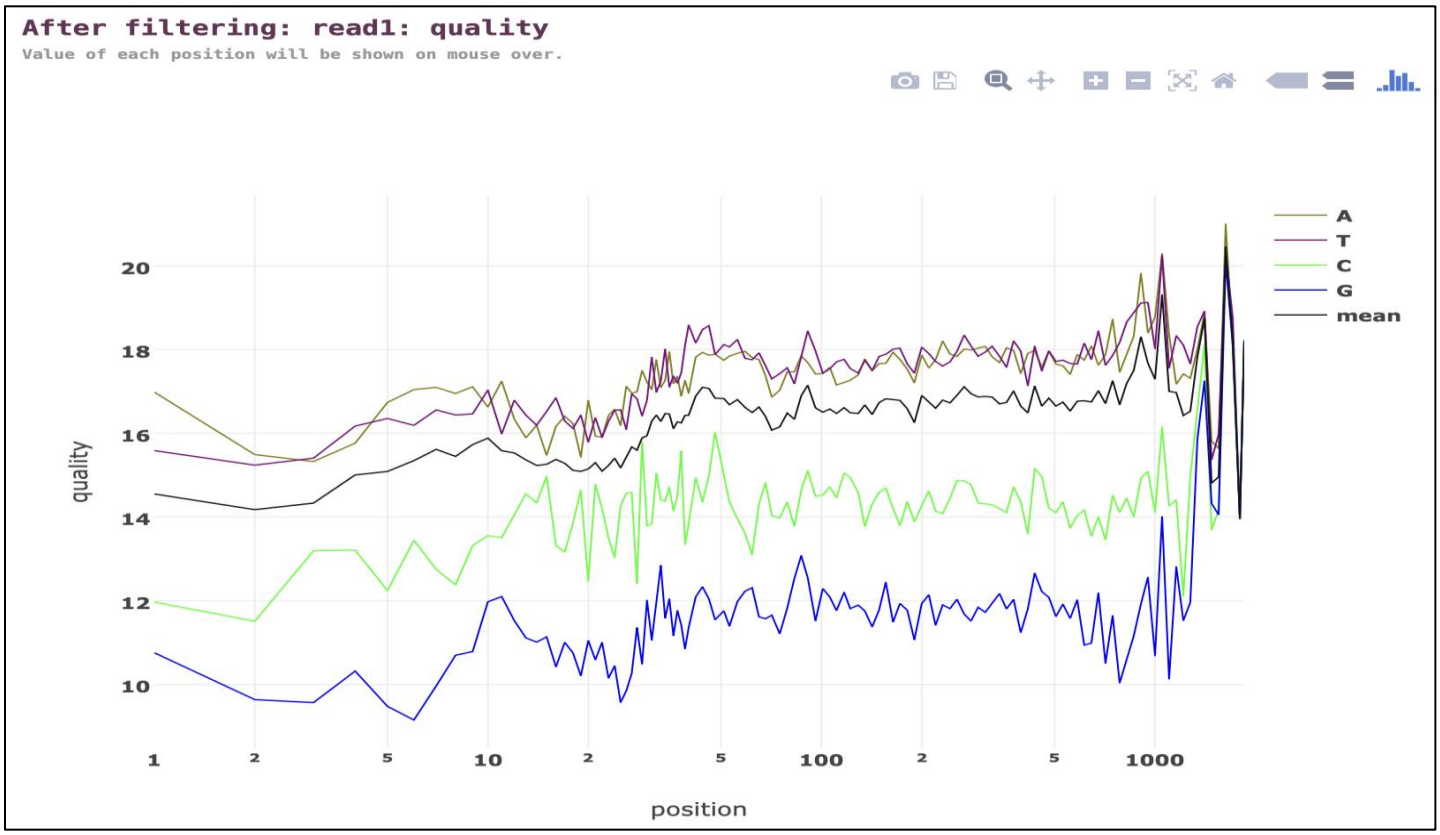

Fig 4: After Filtering Quality

D. Taxonomic Assignment

➢ *Taxonomic Classification using Kraken2*

- Sequences are compared to a reference database that assigns taxonomic labels.
- Kraken2 uses a prebuilt RefSeq index to classify microbial reads with great efficiency.
- The tool provides a report containing taxa along with relative abundance.

➢ *Filtering Low Confidence Taxa*

The dataset is cleansed of potential misidentifications and contaminants, thus providing a more reliable microbial profiling, by filtering out taxonomic groups with insufficient read support to refine classification results. This is achieved by applying a threshold that retains only taxa with a minimum of five assigned reads.

E. Taxonomic Visualization

➢ *Krakentools Helps by:*

- Converting Kraken2 results into structured formats (e.g., Krona, BIOM, or report files) for downstream analysis.
- Preserving taxonomic hierarchy, ensuring accurate representation of microbial relationships.
- Enabling seamless compatibility with visualization and statistical tools for effective interpretation.

F. Interactive Visualization (Krona)

➢ *Understanding Microbial Diversity Requires Intuitive Visualization. Krona Provides:*

- Interactive pie charts that allow hierarchical exploration of taxonomic distributions.
- Zoomable, multi-layered views to analyze microbial composition at different taxonomic levels.
- Improved data interpretation, aiding researchers in identifying dominant taxa and community structures.

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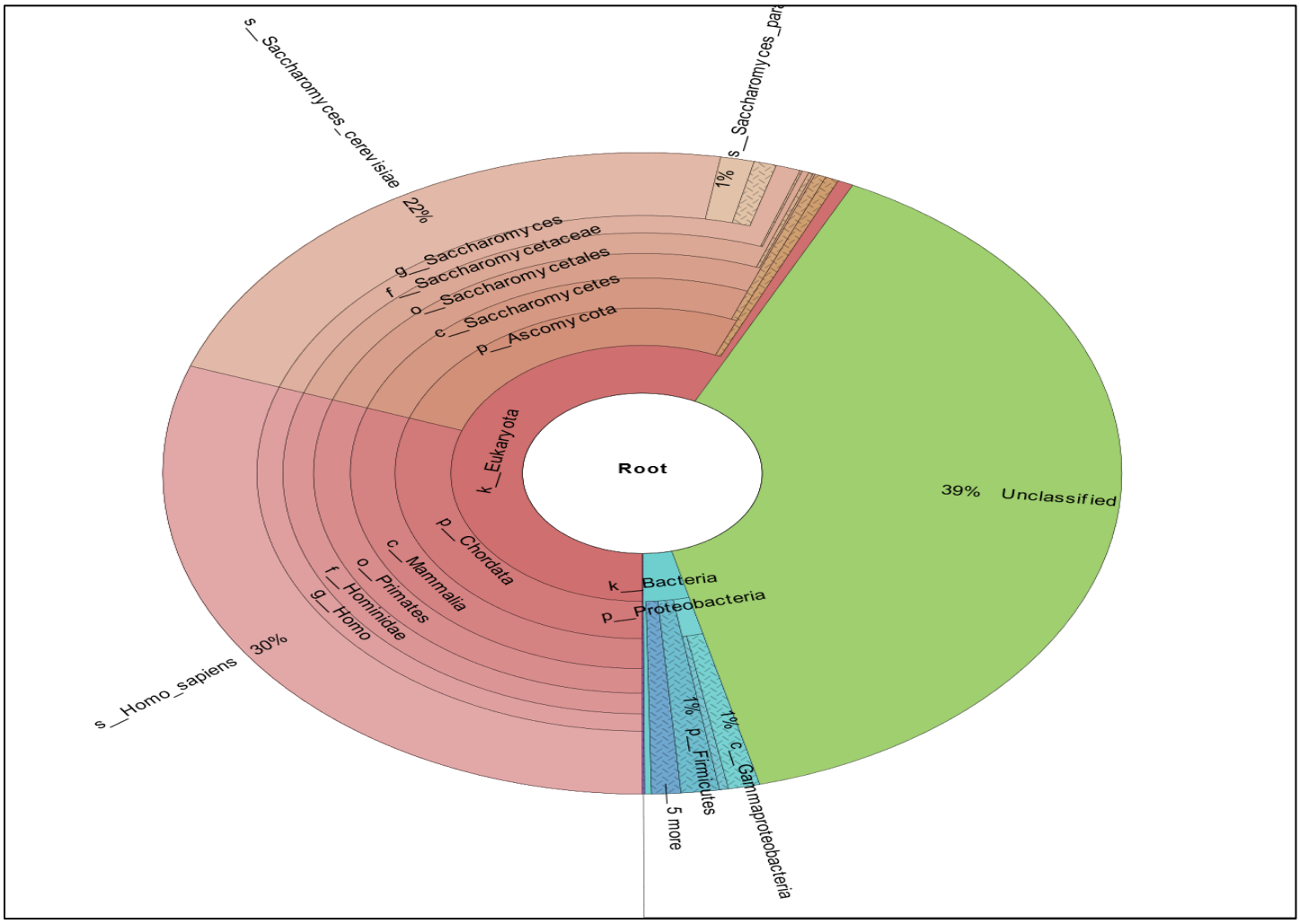

Fig 5: Sunburst Chart Depicting Taxonomic Composition

G. Microbiome Characterization

The final stage involves investigating yeast populations in the beer microbiome. Yeasts primarily belong to the fungal kingdom and are distributed across the Ascomycota and Basidiomycota phyla. The Saccharomycetales order encompasses the "true yeasts," which play a significant role in fermentation. The refined dataset provides insights into the composition and diversity of yeast species present in the sample.

➢ *The Species Identified for Chimay Beers are:*

- Saccharomyces cerevisiae
- Saccharomyces mikatea: a species generally used in winemaking
- Kazachstania martiniae: Kazachstania is a genus from the family Saccharomycetaceaethe.
- Saccharomyces kudriavzevii
- Brettanomyces bruxellensis

Brettanomyces is a non-spore forming genus of yeast in the family Saccharomycetaceae, and is important to both the brewing and wine industries due to the sensory compounds it produces.

Brettanomyces bruxellensis is typically used for the production of the Belgian beers.

- Saccharomyces paradoxus: a wild yeast species closely related to Saccharomyces cerevisiae
- Kazachstania kunashirensis
- Saccharomyces cariocanus: a wild yeast species closely related to Saccharomyces cerevisiae
- Filobasidium magnum
- Malasseria restricta
- Pichia kudriavzevii
- Aureobasidium pullulans
- Sporidiobolus metaroseus

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III. DISCUSSIONS

A. Strengths

- User-Friendly Interface – Galaxy provides an intuitive platform that does not require advanced programming skills, making it accessible to researchers.
- Comprehensive Quality Control – The use of FASTQC, Porechop, and Fastp ensures thorough quality assessment and filtering of sequencing data.
- Efficient Taxonomic Classification – Kraken2 allows rapid and accurate microbial identification using an extensive reference database.
- Clear Data Visualization – Krona's interactive pie charts offer an effective way to explore taxonomic distributions.

B. Limitations

- Reference Database Bias – Kraken2's accuracy is dependent on the completeness and quality of the reference database used.
- Computational Resource Demand – Processing large datasets, especially in taxonomic classification, requires significant memory and computing power.
- Potential Loss of Rare Species – Filtering out low-confidence taxa may remove rare but relevant microorganisms from the analysis.
- Limited Functional Insights – The workflow identifies microbial composition but does not provide functional or metabolic profiling of the microbiome.

IV. CONCLUSIONS

This workflow has been systematic to analyze the beer microbiome in Galaxy and, hence, gives high quality, accurate taxonomic classification, and clear visualization of data. Tools such as FASTQC, Porechop, Fastp, Kraken2, and Krona, the process can effectively filter and classify microbial diversity and present them appropriately. Despite this, accessibility and comprehensive quality control and visualization of the outputs come with its weakness in the biases of databases and computational requirements. This workflow allows for reliable characterization of the microbiome and thus supports fermentation and microbial ecology research in brewing.

- *Data Availabilty*

Zenodo:https://zenodo.org/record/7093173/files/ABJ044_c38189e89895cdde6770a18635db438c8a00641b.fastq

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