The Secrets of Yeasts: exploring the hidden mycobiome of industrial samples from Hungary

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Yeasts have played an important role in food and beverages production since ancient times. Utilization of yeasts, mainly *Saccharomyces cerevisiae*, has evolved to industrial scale throughout the centuries. So much so that many of the strains can be considered domesticated and thus are heavily studied. However, there are certain fermentation environments where the microbial composition and the role of yeasts have not yet been examined in its depth. Two of these special niches are animal feedstuff from agricultural sources and sourdough from the food industry. Both of these fermentation processes are heavily dominated by bacteria, so the studies published so far mainly concentrate on them. Information and data on yeast species and yeast genomic attributes are lagging behind. As of recently, there is a trend to utilize culture-free methods for examination of these communities to get information about species with lower abundance as well.

To examine the microbial composition of samples of these special environments where yeasts closely coexist with bacteria, our aim was to implement Oxford Nanopore Technologies' culture-free solutions for metagenomic/metabarcoding analyses and whole genome sequencing.

Materials and methods

Samples:

- Fermented and not fermented feedstuff (silage and other forage) from hungarian dairy farms
- Traditional and industrial sourdough samples from bakeries

Sequencing methods

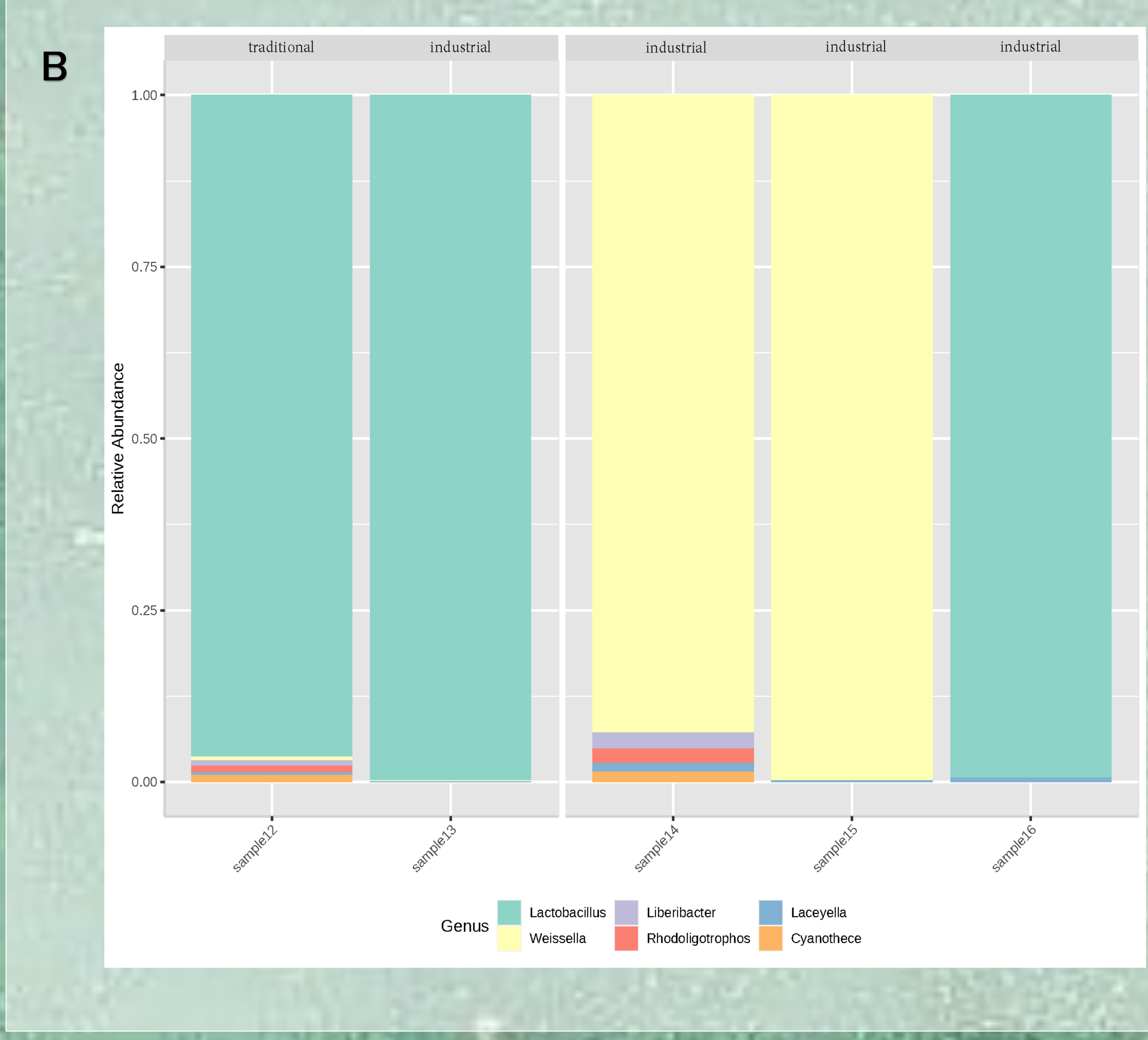
- 16S metagenome sequencing with Oxford Nanopore Technologies 16S long-read metabarcoding kit, R9.4.1 flow cells, MinION Mk1b device
- ITS metagenome sequencing with custom primers, ONT PCR Barcoding kit, ONT Ligation Sequencing kit, R9.4.1 flow cells, MinION Mk1b device based on D'Andreano et al.[1]
- 2 samples' whole genome were also sequenced with Illumina short-read sequencing with NextSeq550 instrument

Downstream analyses

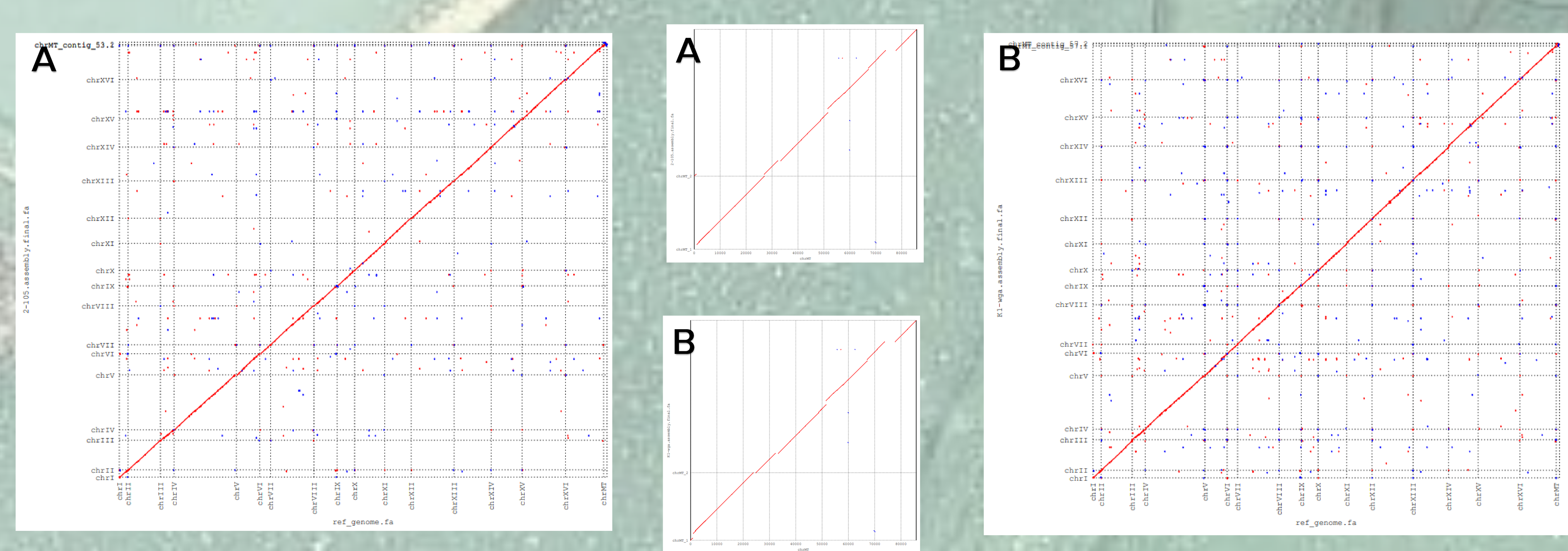
- Species level identification based on 16S region: Emu v3.4.4 default, MicrobiomeAnalyst
- Species level identification based on ITS: comparison between Emu v3.4.4 UNITE database and EPI2ME Fastq WIMP
- Whole genome assembly and annotation: after quality check and read processing: Flye v9.1, Racon-MEDAKA v1.4, POLCA v4 and LRSDAY v1.6 pipline.

Results

Bacterial composition of silage and other forage samples (A) as well as traditional and industruial sourdough samples (B)



Genome wide dotplot of reference genome (S288c) against whole genome and mitochondrial genome assembly of a silage (A) (BUSCO 99.6%, 16 whole scaffolds) and a sourdough (B) (BUSCO 99.7%, 16 whole scaffolds) *Saccharomyces cerevisiae* yeast



Comparison of fungal composition results based on ITS between Emu v3.4.4 UNITE and EPI2ME Fastq WIMP through 3 species with the highest relative abundance in a silage and a sourdough sample

Silage		Sourdough	
Emu	WIMP	Emu	WIMP
Pyronemataceae sp.	*Zygotorulaspora mrakii*	*Saccharomycetales sp.*	*Saccharomyces cerevisiae*
Kazachstania aquatica	*Saccharomyces cerevisiae*	*Naumovozyma baii*	*Actinomyces oris*
Ceratobasidiaceae sp.	*Torulaspora globosa*	-	*Kluyveromyces marxianus*

Conclusion

We were able to create an in-hous database and visualize the microbial communities from both the feedstuff and sourdough samples based on the 16S and ITS metagenomic sequencing. Comparison of ITS analysis results between Emu and EPI2ME WIMP suggests that ITS-based analyses need further optimization, finetuned to the spesifics of the samples and the species they possibly contain. Whole genome assemblies are highly continous with complete chromosomes and BUSCO scores above 90%.

Using Oxford Nanopore Technolgies' solutions, we were able to generate and analize valuable, previously not examined metagenomic and single genomic data of feedstuff and sour dough samples from Hungary. By presenting not only the bacterial, but also the fungal composition of these samples, we created a great base for further discussion and examination of how these yeasts and bacteria coexist. These results shed light on areas that could be interesting for further interaction analyses of the microbes in these environments.

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Reference: [1]D'Andreano S, Cuscó A, Francino O. Rapid and real-time identification of fungi up to species level with long amplicon nanopore sequencing from clinical samples. Biol Methods Protoc. 2020 Dec 23;6(1):bpaa026. doi: 10.1093/biomethods/bpaa026. PMID: 33506108; PMCID: PMC7820110.