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KU LEUVEN GENT

Laboratory of Enzyme, Fermentation, and Brewing Technology (EFBT)

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Influence of steeping and germination temperature on the state of the proteomes of barley-associated microflora

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Abstract

Temperature is one of the **fundamental variables** of the malting process that influences the composition and functionality of microbial communities associated with barley. Temperature changes could alter the microbial diversity and thus impact the quality of the final malt. In this study, we used a **metaproteomics approach** to profile the temporal changes in the proteome of barley-associated microbes under two malting conditions: A standard **industrial malting regime (IMR)** (germination for 120 h at 16°C), and an **accelerated malting regime (AMR)** (germination for 74 h at 20°C). A quantitative comparison of the temporal state of the proteomes of the barley-derived microbes under the two regimes was performed. By this, we gained insights into the effect of changes in the steeping and germination temperature on the state of protein expression within the identified barley-derived microflora. In parallel with the acquired enzymatic activity data, these results give us novel insights into the functionality of the microflora associated with barley during germination as well as providing us information on the specific microflora contributing to the enzyme potential of green malt.

Experimental design

Pilot-scale Malting

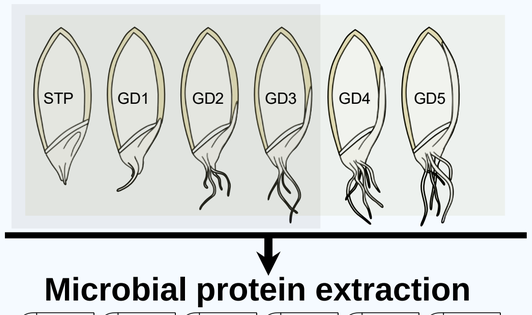
4 barley varieties (Planet, Fandaga, Fantex, and Faro) in triplicates

Regime	Stage	Time (h)	Temperature (°C)
IMR	Steeping (WS/DR/WS/DR)	4/9/4/9	12/22/16/22
	Germination	120	16
AMR	Steeping (WS/DR/WS)	5.5/18/1.25	22/21/20
	Germination	74	20

WS = Wet Steep; DR = Dry Rest; STP = Steep; GD = Germination Day

IMR (STP-GD5)

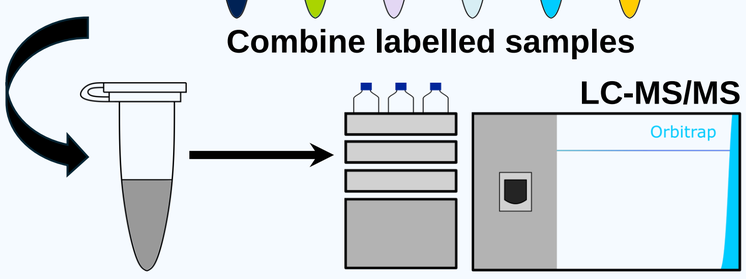
AMR (STP-GD3)



Microbial protein extraction

Trypsinization and Tandem mass tag (TMT) labelling

Combine labelled samples



Database search

Comet [1], tailor-made database: barley, bacteria, fungi

Peptide validation

PeptideProphet & iProphet [2,3]
5% false discovery rate

Taxonomic analysis

Unipept desktop [4]

Statistical analysis

Limma [5]

Functional analysis

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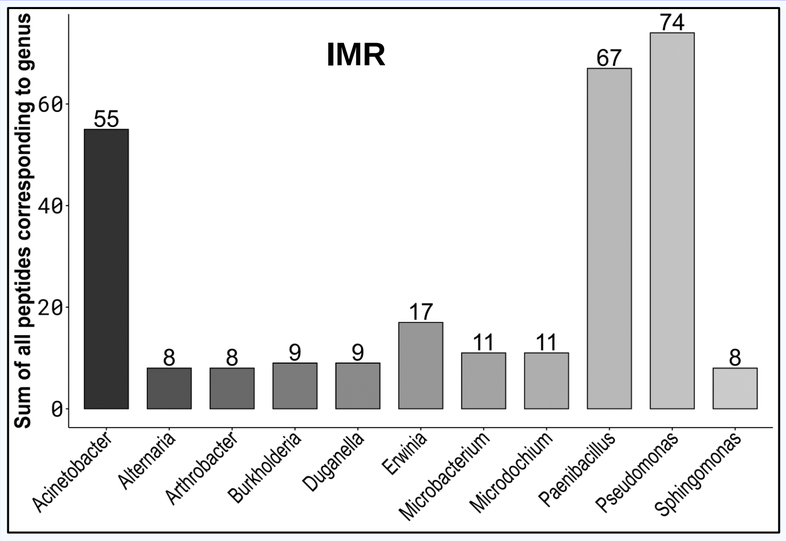
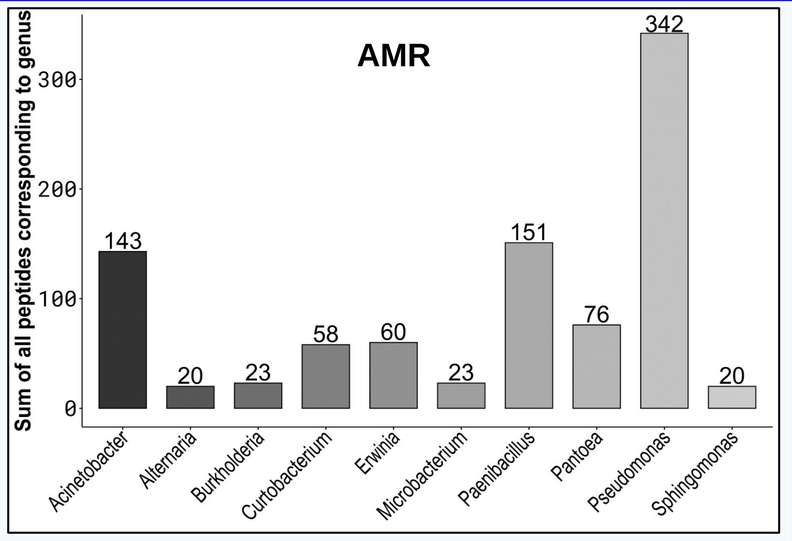
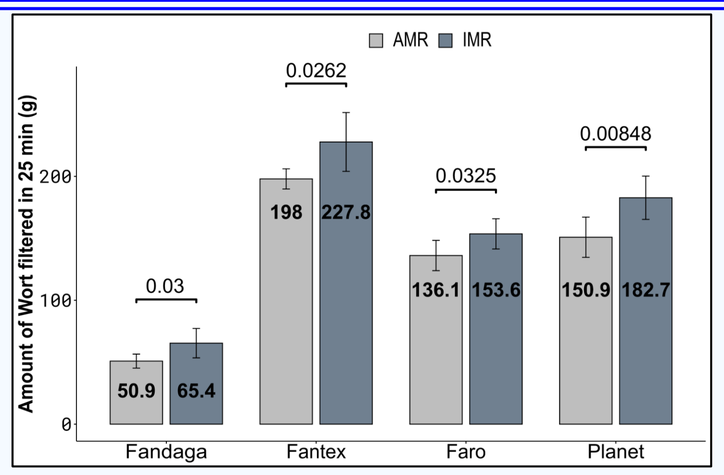
Metaproteomic and Taxonomic analysis



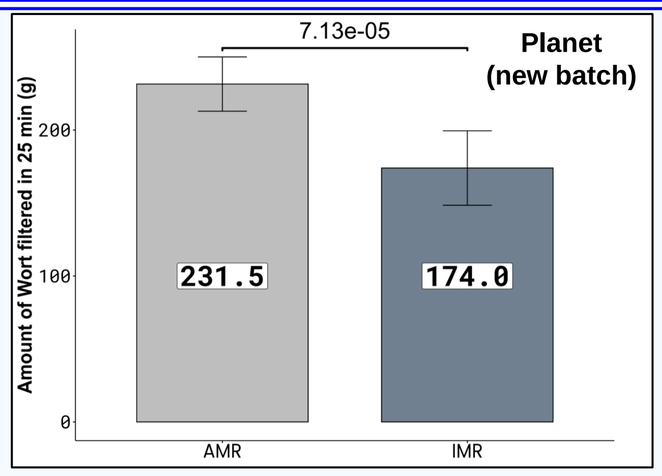


Increased temperatures generically **favoured** the expression of proteins attributed to ***Pantoea, Curtobacterium*, *Erwinia*, and *Pseudomonas*.** The extent of the fold increase for each genus was different among the genotypes.

Impact of malting regime on malt quality



- Reduction in wort filtration rate for the AMR
- Probably due to an **increased abundance** of **potential biofilm-forming bacteria** (no increased β-glucan nor arabinoxylan content in AMR malt)



- Validation experiment with a new batch of Planet
- Slow filtration rate **not an effect of AMR**
- *Pseudomonas* peptides higher abundant in **older batch (56)** versus **newer batch (38)** on GD3

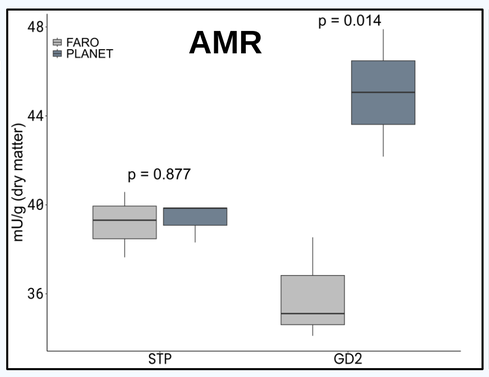
Contribution of microbes to enzymatic activity of malt

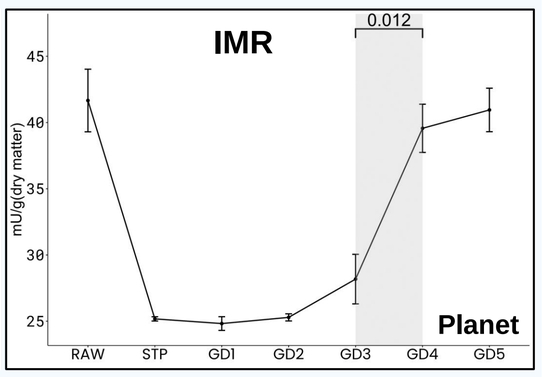
- **GO:004593** (Xylanase catabolic process) **significantly enriched** for the following contrasts:

  (**bold** = protein upregulated as per proteome analysis)

  - **Planet** vs Faro: STP, GD2 (AMR)
  - Planet: GD3 vs **GD4** (IMR)
- Two associated proteins:
  - **Endo-1,4-beta-xylanase (IMR and AMR)**,
  - **Non-reducing end alpha-L-arabinofuranosidase (AMR)**

Endo-1,4-β-D-xylanase activity





- A higher abundance of associated microbial proteins generally reflected in increased endo-1,4-β-D-xylanase activity
- Associated proteins are of fungal origin as per Unipept analysis (**Class Dothideomycetes** or **Order Pleosporales**)

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Conclusions

- Growth of biofilm-forming microbes during malting, could slow down the wort filtrate rate of the final malt
- Barley-associated microflora contribute to the overall activity of enzymes vital to malt quality
- Epiphytic fungi may play a role in barley cell wall degradation during malting

References

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