VIABILITY AND PHYSIOLOGICAL RESPONSES OF YEASTS EXPOSED TO STRESS CONDITIONS OCCURING IN WEST AFRICAN FERMENTED CEREAL DOUGHS

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Background

- African fermented doughs constitutes a important part of daily food intake
- Fermentation lasts from 24-48h, depending on the rate of acidification and the preference of the producer
- Elucidation of the viability and stress resistance responses of yeasts toward conditions mimicking those in cereal doughs can impact the design strategies for improved quality and safety of the doughs

AIM: Get an understanding of how the stress factors in fermented cereal doughs influence the growth and survival of the predominant yeast species (*Saccharomyces* and non-*Saccharomyces*) and to investigate differences in sensitivity at species and strain levels

West African fermented cereal dough

Ethanol

Lactic acid
Acetic acid

The twelve yeast isolates included

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Occurrence of lactic acid bacteria and yeasts at species and strain level during spontaneous fermentation of mawè, a cereal dough produced in West Africa

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Isolate	Identity	Isolate source (cereal dough and fermentation duration)		NCBI GenBank accession no
Sc1	*Saccharomyces cerevisiae*	Undehulled maize mawè	36h	MG245859
Sc2	*Saccharomyces cerevisiae*	Commercial maize mawè	onset	MG245839
Sc3	*Saccharomyces cerevisiae*	Undehulled maize mawè,	36h	MG245858
Cg1	*Candida glabrata*	Commercial maize mawè	6h	MG245841
Cg2	*Candida glabrata*	Commercial maize mawè	onset	Submission in progress
Cg3	*Candida glabrata*	Commercial maize mawè	24h	MG245821
Km1	*Kluyveromyces marxianus*	Commercial maize mawè	onset	MG245826
Km2	*Kluyveromyces marxianus*	Commercial sorghum mawè	6h	MG245824
Km3	*Kluyveromyces marxianus*	Homemade maize mawè	onset	MG245846
Pk1	*Pichia kudriavzevii*	Homemade maize mawè	onset	MG245834
Pk2	*Pichia kudriavzevii*	Commercial sorghum mawè	6h	MG245830
Pk3	*Pichia kudriavzevii*	Homemade maize mawè	12h	MG245831

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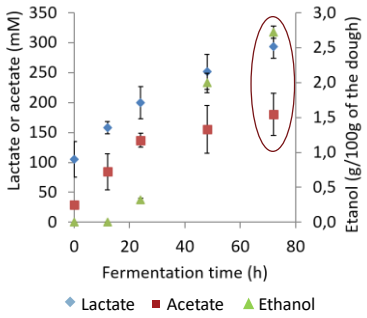
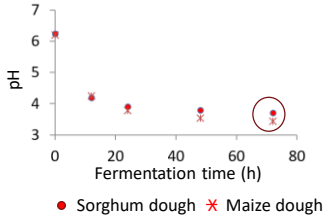
Experiment conditions

Performed in malt, yeast extract, glucose, peptone (MYGP)

1. **No stress** (pH 5.6)
2. **Low pH stress** (pH 3.4)
3. **Ethanol stress (EtOH)** (with 3% (v/v) EtOH, pH 3.4)
4. **Lactic acid stress (LA)** (with 285 mM LA, pH 3.4)
5. **Acetic acid stress (AA)** (with 150 mM AA, pH 3.4)
6. **LA + AA stress** (with 285 mM LA and 150 mM AA, pH 3.4)
7. **LA + AA + EtOH stress** (with 285 mM LA, 150 mM AA, 3% (v/v) EtOH, pH 3.4)

DOUGH CONDITIONS



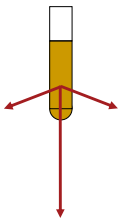


Growth and viability

Cells suspension in stress conditions, analyzed at 6, 24, 48 and 72h)

Plate counting

(Log_{10} CFU)

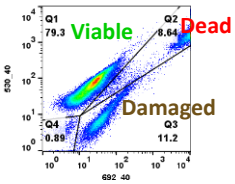


Growth curve

(μ_{max}, h^{-1})

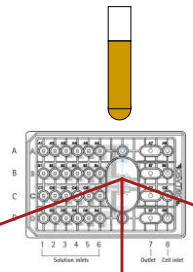
SYTO 13 and PI staining

Flow cytometry

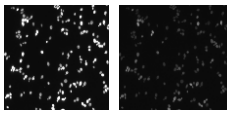


pHi, membrane permeability and micro colony formation of stressed single cells

Cells exposed to LA+AA+EtOH at pH 3.4 for 6h followed by staining with CFDA-se



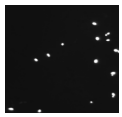
pHi determination



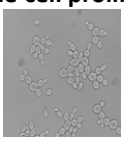
490 nm 435 nm

PI staining

Membrane permeability

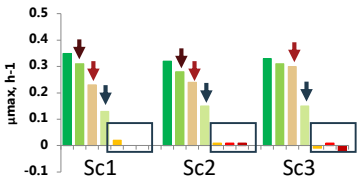


Single cell proliferation

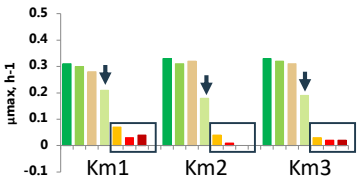


Maximum specific growth rate (μ_{max}, h^{-1})

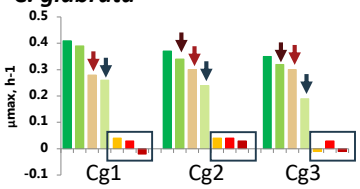
S. cerevisiae



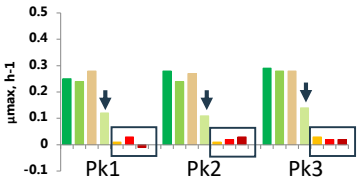
K. marxianus



C. glabrata



P. kudriavzevii



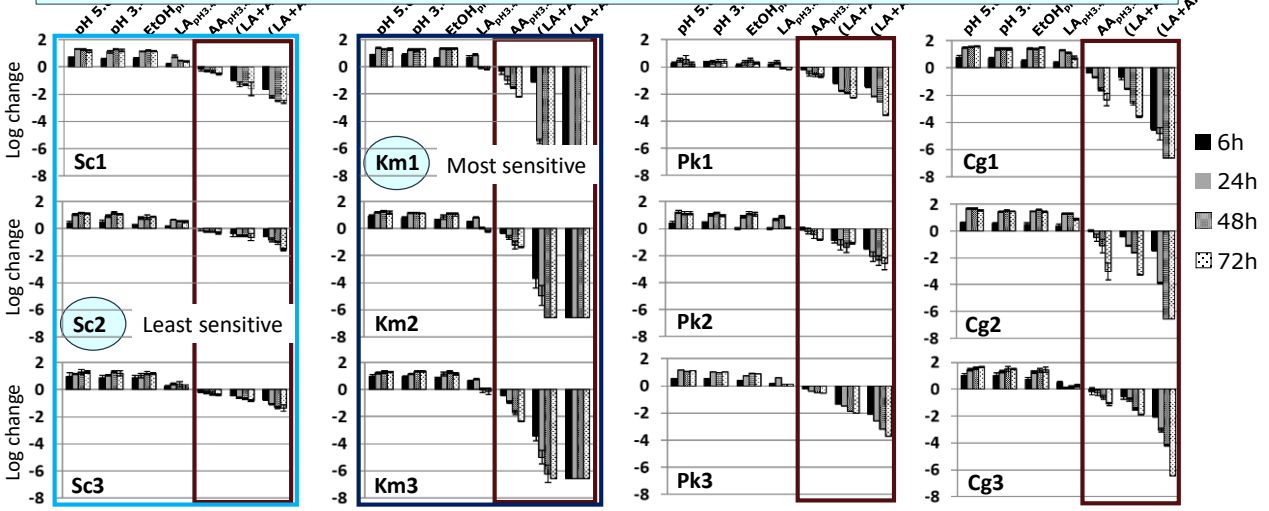
Maximum specific growth rate:

Decreased significantly (h^{-1}) for all strains of *S. cerevisiae* exposed to
- Lactic acid (pH 3.4)
- Combination of lactic and acetic acid (pH 3.4)
- Ethanol stress (pH 3.4)
- Combination of lactic, acetic acid and ethanol (pH 3.4)

- No-stress condition, pH 5.6
- Low pH stress, pH 3.4
- Ethanol stress, $EtOH_{pH3.4}$
- Lactic acid stress, $LA_{pH3.4}$
- Acetic acid stress, $AA_{pH3.4}$
- Combination of lactic and acetic acid stresses, $(LA+AA)_{pH3.4}$
- Combination of lactic, acetic acid and ethanol stresses, $(LA+AA+EtOH)_{pH3.4}$

Survival in the various stress conditions assesed by plate counting

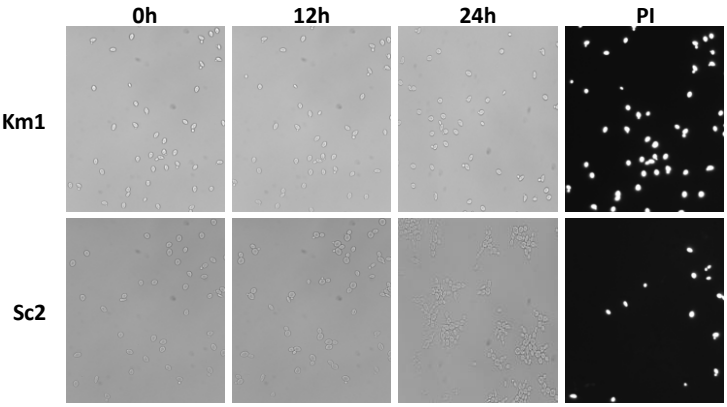
- Acetic acid and the combinations of stress conditions decreased survival of all strains
- *Klyuveromyces marxianus* was the most sensitive species compared to the other yeast species
- Both *S. cerevisiae* and *K. marxianus* showed slight differences in sensitivity between the three strains



Ability of yeast cells to recover from the stress damages determined by fluorescent microscopy

98% of stressed cells of Km1 had acidic pH_i ≤5.0

38% of stressed cells of Sc2 maintained pH_i between 6 and 7.4



- 100% of Km1 cells were membrane permeable and did not grow
- 46.5% of Sc2 cells maintained membrane integrity and resumed proliferation after 3-24h

Conclusions and perspectives

- The combined approach of flow cytometry and fluorescent microscopy provided qualitative and quantitative information in near real time on the sensitivity between and within species and strains
- The sensitivity of examined yeasts was species, strain and cell dependent
- *S. cerevisiae* strains were less sensitive followed by *P. kudriavzevii*, while *C. glabrata* and *K. marxianus* were more sensitive
- pHi and plasma membrane integrity were found to be inter-related physiological parameters, which together determine yeast cell sensitivity, viability and culturability
- There is the need for optimizing the fermentation process in order to change the environment of fermented dough in favor of *K. marxianus* strains as well as other less resistant *S. cerevisiae* strains

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