



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

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Objectives

To get an understanding of how various stress factors in fermented cereal doughs influence the growth and survival of the predominant yeast species and to discover differences in sensitivity at species and strain levels

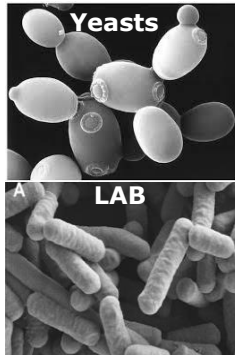


Cereal dough fermentation in West Africa

Spontaneous fermentation lasting 24-72h



Involvement of yeasts and lactic acid bacteria



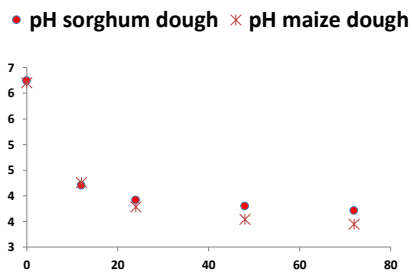
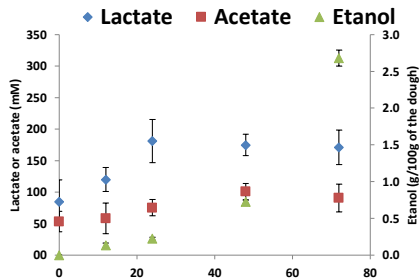
Production of alcohols and organic acids

⇒ Ethanol

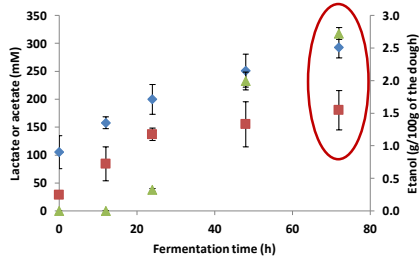
⇒ Lactic acid and acetic acid

Stress factors of fermented cereal doughs

Sorghum dough



Maize dough



Most stressful condition

pH 3.4
 Total lactate 285 mM
 Total acetate 150 mM
 Ethanol 3%

Six stress conditions and one non-stress condition were defined

Abbreviation	Definition	Preparation
pH 5.6	Non-stress condition	MYGP medium, pH 5.6
pH 3.4	Low pH stress	MYGP medium, pH 3.4
EtOH _{pH3.4}	Ethanol stress	MYGP medium with ethanol 3% (v/v), pH 3.4
LA _{pH3.4}	Lactic acid stress	MYGP medium with 285 mM lactic acid, pH 3.4
AA _{pH3.4}	Acetic acid stress	MYGP medium with 150 mM acetic acid, pH 3.4
(LA+AA) _{pH3.4}	Combination of lactic and acetic acid stresses	MYGP medium with 285 mM lactic acid and 150 mM acetic acid, pH 3.4
(LA+AA+EtOH) _{pH3.4}	Combination of lactic, acetic acid and ethanol stresses	MYGP medium with 285 mM lactic acid, 150 mM acetic acid and ethanol 3% (v/v), pH 3.4

Twelve yeast strains were tested

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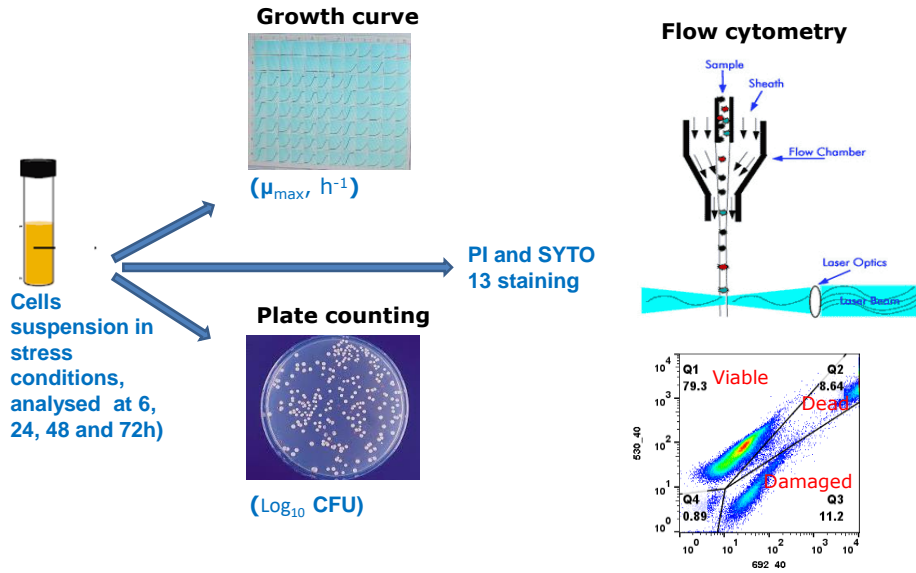
Occurrence of lactic acid bacteria and yeasts at species and strain level during spontaneous fermentation of mawè, a cereal dough produced in West Africa



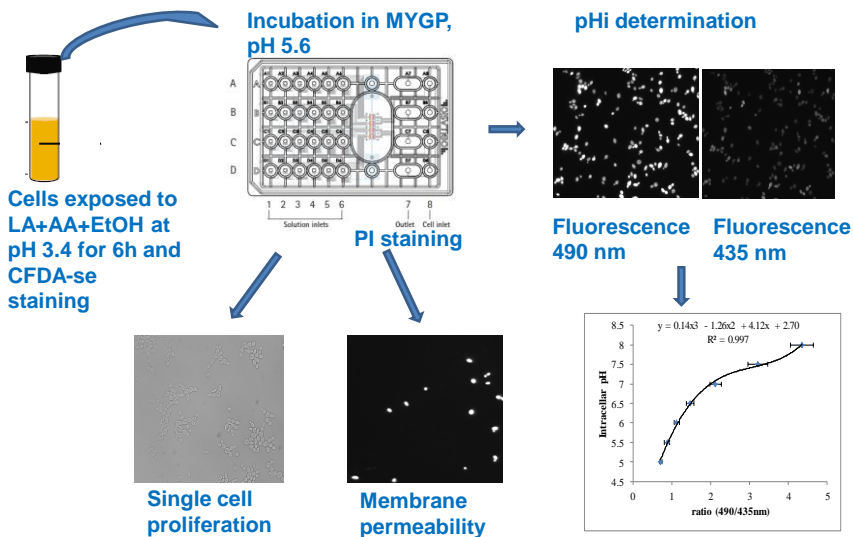
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Isolate	Identity	Isolate source (cereal dough and fermentation duration)	NCBI GenBank accession no
Sc1	<i>Saccharomyces cerevisiae</i>	Undehulled maize mawè 36h	MG245859
Sc2	<i>Saccharomyces cerevisiae</i>	Commercial maize mawè onset	MG245839
Sc3	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

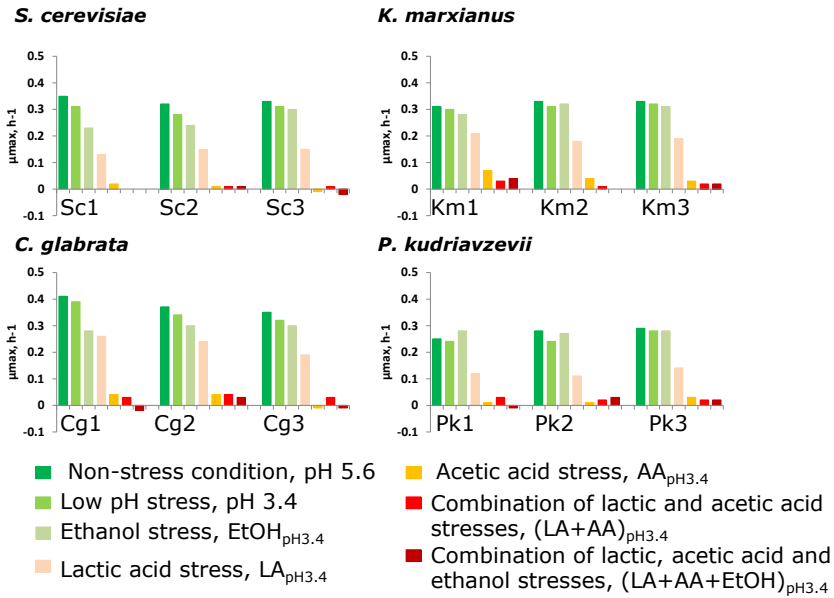
Growth and viability assessment



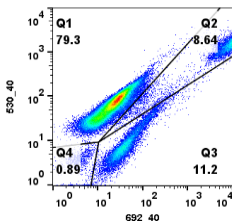
pHi, membrane permeability and micro colony formation of stressed single cells with fluorescent microscopy



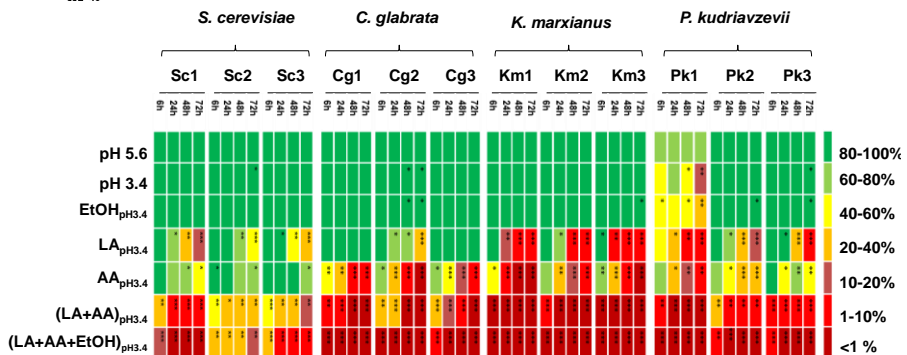
Maximum specific growth rate (μ_{max} , h⁻¹)



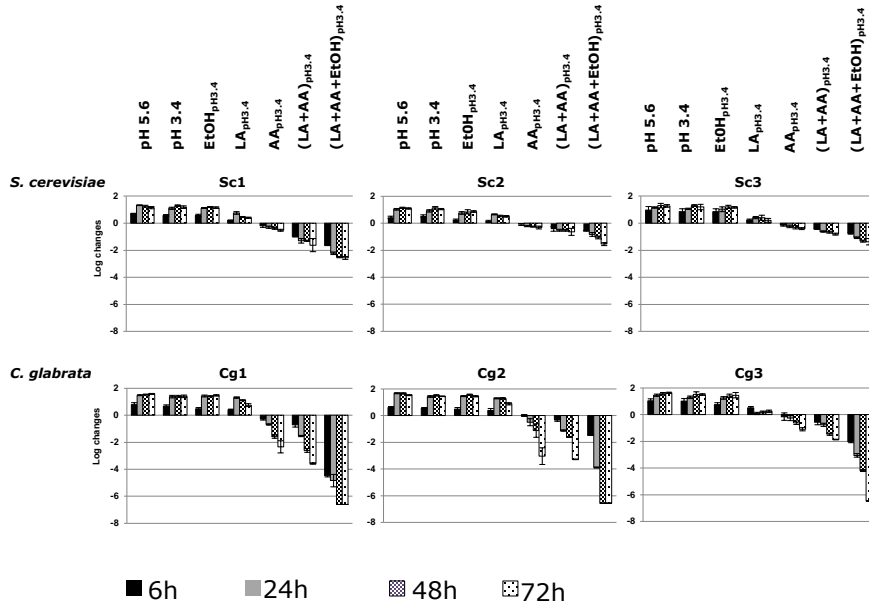
Viability as determined by flow cytometry



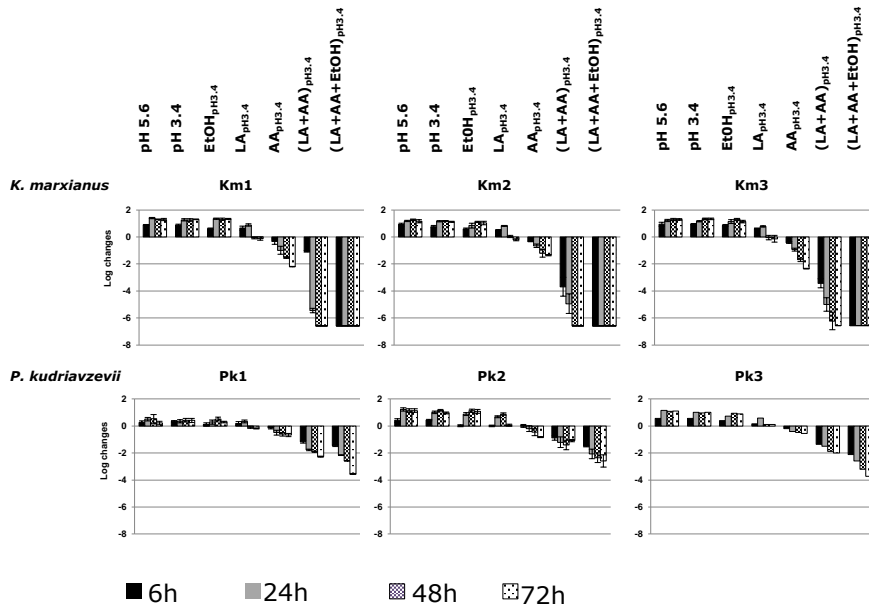
Q1 Viable cells with intact membrane (SYTO 13 fluorescent)
 Q2 Dead cells (PI fluorescent)
 Q3 Intermediate state with damaged cell membrane (SYTO and PI fluorescent)
 Q4 weakly stained cells (No fluorescent)



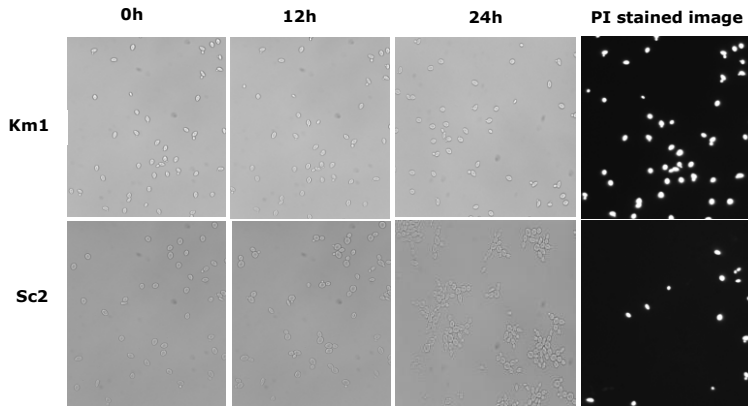
Viability as determined by plate counting



Viability as determined by plate counting



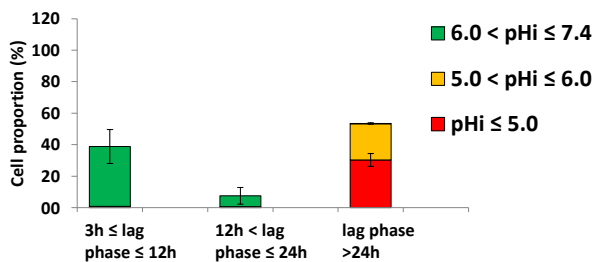
Micro colony formation and membrane permeability of stressed single cell



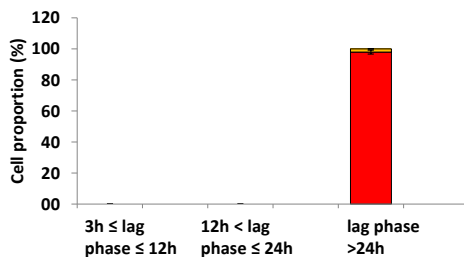
100% of Km1 cells were membrane permeable and did not grow

46.5 % of Sc2 cells maintained membrane integrity and resumed proliferation after 3 -24h

Intracellular pH and lag phase of stressed single cells transferred in non-stress condition



38% of stressed cells of Sc2 maintained pHi between 6 and 7.4 and resumed proliferation between 3-24h



98% of stressed cells of Km1 had acidic pHi ≤ 5.0 and did not proliferate till 24h in non-stress condition

Take home messages

The most important stressful factors in West African fermented cereal doughs are lactic acid, acetic acid and ethanol

Acetic acid is the most stressful factor and the combination with lactic acid and ethanol is even more toxic to yeast cells

S. Cerevisiae strains were the less sensitive following by *P. kudriavzevii*, while *C. glabrata* and *K. marxianus* were more sensitive

38% of resistant cells of *S. cerevisiae* could maintain pH_i to physiological range and could also maintain plasma membrane integrity

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