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Yeast diversity and susceptibility to organic acids during spontaneous fermentation of mawè, a cereal-based dough produced in West Africa

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Introduction

Mawè is a cereal dough prepared by spontaneous fermentation lasting 12-24 h (non-acidic mawè) or 48-72 h (sour mawè). The fermentation is dominated by adventitious LAB and yeasts species, as well as some opportunistic pathogenic yeasts. The ability of these microorganisms to survive under the ecological environment of mawè is of great interest regarding the safety and quality of mawè based food. Sour mawè is characterized by a low pH of 3.3, an ethanol content of 3% (v/w) as well as high lactic acid and acetic acid contents of 288 µM and 150 µM, respectively. Such an environment could be a relative hostile medium for both beneficial and pathogenic microorganisms. This study will investigate the yeast diversity of mawè and the survival of yeasts at the ecological environment of sour mawè.

Materials and methods

Yeast identification

A total of 333 yeasts were isolated from eight different mawè samples, and isolates were grouped by $(GTG)_5$-based repetitive PCR followed by D1/D2 domain of the 26S rRNA gene sequencing.

Yeast strains and stressful conditions

Three strains of *Candida glabrata* (CG1, CG2 and CG3) and two strains of *Saccharomyces cerevisiae* (SC1 and SC2) were tested under six different stressful conditions of sour mawè. The stressful conditions are defined as follow: MYGP lowered to pH 3.3 by HCl (**pH 3.3**), MYGP pH 3.3 containing 3% (v/v) of ethanol (**EtOH**), MYGP pH 3.3 containing total lactic acid of 288 µM (**LA**), MYGP pH 3.3 containing total acetic acid of 150 µM (**AA**), MYGP pH 3.3 containing total lactic acid of 288 µM and total acetic acid of 150 µM (**LA+AA**), and MYGP pH 3.3 containing total lactic acid of 288 µM plus total acetic acid of 150 µM plus 3% (v/v) of ethanol (**LA+AA+EtOH**).

Determination of growth performance and survival

Growth performance was assessed in 96 wells microplate by measuring OD_{600} using a VarioskanTM Flash for 24 h and growth parameters were determined using the DMFiT software.

Physiological states of cells were accessed by fluorescent staining with SYTO 13 and propidium iodide (PI) followed by flow cytometric analysis using a BD FACS Jazz cell Sorter with an 488 nm argon ion laser.

Culturability of cells after exposure to the above mentioned stress conditions was assessed by CFU count on MYGP agar, pH 5.6 incubated at 25ºC for up to 72 h. Log reduction was defined as the difference between the log CFU counts at 0 h and at the end of exposure time (6 h, 12 h, 24 h, 48 h and 72 h) .

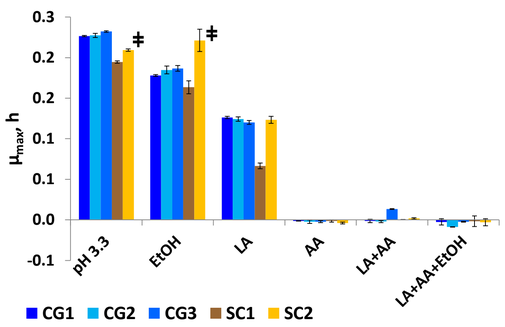

Fig. 1. Maximum specific growth rate (μ_{max}, h) of three strains of *C. glabrata* (CG1, CG2, and CG3) and two strains of *S. cerevisiae* (SC1 and SC2) grown in MYGP under different stressful conditions. The mean values and SD (bars) were calculated from two biological replicates. ‡ denotes no statistical significant difference (P = 0.05).

Results

Yeast diversity

Table 2 shows the yeast diversity in mawè. *P. kudriavzevii*, *K. marxianus* and *S. cerevisiae* were the dominant species. Though *C. glabrata* was detected at low incidence (1%), it is preferable that *C. glabrata* is absent, as this species is considered as an opportunistic pathogen. Therefore, we investigated the growth performance and the survival of *C. glabrata* under the stressful condition of mawè. For comparison, strains of *S. cerevisiae* were included in the study.

Table 2. Yeast diversity of mawè

Group	Identity	Number of isolates	Percentage
I	*P. kudriavzevii*	221	66%
II	*K. marxianus*	80	24%
III	*S. cerevisiae*	21	6%
IV	*O. polymorpha*	7	2%
V	*C. glabrata*	4	1%

Growth performance

All the three strains of *C. glabrata* and the two strains of *S. cerevisiae* grew well at low pH (pH 3.3). For all the strains, growth occurred at 3% (v/v) of ethanol (EtOH) and at 288 µM of total lactic acid (LA), but these two treatments exhibited significant increase of lag phase time (λ) (data not shown). Moreover, the two treatments induced significant decrease of maximum specific rate (μ_{max}) for all strains; except for SC2 which was not affected by EtOH. No growth was observed for all the tested strains at 150 µM of total acetic acid (AA), at 288 µM of total lactic acid plus 150 µM of total acetic acid (AA+LA) and at 288 µM of total lactic acid plus 150 µM of total acetic acid plus 3% (v/v) of ethanol (LA+AA+EtOH). These three treatments resulted in negative or zero values of μ_{max} for all the tested strains showing total growth inhibition. These results show that the exposures to AA, LA+AA and LA+AA+EtOH prevented cells in growing but viable cells would still be present in different physiological states (**Fig. 1**).

Physiological state

The physiological state of exposed cells of CG1 and SC1 was investigated by flow cytometric analysis. Four subpopulations were found: viable cells with intact cell membrane (Q1), intermediate cells with damaged cell membrane (Q2), dead cells (Q3) and unstained cells (Q4) **(Fig. 2).** Data of the density plot images of Fig. 2 were used to calculate the cell proportions of each subpopulation and results are shown in **Fig. 3.** For *C. glabrata* (CG1), pH 3.3, EtOH and LA did not significantly influence the proportion of viable cells. However, significant effects were observed for AA, LA+AA and LA+AA+EtOH. The same observation was found for *S. cerevisiae* (SC1) under pH 3.3, EtOH, LA+AA and LA+AA+EtOH.

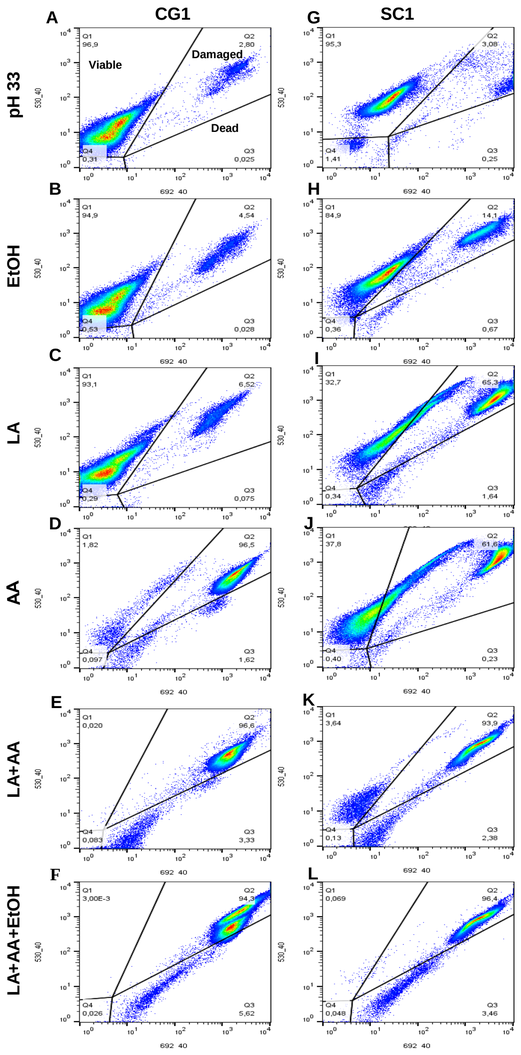

Fig. 2. Density plot images of *C. glabrata* (A–F) and *S. cerevisiae* (G–L) exposed to pH 3.3, EtOH, LA, AA, LA+AA, and LA+AA+EtOH in MYGP for 72 h and analyzed by flow cytometry. The SYTO 13 signal was recorded in the green channel (530_40), the PI signal was recorded in the red channel (692_40). Events in different quadrants correspond to different populations: viable cells (Q1), intermediate population with damaged cell membranes (Q2), dead cells (Q3) and weakly stained cells (Q4), The number in each quadrant represents the relative percentage of each population.

In general, more damaged cells and dead cells were recorded for *C. glabrata* (CG1) compared to *S. cerevisiae* (SC1) for AA, LA+AA and LA+AA+EtOH. Surprisingly, by the flow cytometric analysis, *S. cerevisiae* (SC1) seemed to be more affected by LA than AA. However, only one strain of *S. cerevisiae* was investigated in this case.

Flow cytometric results did not provide whether the damaged cells could get repaired and survive when the ecological environment become better. Therefore, CFU counts were assessed in order to evaluate the culturability of exposed cells.

Culturability of exposed cells

Fig 4 shows the log reduction during the exposure time of 6 h to 72 h for AA, LA+AA and LA+AA+EtOH. The initial count was 6.5 ± 0.08 log CFU. In general, *S. cerevisiae* appeared to be more stress tolerant than *C. glabrata*. For both species, the lowest log reduction was found for AA followed by LA+AA. As expected, LA+AA+EtOH resulted in the highest log reduction for both species. This treatment induced 100% log reduction for CG1 after 48 h, showing that CG1 cells exposed to LA+AA+EtOH for 48 h could not get repaired and divide on normal MYGP after 72 h of incubation. 39% log reduction was recorded for SC1 after 72 h exposure to LA+AA+EtOH, showing that 61% of SC1 cells were able to survive after 72 h exposure to LA+AA+EtOH.

The flow cytometric analysis fitted well with the determination of μ_{max} in liquid media. However, no clear correlation was found between the CFU counts on solid media and the flow cytometric analyses.

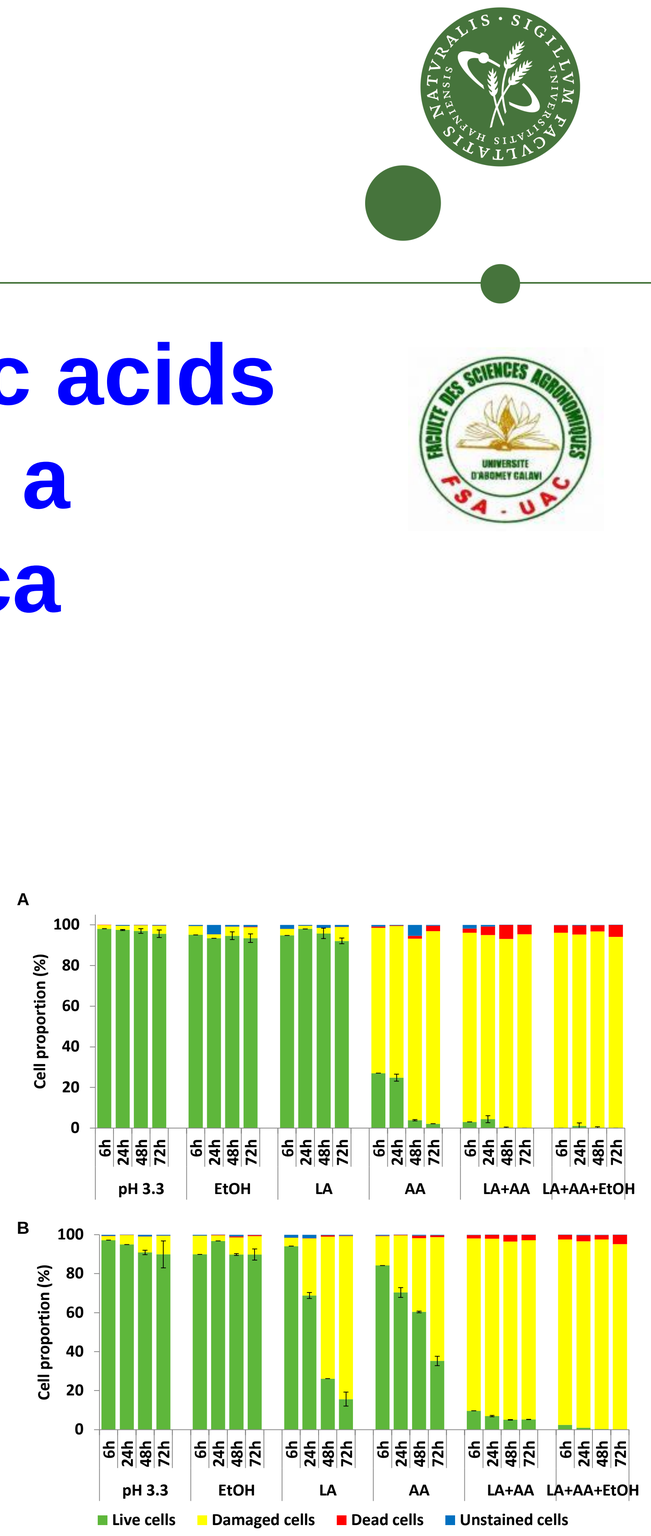

Fig. 3. The proportions of viable cells, damaged cells, dead cells and unstained cells of *C. glabrata* (CG1) (A) and *S. cerevisiae* (SC1) (B) calculated using dada of the density plot images from flow cytometric analysis. Examples of density plot images are shown in Fig. 2. The mean values and SD (bars) were calculated from two biological replicates.

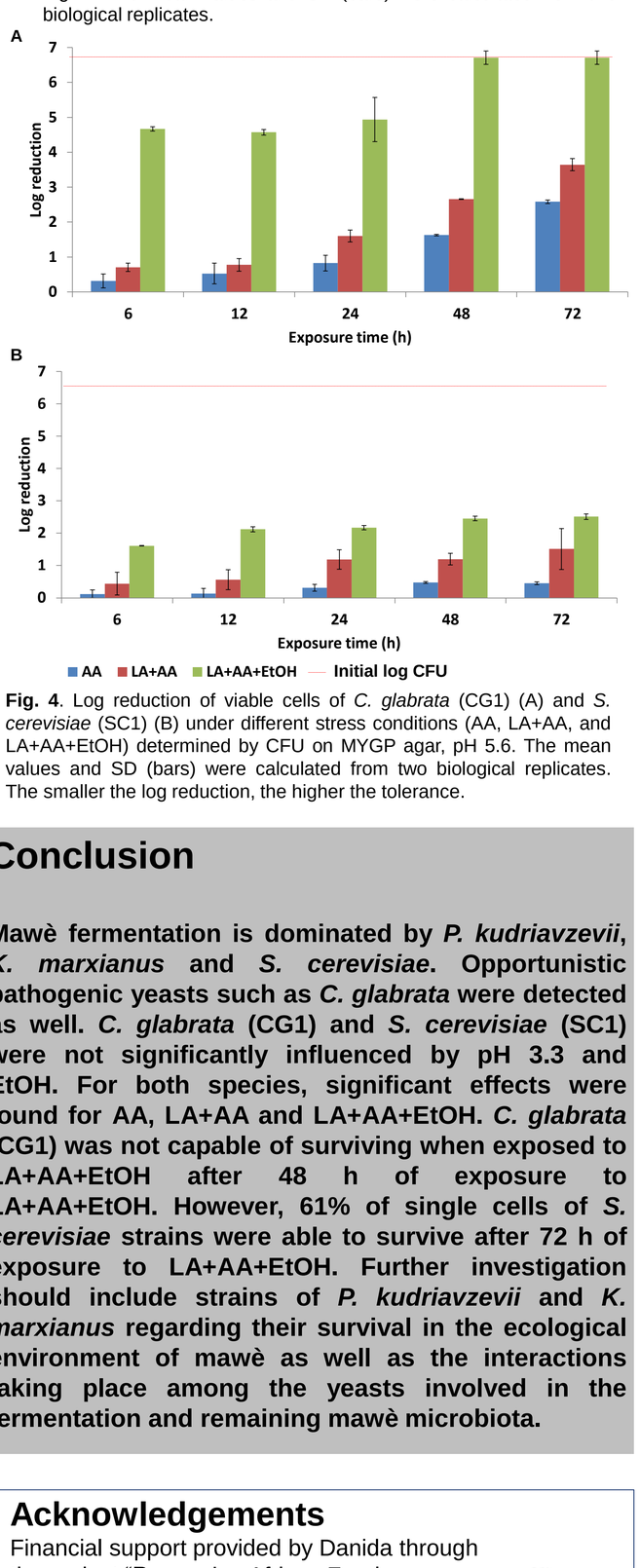

Fig. 4. Log reduction of viable cells of *C. glabrata* (CG1) (A) and *S. cerevisiae* (SC1) (B) under different stress conditions (AA, LA+AA, and LA+AA+EtOH) determined by CFU on MYGP agar, pH 5.6. The mean values and SD (bars) were calculated from two biological replicates. The smaller the log reduction, the higher the tolerance.

Conclusion

Mawè fermentation is dominated by *P. kudriavzevii*, *K. marxianus* and *S. cerevisiae*. Opportunistic pathogenic yeasts such as *C. glabrata* were detected as well. *C. glabrata* (CG1) and *S. cerevisiae* (SC1) were not significantly influenced by pH 3.3 and EtOH. For both species, significant effects were found for AA, LA+AA and LA+AA+EtOH. *C. glabrata* (CG1) was not capable of surviving when exposed to LA+AA+EtOH after 48 h of exposure to LA+AA+EtOH. However, 61% of single cells of *S. cerevisiae* strains were able to survive after 72 h of exposure to LA+AA+EtOH. Further investigation should include strains of *P. kudriavzevii* and *K. marxianus* regarding their survival in the ecological environment of mawè as well as the interactions taking place among the yeasts involved in the fermentation and remaining mawè microbiota.

Acknowledgements

Financial support provided by Danida through the project “Preserving African Food Microorganisms for Green Growth” (DFC No 13-04 KU) is gratefully acknowledged.

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Poster presented at the 33rd International Specialised Symposium on Yeast (ISSY33), Cork, Ireland, 2017