UNIVERSITE D'ABOMEY-CALAVI

FACULTE DES SCIENCES AGRONOMIQUES

Preserving African Food Microorganisms for Green Growth
(Green Growth for Africa)

Report of

Test of starter cultures of relevant QPS microorganisms with optimal technological properties in fermentation trials

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Introduction

In the frame of the research project funded by DANIDA entitled "*Preserving African Food Microorganisms for Green Growth*", partners, including the University of Abomey-Calavi (UAC) in Benin, have already isolated microorganisms from their traditional foods and stored in biobanks. One of the objectives of the research project was to select promising microorganisms in order to develop multifunctional starter culture that will be transferred to local small and medium enterprises (SMEs) in food sector. Hence, selected strains of lactic acid bacteria and yeasts were investigated for their ability to growth under low pH acid condition mimicking the condition of fermented cereal dough (mawè), ability to ferment mawè (rapid acidification), ability to inhibit the growth of opportunistic pathogen *Candida glabrata* and ability to improve free amino acid content during mawè production. Furthermore, the QPS status of the promising strains was investigated and the development of starter culture using the relevant QPS microorganisms and test of the defined starter culture in fermentation trails are in progress.

Microorganisms examined

From the lactic acid bacteria (n=321) and yeasts (n=298) isolated during the fermentation of four mawè types in rural and urban areas in Benin (Houngbédji *et al*., 2018), representative strains have been selected and tested for their technological property and QPS status. A complete list of the examined strains is provided in table 1.

Table 1: List of examined strains

		Identity	Isolate source*	Strain code*	NCBI GenBank accession no*
LAB	Lf1	*Lactobacillus fermentum*	Commercial maize mawè, fermented for 36h	C2-36-L5	MG245812
	Lf2	*Lactobacillus fermentum*	Commercial maize mawè, fermented for 6h	C2-6-L3	MG245804
	Lf3	*Lactobacillus fermentum*	Homemade maize mawè, fermented for 24h	H2-24-L1	MG245801
	Lf4	*Lactobacillus fermentum*	Commercial sorghum mawè, at onset of fermentation	S2-0-L7a	MG245806
	Lf5	*Lactobacillus fermentum*	Undehulled maize mawè, fermented for 12h	Cm2-12-L5	MG245798
	Lp1	*Lactobacillus plantarum*	Commercial sorghum mawè, fermented for 24h	S1-0-L10a	MG245815
	Lp2	*Lactobacillus plantarum*	Homemade maize mawè, at onset of fermentation	S1-0-L7a	MG245814
	Wc1	*Weissella confusa*	Commercial sorghum mawè, fermented for 24h	C1-0-L5a	MG245793
	Wc2	*Weissella confusa*	Commercial maize mawè, fermented for 36h	H2-6-L8	MG245791
	Wc3	*Weissella confusa*	Commercial maize mawè, at onset of fermentation	H2-0-L2	MG245794
	Pp1	*Pediococcus pentasaceus*	Homemade maize mawè, fermented for 36h	S2-24-L4	-
	Pp2	*Pediococcus pentasaceus*	Homemade maize mawè, at onset of fermentation	H2-0-L3	MG245820
	Pa1	*Pediococcus acidilactici*	Commercial sorghum mawè, at onset of fermentation	S2-24-L7	MG245817
	Pa2	*Pediococcus acidilactici*	Commercial sorghum mawè, at onset of fermentation	C1-36-L4a	MG245819
Yeast	Pk1	*Pichia kudriavzevii*	Homemade maize mawè, fermented for 6h	Cm2-6-Y4	MG245854
	Pk2	*Pichia kudriavzevii*	Commercial maize mawè, at onset of fermentation	C2-0-Y6	MG245829

Pk3	*Pichia kudriavzevii*	Commercial maize mawè, fermented for 36h	C2-36-Y3	MG245856
Pk4	*Pichia kudriavzevii*	Homemade maize mawè, fermented for 24h	Cm2-24-Y4	MG245852
Pk5	*Pichia kudriavzevii*	Homemade maize mawè, at onset of fermentation	H1-0-Y5	MG245834
Pk6	*Pichia kudriavzevii*	Commercial sorghum mawè, fermented for 6h	S2-6-Y4	MG245830
Km1	*Kluyveromyces marxianus*	Commercial sorghum mawè, fermented for 6h	S1-6-Y4a	MG245824
Km2	*Kluyveromyces marxianus*	Commercial maize mawè, fermented for 36h	C1-36-Y2	MG245845
Km3	*Kluyveromyces marxianus*	Commercial maize mawè, at onset of fermentation	C1-0-Y6	MG245826
Sc1	*Saccharomyces cerevisiae*	Undehulled maize mawè, fermented for 36h	Cm1-36-Y6	MG245858
Sc2	*Saccharomyces cerevisiae*	Undehulled maize mawè, fermented for 36h	Cm2-36-Y11	MG245859
Sc3	*Saccharomyces cerevisiae*	Commercial maize mawè, at onset of fermentation	C2-0-Y2	MG245839
Cg1	*Candida glabrata*	Commercial maize mawè, fermented for 6h	C2-6-Y2	MG245841

*Houngbédji *et al*. (2018)

Preparation of decontaminated mawè flour used in fermentation trials

A commonly consumed maize variety (small grain and white color) purchased in Dantokpa market in Benin was used for preparation of fresh dough (mawè) samples. Sorted and wet cleaned maize grains were soaked in ethanol solution (70%, v/v) for 05 min and rinsed with sterilized distilled water in order to reduce the microbial flora of the raw material. The decontaminated maize grains were grounded and immediately milled into fresh mawè (pH = 6.5 ± 0.45, water content = 41 ± 1.8 %). The fresh mawè was dried at 48-50 °C for 4-5 h until it reached <10% water content. The resulted dried mawè was then decontaminated by autoclaving at 110°C for 20 min, which decreased the endogenous flora to <2.5 log cfu per g. The decontaminated mawè was inoculated with single culture of the examined LAB and yeast strains.

Acidification ability

As seen in Table 2 lactic acid bacteria had strong ability to reduce pH during mawè fermentation. All the *L. fermentum* strains yielded pH < 4 after only 12 h of fermentation, except Lf4 for which the pH of mawè at 12 h was slightly higher (4.1± 0.15). Contrary, *W. confusa* strains had lowest acidification ability among LAB species. The ability of yeast strains to decrease pH is significantly lower compared with LAB strains.

Table 2: pH changes during fermentation of mawè by single strains of LAB and yeast species

			0h	12h	24h	48h	72h
LAB	*L. fermentum*	Lf1	6.2 ± 0.15^{a}	3.9 ± 0.03^{abC}	3.8 ± 0.15^{cdB}	3.6 ± 0.08^{deA}	3.6 ± 0.08^{cA}
		Lf2	6.2 ± 0.15^{a}	3.9 ± 0.15^{aC}	3.7 ± 0.07^{abB}	3.6 ± 0.02^{bcAB}	3.5 ± 0.13^{bA}
		Lf3	6.2 ± 0.15^{a}	3.9 ± 0.04^{abB}	3.9 ± 0.05^{deB}	3.7 ± 0.05^{efA}	3.6 ± 0.11^{cA}
		Lf4	6.2 ± 0.15^{a}	4.1 ± 0.15^{cdC}	3.8 ± 0.06^{cdB}	3.6 ± 0.01^{bbdA}	3.6 ± 0.02^{bA}
		Lf5	6.2 ± 0.15^{a}	3.9 ± 0.17^{aB}	3.6 ± 0.14^{aA}	3.4 ± 0.10^{aA}	3.4 ± 0.07^{aA}

	L. plantarum	Lp1	6.2 ± 0.15^{a}	4.0 ± 0.05^{abC}	3.6 ± 0.12^{aB}	3.6 ± 0.06^{bAB}	3.5 ± 0.04^{bA}
		Lp2	6.2 ± 0.15^{a}	4.1 ± 0.12^{cdC}	3.7 ± 0.08^{bcB}	3.6 ± 0.07^{bdBA}	3.6 ± 0.05^{bcA}
	W. confusa	Wc1	6.2 ± 0.15^{a}	4.3 ± 0.04^{eC}	4.2 ± 0.00^{gB}	4.1 ± 0.02^{iAB}	4.1 ± 0.04^{fA}
		Wc2	6.2 ± 0.15^{a}	4.2 ± 0.03^{deC}	4.1 ± 0.01^{ghB}	4.0 ± 0.01^{hA}	4.0 ± 0.02^{eA}
		Wc3	6.2 ± 0.15^{a}	4.1 ± 0.02^{cdB}	4.0 ± 0.01^{fgA}	4.0 ± 0.01^{hA}	4.0 ± 0.01^{eA}
	P. pentosaceus	Pp1	6.2 ± 0.15^{a}	4.2 ± 0.06^{deC}	3.8 ± 0.01^{cdB}	3.7 ± 0.01^{fA}	3.7 ± 0.01^{dA}
		Pp2	6.2 ± 0.15^{a}	4.2 ± 0.04^{deD}	3.9 ± 0.02^{efC}	3.8 ± 0.02^{gB}	3.7 ± 0.02^{dA}
	P. acidilactici	Pa1	6.2 ± 0.15^{a}	3.9 ± 0.03^{abB}	3.8 ± 0.02^{cdB}	3.6 ± 0.06^{bcdA}	3.5 ± 0.04^{bA}
		Pa2	6.2 ± 0.15^{a}	4.0 ± 0.09^{bcC}	3.8 ± 0.01^{cdBC}	3.6 ± 0.03^{bbdAB}	3.5 ± 0.04^{bA}
Yeasts	*P. kudriavzevii*	Pk1	6.3 ± 0.20^{a}	5.6 ± 0.31^{bC}	4.4 ± 0.04^{aB}	4.2 ± 0.06^{dAB}	4.0 ± 0.22^{bA}
		Pk2	6.3 ± 0.20^{a}	4.3 ± 0.05^{dC}	4.2 ± 0.11^{aBC}	4.0 ± 0.14^{aAB}	4.0 ± 0.18^{bA}
		Pk3	6.3 ± 0.20^{a}	5.7 ± 0.15^{cC}	4.5 ± 0.08^{fB}	4.2 ± 0.12^{dA}	4.1 ± 0.14^{aA}
		Pk4	6.3 ± 0.20^{a}	5.9 ± 0.24^{hC}	4.6 ± 0.10^{bB}	4.0 ± 0.06^{aA}	$4,0 \pm 0.15^{bA}$
		Pk5	6.3 ± 0.20^{a}	4.9 ± 0.75^{eB}	4.3 ± 0.06^{aA}	4.2 ± 0.11^{dA}	$4,0 \pm 0.19^{bA}$
		Pk6	6.3 ± 0.20^{a}	5.7 ± 0.05^{bC}	$4.3 \pm 0.11aA$	4.2 ± 0.11^{dA}	4.0 ± 0.06^{bA}
	K. marxianus	Km1	6.3 ± 0.20^{a}	6.0 ± 0.04^{iD}	4.6 ± 0.10^{bC}	4.1 ± 0.22^{dB}	3.9 ± 0.19^{cA}
		Km2	6.3 ± 0.20^{a}	5.8 ± 0.10^{gD}	4.7 ± 0.36^{cC}	4.3 ± 0.05^{eB}	3.9 ± 0.06^{bA}
		Km3	6.3 ± 0.20^{a}	5.6 ± 0.35^{fC}	4.7 ± 0.56^{cB}	4.3 ± 0.07^{eAB}	4.1 ± 0.11^{aA}
	S. cerevisiae	Sc1	6.3 ± 0.20^{a}	5.2 ± 0.16^{aC}	4.8 ± 0.10^{dB}	4.5 ± 0.19^{fAB}	4.4 ± 0.12^{eA}
		Sc2	6.3 ± 0.20^{a}	$5,3 \pm 0.09^{aB}$	5.2 ± 0.14^{gAB}	4.9 ± 0.19^{gAB}	4.8 ± 0.52^{gA}
		Sc3	6.3 ± 0.20^{a}	$5,3 \pm 0.12^{aB}$	5.1 ± 0.09^{gAB}	4.6 ± 0.17^{gAB}	4.4 ± 0.10^{gA}

Antifungal effect against opportunistic pathogenic yeast *Candida glabrata*

Antifungal effect of the LAB strains against *C. glabrata* was assessed. For each LAB species, result of the strain exhibiting the highest antifungal effect is showed in Figure 1. All the tested LAB strains are able to delay the growth of *C. glabrata*. The highest antifungal effect is observed for *W. confusa* strain Wc2 followed by *L. fermentum* strain Lf2. These two strains could be included in the development of starter culture for safe mawè fermentation.

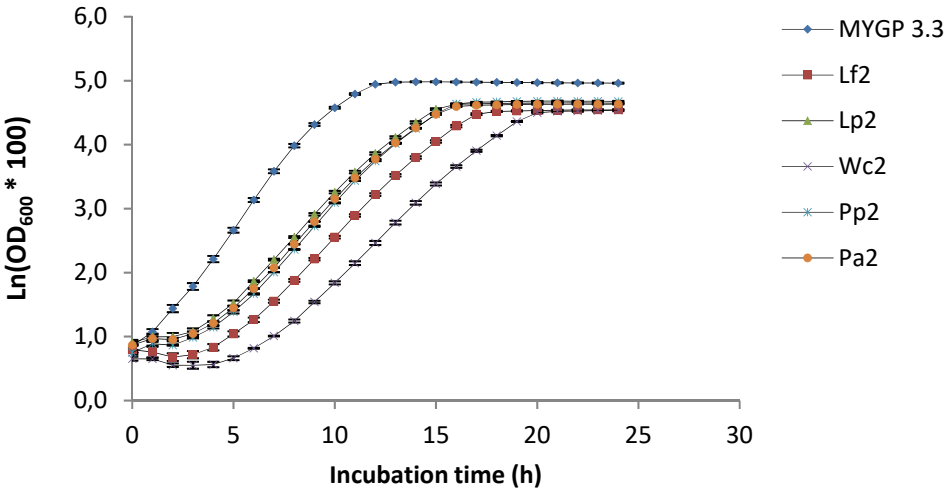

Figure 1: Growth inhibition of *C. glabrata* strain (Cg1) growing in MYGP, pH 3.3 supplemented with supernatant of single culture of LAB strains. Only the result of the strain exhibiting the highest antifungal effect in each LAB species is shown.

Ability to release free amino acid

As shown in figure 2, LAB strains did not contribute to free amino acid content during mawè fermentation except *W. confusa* strains which increased free proline content during fermentation. Contrary, yeast strains showed high free amino acid release ability except the *S. cerevisiae* strains and *P. kudriavzevii* strain Pk4. The highest free amino acid release ability was found for *P. kudriavzevii* Pk3, though Pk1, Pk2, Pk6 as well as *K. marxianus* strain Km3 also improved markedly free amino acid content during mawè fermentation.

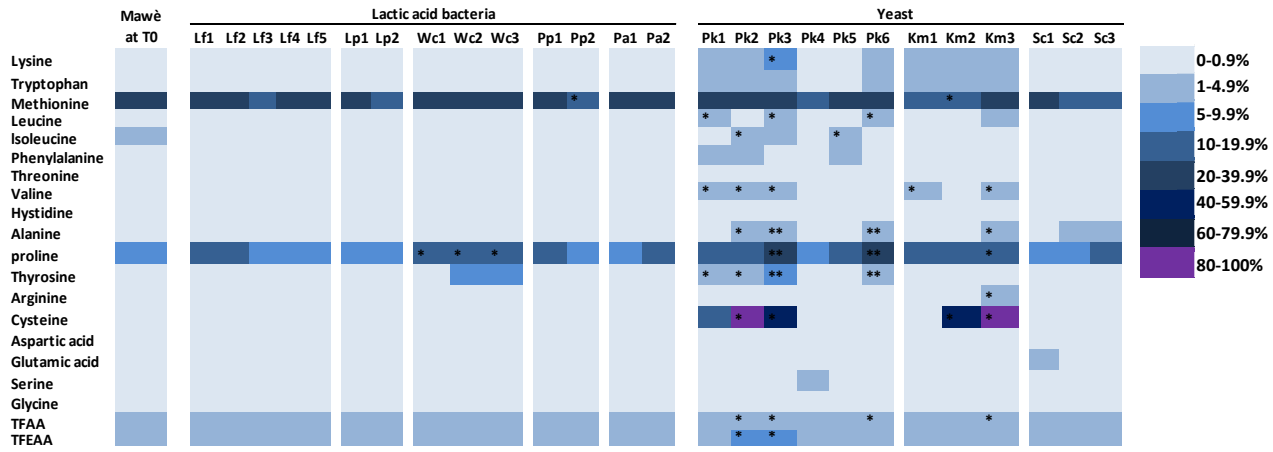

Figure 2: Proportion of free amino acid (%) released at 0 h (Mawè T0) and at 48 h of mawè fermentation using single culture of LAB and yeasts strains.

QPS status of promising strains

We aimed at developing a defining multifunctional starter culture containing at least one strain from each species involved in spontaneous fermentation of mawè. Based on above results, we evaluated QPS status of two or three strains of each species that showed the most interesting multifunctional properties. For LAB strains, the QPS analysis were evaluated by testing resistance to five antibiotics including ampicillin, chloramphenicol, erythromycin, streptomycin and tetracycline and results are presented in table 3. The experiments for yeast strains are in progress. Almost all the LAB strains examined were susceptible to ampicillin, chloramphenicol, erythromycin and tetracycline. However, these strains had phenotypic resistance to streptomycin except strains Lf2, Lf3 and Wc2. Based on the results, Lf2, Lp2, Wc2 and Pp2 could be selected for the preparation of a defined multifunctional starter. However, the absence of transferability to pathogenic microorganisms of the erythromycin and tetracycline resistance of Wc2, streptomycin resistance of Pp2 and Pa2 need to be demonstrated and experiments are therefore in progress.

Table 3: Minimum inhibitory concentration (MIC; in µg/ml) of five antibiotics against the LAB strains. Number in brackets indicates the Break Point (BP) level (in µg/ml) inherent the species for the tested antibiotic. Strains with MICs higher than the BP are considered as resistant (EFSA, 2007). N.r, Not required (EFSA, 2007).

		Ampicillin	Chloramphenicol	Erythromycin	Streptomycin	Tetracycline
L. fermentum	Lf1	<1 (2)	16 (4)	1 (1)	512 (64)	8 (8)
	Lf2	<1	<1	<1	64	8
	Lf3	1	<1	<1	64	4
L. plantarum	Lp1	<1 (2)	4 (8)	2 (1)	512 n.r	32 (32)
	Lp2	1	8	1	512	16
W. confusa	Wc1	<1 (1)	<1 (2)	<0.5 (0.5)	32 (8)	2 (2)
	Wc2	<1	2	1	4	8
P. pentasaceus	Pp1	1 (4)	<1 (4)	1 (1)	128 (64)	8 (8)
	Pp2	2	<1	<1	512	4
P. acidilactici	Pa1	<1 (4)	<1 (4)	2 (1)	512 (64)	8 (8)
	Pa2	<1	<1	<1	512	<1

Fermentation trials with the relevant QPS starter culture

Experiments will be conducted to evaluate the fermentation ability and technological performance of the defined starter culture which will be developed using relevant QPS LAB strains, as e.g. Lf2, Lp2, Wc2 and Pp2 and yeasts strains with promising multifunctional properties.

Conclusion

This report summarizes multifunctional property and QPS status of LAB and yeasts involved in cereal dough (mawè) fermentations in Benin. The relevant QPS microorganisms with promising multifunctional property are selected and will be used for the preparation of a defined multifunctional and safe starter culture that could be used at SMEs level for fermented foods production.

References

Houngbédji, M., Johansen, P., Padonou, S. W., Akissoé, N., Arneborg, N., Nielsen, D. S., Hounhouigan, D. J. Jespersen, L. (2018). Occurrence of lactic acid bacteria and yeasts at species and strain level during spontaneous fermentation of mawè, a cereal dough produced in West Africa. *Food Microbiology*, 76 267-278.

EFSA. (2007). Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *The European Food Safety Autorithy (EFSA) Journal* 587, 1-16.