Non-*Saccharomyces* yeasts for the fermentation of lignocellulosic biomass - tolerance to inhibitors and fermentation to ethanol

Stefan Ruyters, Vaskar Mukherjee, Kris Willems and Bart Lievens

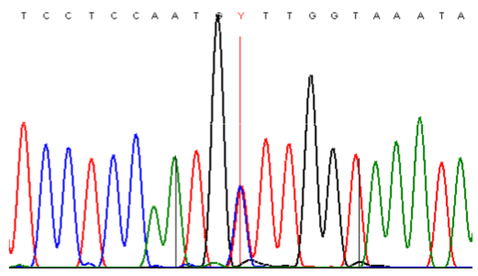
Lab of Process Microbial ecology and Bioinspirational Management (PME&BIM), Cluster of Bioengineering Technology, KU Leuven
Technologiecampus De Nayer, Fortsesteenweg 30A, 2860 Sint-Katelijne-Waver, Belgium

Introduction

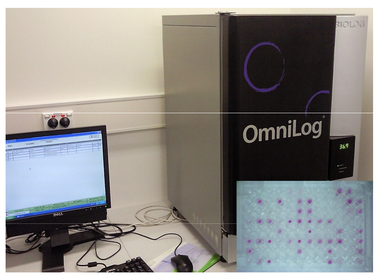
Lignocellulosic biomass has received increasing attention as carbon source for microbial fermentations. Pretreatment of the biomass is needed to liberate sugars with intense treatments yielding higher sugar concentrations (which are economically preferred). However, these intense treatments also result in the **formation of several undesired compounds** in concentrations which may reduce the fermentation efficiency. The main inhibitors are solutes (osmostress), furans (5-hydroxymethylfurfural (HMF), furfural), weak acids (acetic acid, levulinic acid, formic acid) and phenolic and aromatic compounds originating from the lignin fraction (e.g. vanillin). Microorganisms are therefore exposed to a **new, challenging and diverse fermentation medium** due to the large diversity of lignocellulosic biomass sources and hydrolysis conditions. It is therefore required to align the hydrolysate with a microorganism possessing the best characteristics in terms of tolerance to inhibitors and sugar consumption profile.

Technology Platform

Collection of microorganisms
Yeasts isolated from plant nectar and beet sugar thick juice



Identification
PCR and sequencing using primers targeting the D1/D2 domain of the large subunit as taxonomic marker



Two-stage phenotypic screening using Omnilog reader of Biolog
Metabolic activity in the presence of increasing concentrations of glucose, HMF, ethanol and in a second stage weak acids, furfural and vanillin in liquid medium

Fermentation using Eppendorf BioFlo Bioreactors
Ethanol production and sugar consumption of tolerant yeasts in 25% glucose and in the presence of inhibitors at 30° C, 300 rpm and pH 4.5

Results & Conclusions

Species	#	Source	Osmostress (% Glucose) 40	50	55	70	Ethanol (%) 5	7	10	HMF (g l^{-1}) 4	5	6	7
Candida bombi	14	Nectar	62 (3)	35 (2)	23 (1)	24 (2)	52 (3)	27 (2)	0	108 (3)	87 (4)	68 (4)	51 (3)
Hanseniaspora uvarum	5	Nectar	27 (1)	8 (1)	0	0	18 (3)	0	0	12 (2)	0	0	0
Metchnikowia reukauffii	11	Nectar	31 (1)	17 (1)	10 (1)	0	0	0	0	**17** (6)	0	0	0
Starmerella bombicola	9	Nectar	58 (1)	35 (1)	24 (1)	0	18 (2)	**11** (2)	0	62 (9)	25 (8)*	**39** (2)	0
Metchnikowia pulcherrima	2	Soil	75 (1)	36 (5)	37 (2)	9	15 (3)	0	0	132 (31)	13 (0)	0	0
Pichia kudriavzevii	1	Soil	114	0	0	0	116	120	85	71	57	46	39
Citeromyces matritensis	5	Thick juice	76 (3)	51 (5)	36 (4)	12 (2)*	26 (3)*	**19**	0	0	0	0	0
Torulaspora delbrueckii	4	Thick juice (3)	87 (31)	22 (7)	29 (3)	0	57 (5)	36 (4)	**19**	30 (14)*	**22**	**8**	0
Wickerhamomyces anomalus	4	Thick juice	161 (37)	28 (6)	28 (4)	0	80 (10)	59 (7)	32 (4)	69 (15)	24 (9)	11 (4)*	0
Saccharomyces cerevisiae	1	Bioethanol	42	15	0	0	101	102	85	129	33	0	0
Saccharomyces cerevisiae	1	oak	39	17	0	0	94	95	79	87	86	38	15

Table 1: Relative growth of isolates in medium with increasing concentrations of glucose, ethanol or HMF (relative to growth on 2% glucose). Standard error given.

- ***S. cerevisiae*** isolated **from oak** showed **increased tolerance to HMF** and similar tolerance to glucose and ethanol compared to a *S. cerevisiae* strain used in bioethanol production.
- In general **non-*Saccharomyces*** yeasts showed **higher osmotolerance**, **but lower ethanol tolerance** compared to *S. cerevisiae*. One *T. delbrueckii* isolate, *P. kudriavzevii* and 4 *W. anomalus* isolates showed the highest ethanol tolerance.
- ***C. bombi* and *P. kudriavzevii* showed very high HMF tolerance** .

Table 2: Relative growth of selected isolates in medium containing weak acids, furfural or vanillin. Maximal ethanol yield and corresponding fermentation time in fermentation experiments.

Species	Isolation source	Acetic acid 2.5 g l^{-1}	Formic acid 0.5 g l^{-1}	Levulinic acid 2.3 g l^{-1}	Furfural 1.44 g l^{-1}	Vanillin 0.76 g l^{-1}	High Gravity (25% glucose) Max. EtOH (%)	Time (h)	Lignocellulosic fermentation Without inhibitors Max. EtOH (%)	Time (h)	With inhibitors Max. EtOH (%)	Time (h)
S. cerevisiae	Bioethanol	94	89	94	6	86	49	114	54	19	56	19
S. cerevisiae	Oak	106	105	125	9	88	50	52	62	22	61	22
M. pulcherrima	Soil	110	83	90	0	30	38	119	39	42	0	42
W. anomalus	Thick juice	89	94	89	0	99	50	127	55	41	59	88
T. delbrueckii	Thick juice	89	94	83	43	53	31	120	42	42	42	42
P. kudriavzevii	Soil	130	114	121	36	55	26	138	43	22	45	22

Due to their very low ethanol and/or HMF tolerance isolates of C. bombi, H. uvarum, S. bombicola, C. matritensis and M. reukauffii were abandoned for further experiments.

- The **ethanol yield** in all fermentation experiments was below the theoretical yield of 51% (g ethanol/g glucose) for *M. pulcherrima*, *T. delbrueckii* and *P. kudriavzevii*.
- Fermentation to ethanol was only inhibited in the presence of inhibitors for the *M. pulcherrima* strain.

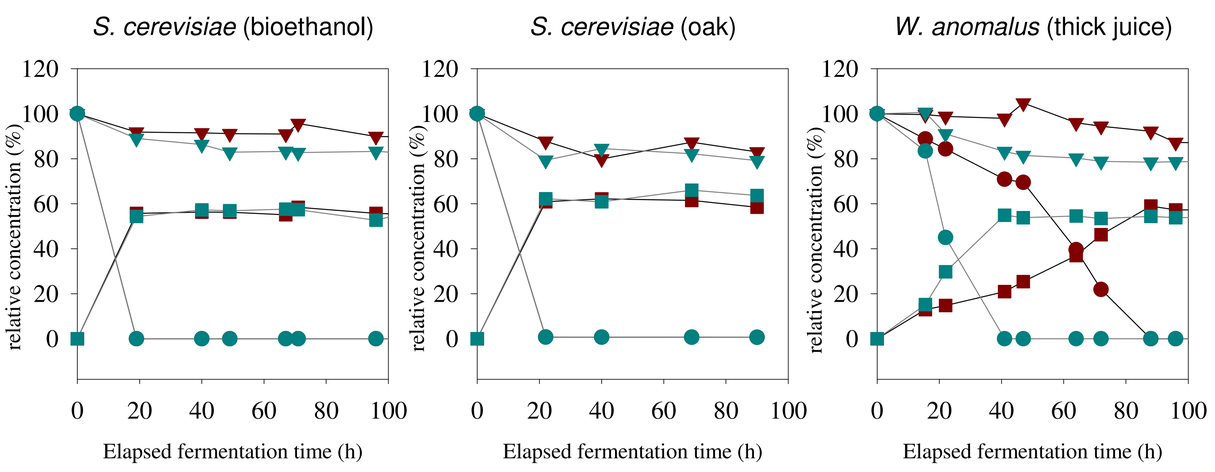


Fig. 1: Glucose (●) and xylose (▼) consumption, and ethanol yield (■) during fermentation by W. anomalus and S. cerevisiae without and with inhibitors.

- No difference in fermentation profile was observed between both S. *cerevisiae* strains. This suggests that bioethanol production strains might be **omnipresent in nature**.
- ***W. anomalus*** was considered as the best performing non-*Saccharomyces* yeast based on its ethanol yield and tolerance profile. However, fermentation time is longer for *W. anomalus*. In contrast to its observed growth on xylose, **xylose was not fermented** to ethanol by *W. anomalus*.