SynBioForum 2019
第二届国际合成生物学论坛

Improved tolerance of *Saccharomyces cerevisiae* to lignin-derived phenolic acid inhibitors by adaptive laboratory evolution

Hanqi Gu*, Xiaoguang Liu, Yanyan Xu, Ran Liu, Yanfang Peng, Jie Li

Department of Biology and Food Science, Hebei Normal University for Nationalities, Hebei 067000, China

Summary

Phenolic acids are the lignin-derived fermentation inhibitors and wildly exist in the various pretreated lignocellulosic hydrolysates.

In this study, the effect of phenolic acids (vanillic, *p*-hydroxybenzoic and syringic acids as model compounds) on *S. cerevisiae* was investigated. Adaptive laboratory evolution was used to improve tolerance to the phenolic acid inhibitors. Tolerant mechanism was analyzed at the morphological and physiological level.

The results show that the phenolic acids caused the synergistic inhibitory effect on the yeast cell growth and ethanol fermentation. The evolved strains presented more tolerance than those of the parental strain by comparing the kinetic parameters of growth and fermentation in synthetic media with different phenolic acids.

Inhibitory effect of individual phenolic acids on S. cerevisiae

Phenolic acids	Concentration (g/L)	μ_{max} [b] (h^{-1})	GI[b] (%)	Lag phase (h)	$Y_{x/s}$ [b] (g/g)	$Q_{glucose}$ (g/L/h)	Q_{EtOH} (g/L/h)	Y_{EtOH} (%)
Vanillic acid	0	0.38	0	3	0.24	3.03	1.37	84.34
	0.33	0.35	7.78	3	0.21	3.07	1.25	81.17
	0.75	0.36	6.64	3	0.19	2.68	1.13	80.80
	1.5	0.33	13.99	3	0.16	2.47	0.88	84.65
	2.25	0.18	53.60	9	0.11	1.15	0.51	80.55
	3.0	0.00	99.55	15	0.03	0.78	0.24	61.14
p-hydroxybenzoic acid	0	0.37	0	3	0.25	3.00	1.27	82.79
	2.0	0.36	5.48	3	0.20	3.07	1.23	78.01
	2.5	0.33	12.20	3	0.18	3.00	1.25	80.36
	3.0	0.30	21.63	3	0.17	2.02	0.82	79.29
	4.0	0.20	45.88	9	0.14	1.17	0.50	82.62
	5.0	0	100	15	0.02	0.75	0.19	49.02
Syringic acid	0	0.38	0	3	0.26	2.87	1.26	85.96
	0.25	0.36	6.01	3	0.26	2.80	1.23	86.25
	0.5	0.36	5.05	3	0.23	2.62	1.17	84.62
	1.0	0.34	11.19	3	0.22	2.45	0.82	86.48
	1.5	0.30	20.98	3	0.20	2.40	0.61	83.11
	2.0	0.27	29.03	3	0.18	2.23	0.63	87.77
V-H-S [a]	0	0.37	0	3	0.26	2.82	1.27	86.06
	0.5-0.3-0.25	0.33	9.83	3	0.25	2.82	1.09	75.76
	1.0-0.6-0.5	0.23	38.77	3	0.20	1.92	1.05	71.06
	1.5-0.9-0.75	0.16	57.39	9	0.08	0.72	0.22	59.62
	2.0-1.2-1.0	0	100	15	0.03	0.10	0.08	21.36
	2.5-1.5-1.25	0	100	24	0.02	0	0	0

The results show that the phenolic acids caused the synergistic inhibitory effect on the yeast cell growth and ethanol fermentation.

Adaptive laboratory evolution

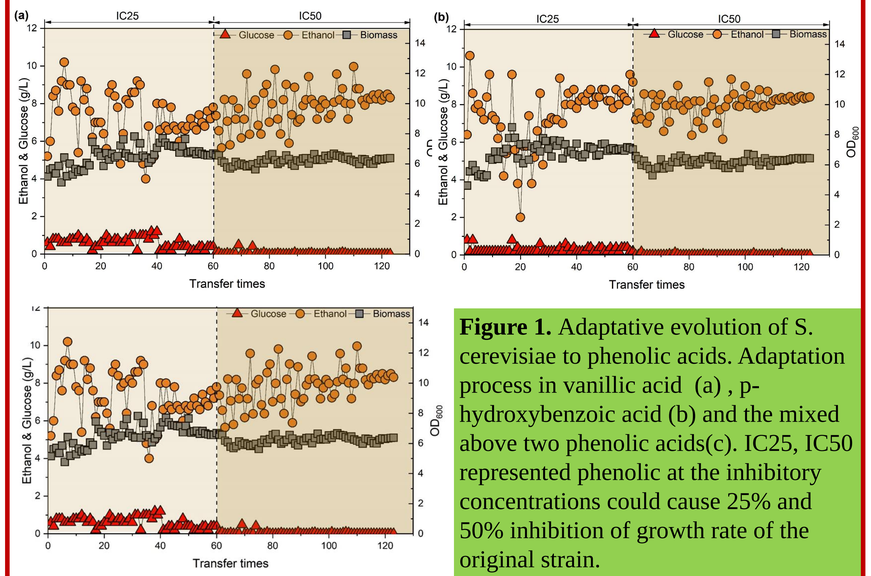
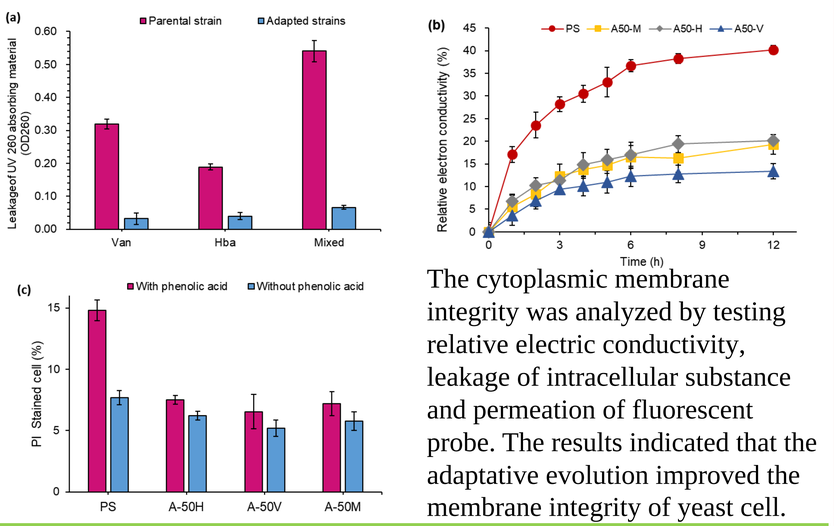


Figure 1. Adaptative evolution of S. cerevisiae to phenolic acids. Adaptation process in vanillic acid (a) , p-hydroxybenzoic acid (b) and the mixed above two phenolic acids(c). IC25, IC50 represented phenolic at the inhibitory concentrations could cause 25% and 50% inhibition of growth rate of the original strain.

The adaptative evolution under stress of phenolic acids could efficiently improve the growth and fermentation performance of the yeast strain not only in the synthetic media with phenolic acids

Cytoplasmic membrane integrity



The cytoplasmic membrane integrity was analyzed by testing relative electric conductivity, leakage of intracellular substance and permeation of fluorescent probe. The results indicated that the adaptative evolution improved the membrane integrity of yeast cell.

Figure 2. Cytoplasmic membrane integrity of yeast strains under phenolic acids stress. (a) leakage of intracellular 260 nm-absorbing substances; (b) relative electric conductivity; (c) PI stained cells rate; **PS** represents the parental strain cells; **A-50H, A-50V and A-50M** were the adapted strains which obtained by adaptative evolution under stress of p-hydroxybenzoic acid, vanillic acid and mixed phenolic acids at 50% inhibitory concentration.

Morphology analysis of adapted strain

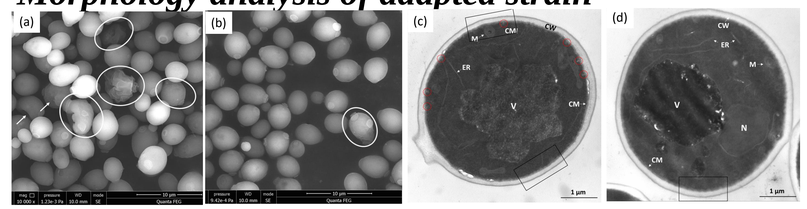


Figure 3. SEM and TEM of *S. cerevisiae* after treatment of phenolic acids. the parental strain cells (a, c) and the adapted strain (b, d). All above strain cells were inoculated in the medium with phenolic acids mixture at concentration of IC50 at 30 °C with agitation at 150 rpm for 9 h.

Phenolic acids caused the parental strain to generate many cytoplasmic membrane invaginations with crack at the top of these sites.

Lipid composition of cytoplasmic membrane

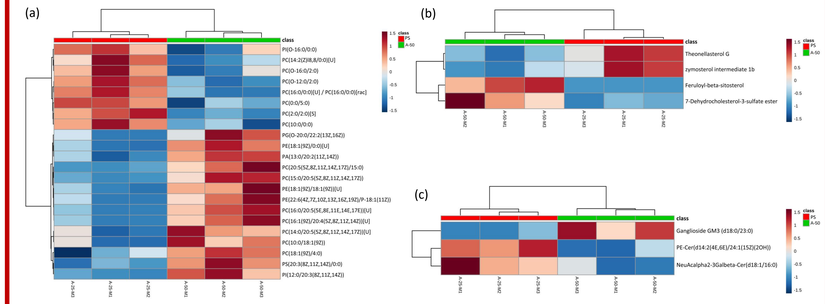


Figure 4. Heat map of phospholipid profiles from parental (PS) and adapted (A-50M) strains of yeast.(a) Phospholipid; (b) sterol; (c) sphingolipids

Conclusion

The cytoplasmic membrane integrity of yeast strain was disrupted by the phenolic acids and the evolved strain exhibited change in cell structure, especially, the cytoplasmic membrane and wall. The evolved strains improved the cytoplasmic membrane integrity of yeast cell under the phenolic acids stress was found by testing the leakage of intracellular substance and the permeability of fluorescent probe. The change of lipid composition induced the structural change of cell membrane might contribute the improvement membrane integrity. The adaptive laboratory evolution will contribute to the development of robust microbials for biofuels production from lignocellulosic biomass.

Acknowledgement

NSFC

河北省科学技术厅
Hebei Provincial Department of Science and Technology