

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

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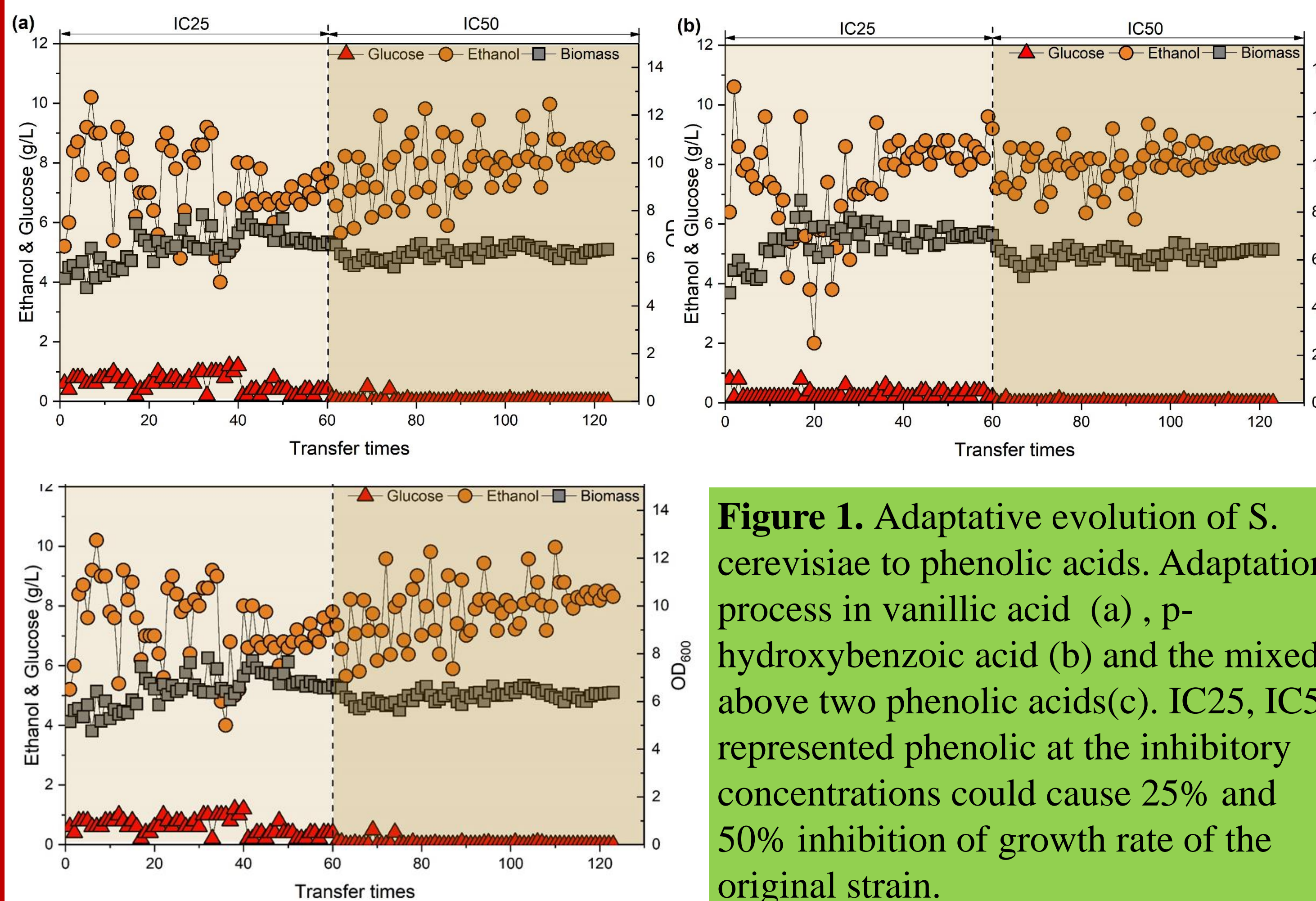
## Summary

Phenolic acids are the lignin-derived fermentation inhibitors and widely 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.

## Adaptive laboratory evolution



The adaptive 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

## Morphology analysis of adapted strain

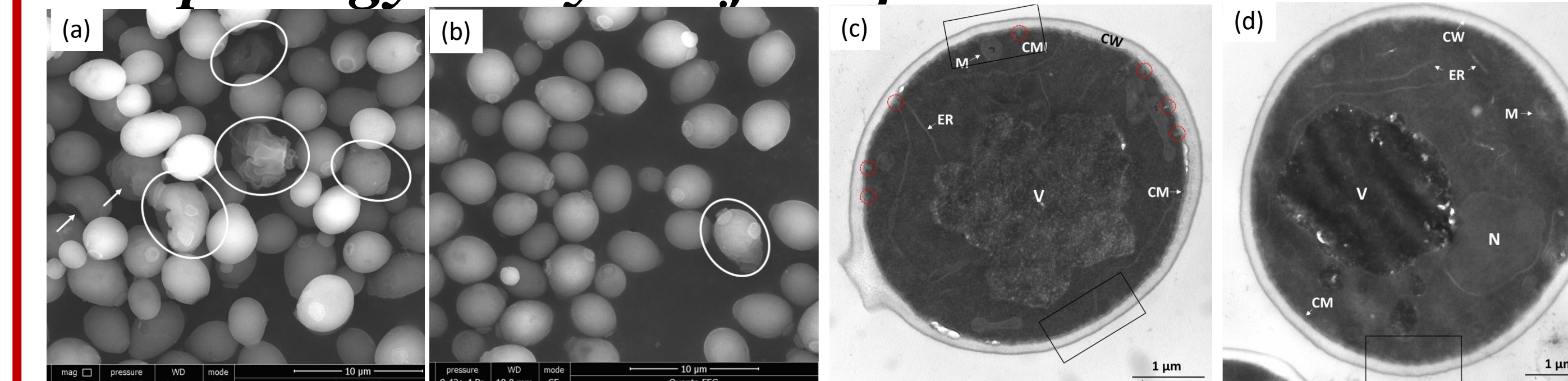


Figure 3. SEM and TEM of *S. cerevisiae* after treatment of phenolic acids. the parental strain cells (a, c) and the adapted strain (b, d). All above strain cells were inoculated in the medium with phenolic acids mixture at concentration of IC50 at 30 °C with agitation at 150 rpm for 9 h.

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

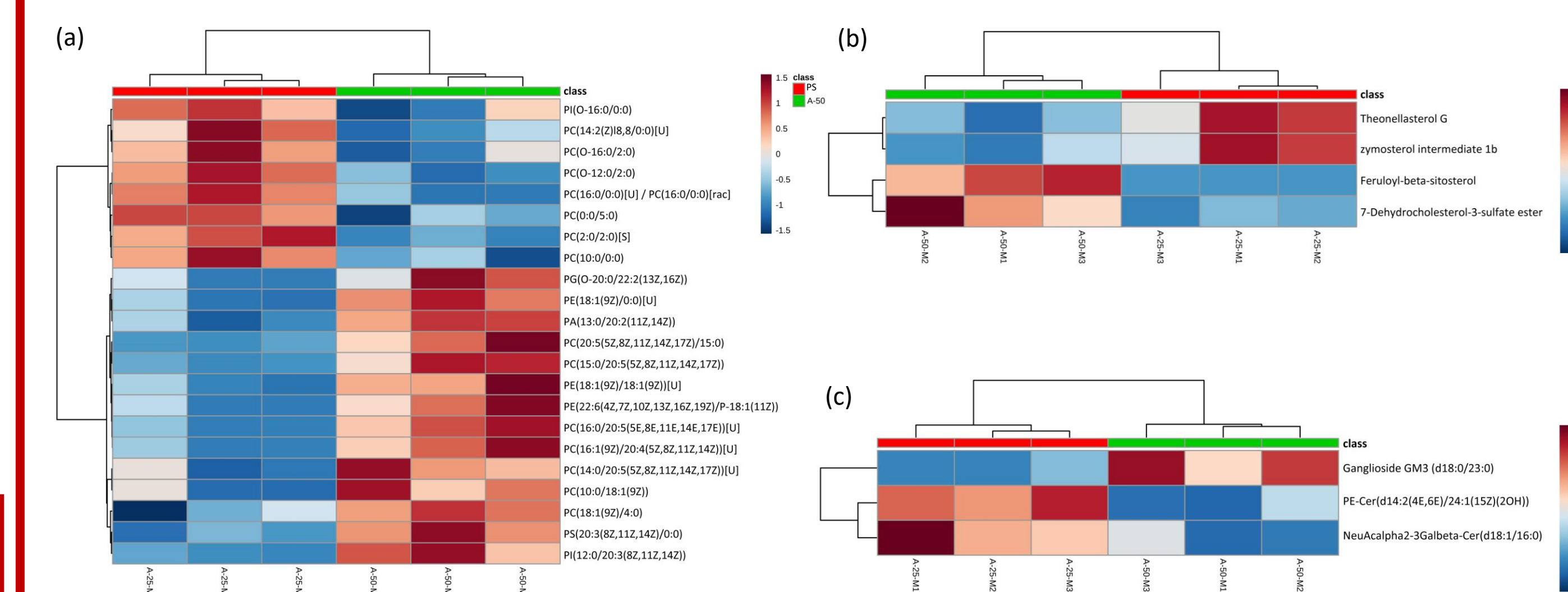


Figure 4. Heat map of phospholipid profiles from parental (PS) and adapted (A-50M) strains of yeast. (a) Phospholipid; (b) sterol; (c) sphingolipids

## 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



## Inhibitory effect of individual phenolic acids on *S. cerevisiae*

Phenolic acids	Concentration (g/L)	$\mu_{max}^b$ ( $h^{-1}$ )	GI <sup>b</sup> (%)	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
<i>p</i> -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
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
V-H-S <sup>a</sup>	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.

## Cytoplasmic membrane integrity

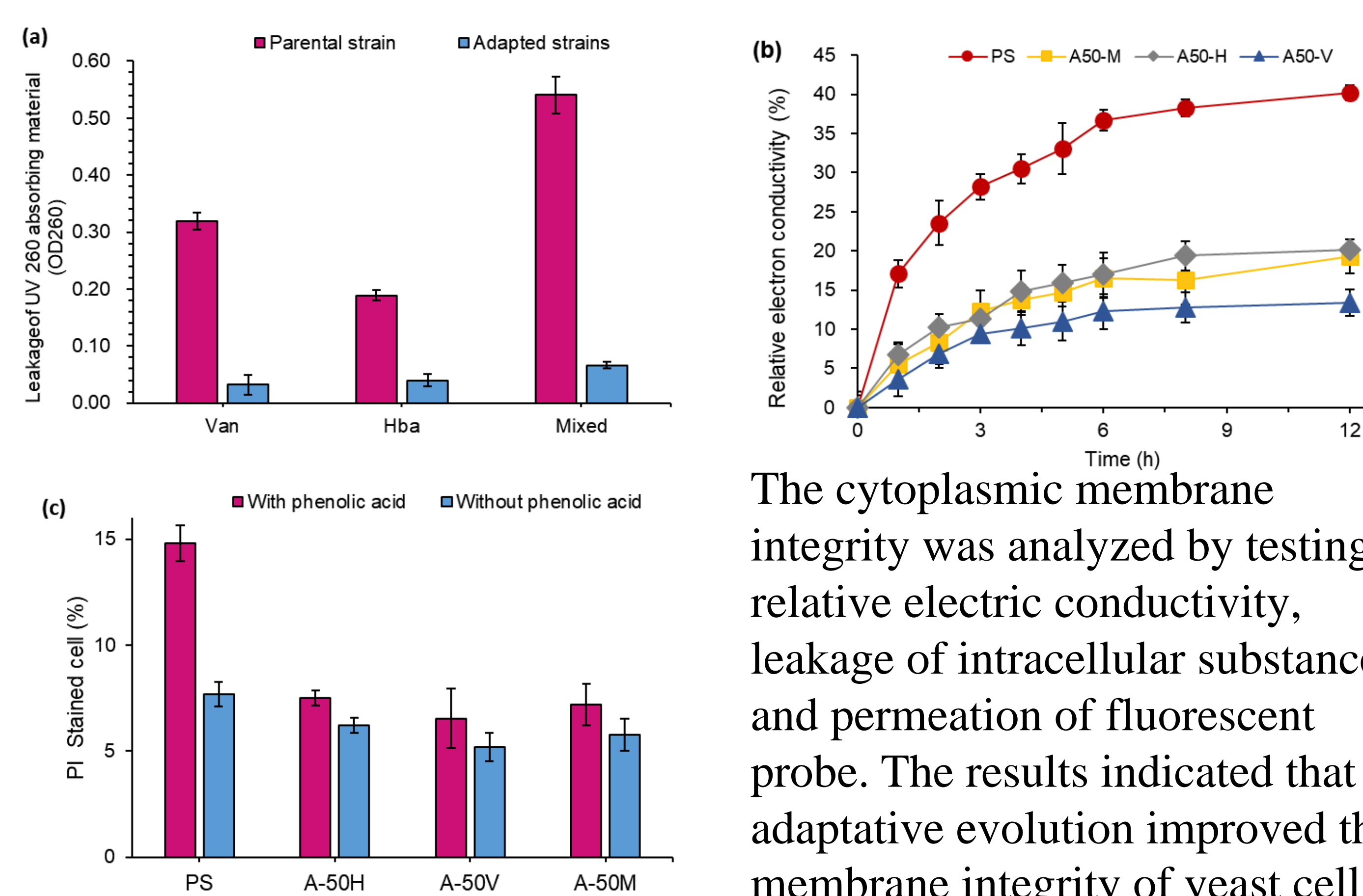


Figure 2. Cytoplasmic membrane integrity of yeast strains under phenolic acids stress. (a) leakage of intracellular 260 nm-absorbing substances; (b) relative electric conductivity; (c) PI stained cells rate; PS represents the parental strain cells; A-50H, A-50V and A-50M were the adapted strains which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