Article

A High-Throughput Screening Procedure (Py-Fe3+) for Enhancing Ethanol Production by *Saccharomyces cerevisiae* Using ARTP Random Mutagenesis

Wei-Yang Wang [1], Shi-Hua Wu [1], Yuan-Han Xie [1], Miao Zhong [1], Man-Li Wei [1], Ze-Yang Li [2], Xiu-Feng Long [1,*] and Fu-Xing Niu [1,*]

[1] Guangxi Key Laboratory of Green Processing of Sugar Resources, Guangxi University of Science and Technology, Liuzhou 545006, China
[2] Key Laboratory for Zhuang and Yao Pharmaceutical Quality Biology, Guangxi Science & Technology Normal University, Laibin 546199, China
* Correspondence: longxiufeng@gxust.edu.cn (X.-F.L.); niufx3@gxust.edu.cn (F.-X.N.)

Supplement Figure S1

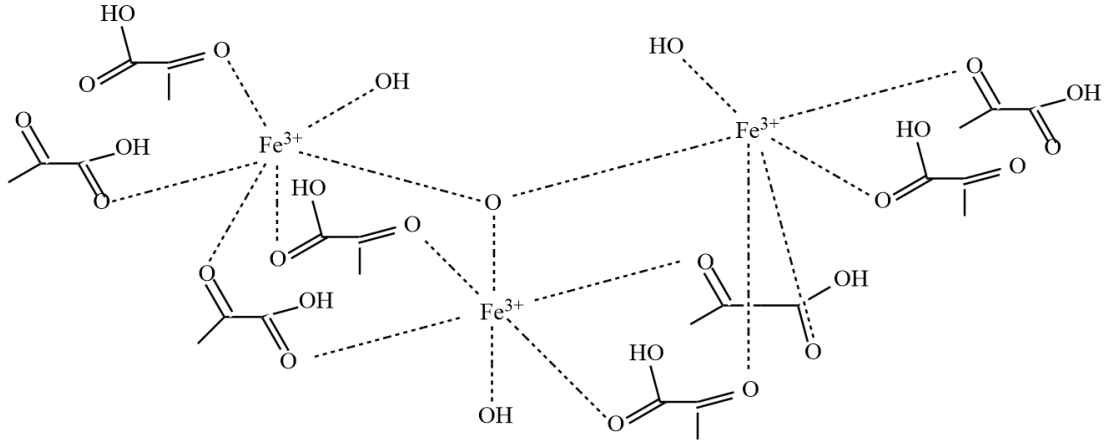
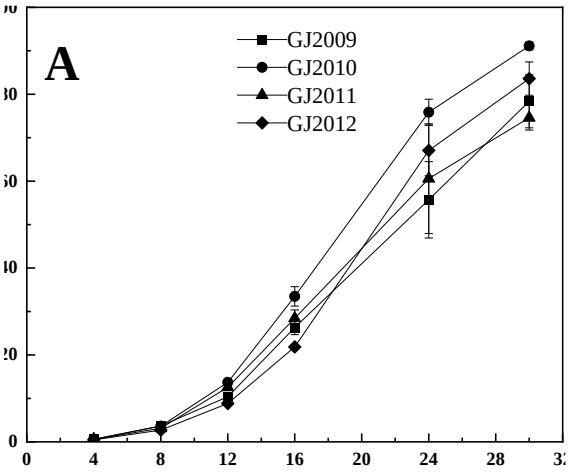
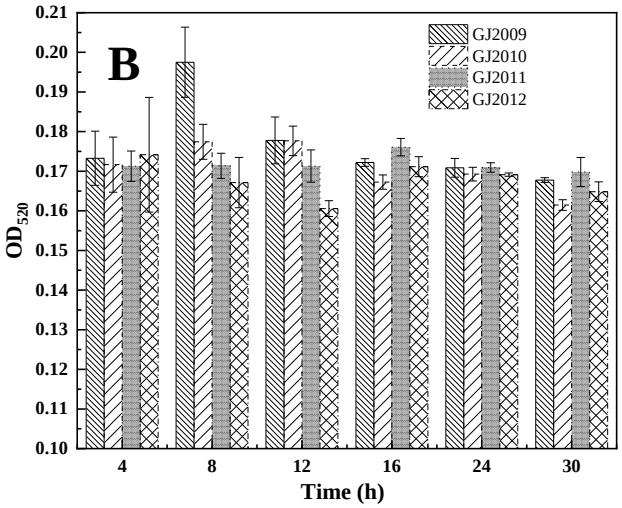


Figure S1 Structure of $[Fe_3O(CH_3COCO_2)_6(H_2O)_3]^+$[1]

Supplement Figure S2

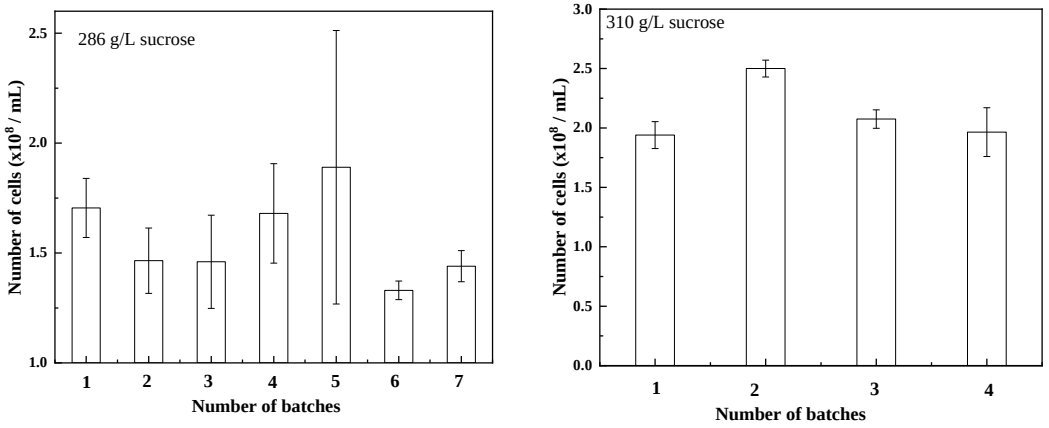
(A) Four *S. cerevisiae* strains, GJ2009, GJ2010, GJ2011, and GJ2012 (derived from GJ2008) with different ethanol synthesis capacities, were verified by fermentation under 190 g/L sucrose. (B) 120 μL of these four *S. cerevisiae* strains' fermentation supernatant (Five times dilution) was transferred to a 96-well enzyme label plate, and 80 μL 1M $Fe(NO_3)_3$was added for reaction at room temperature for 10 min. Then the absorbance was measured at OD_{520} nm.

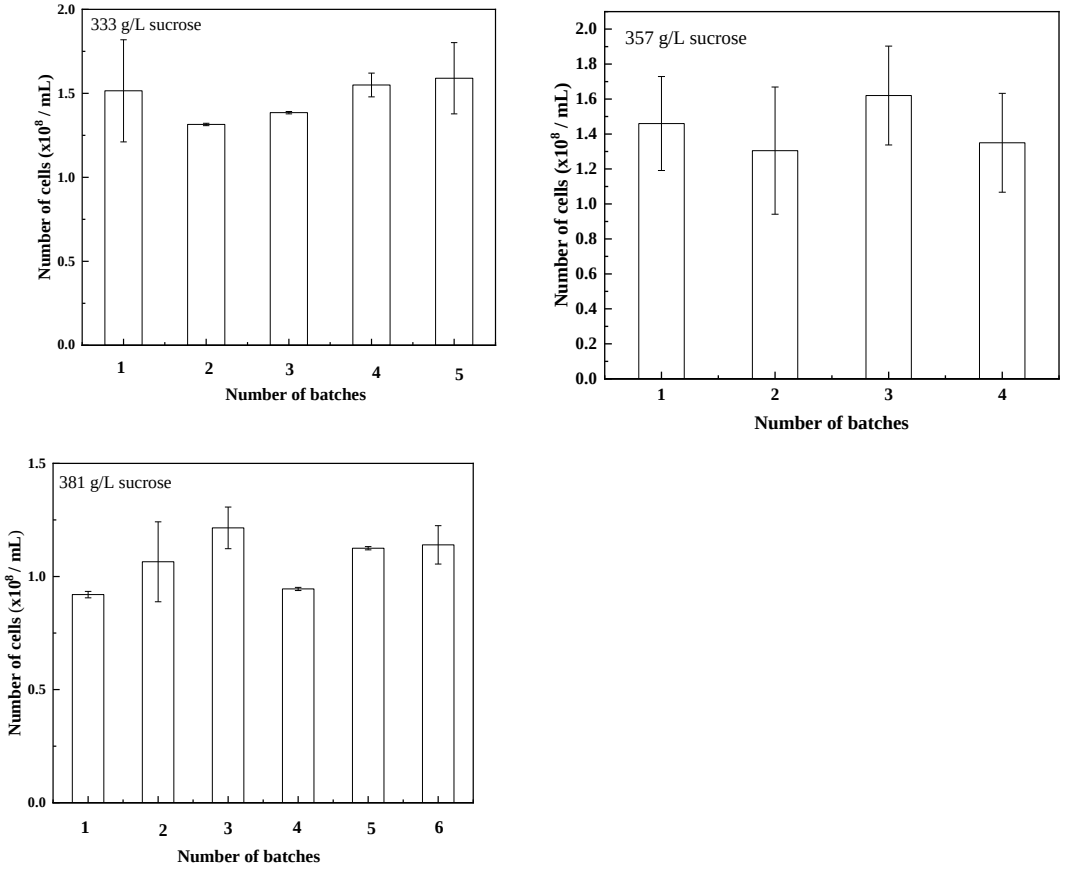




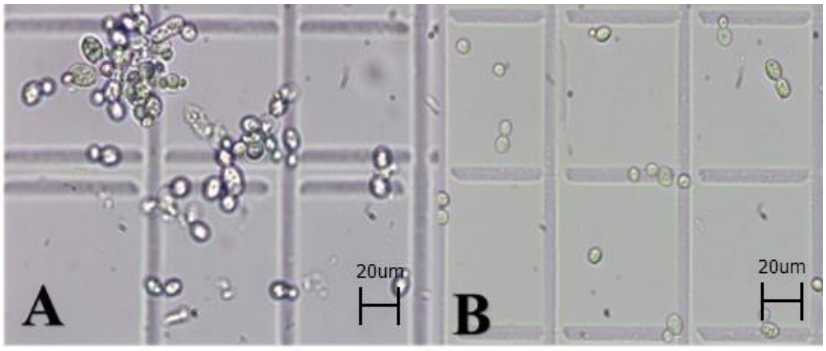
Supplement Figure S3 ARTP mutation and adaptive evolution

After ARTP mutagenesis, the mutant bacterial library obtained was firstly cultured and subcultured in 286 g/L sucrose fermentation medium. After stable growth, the next round of mutagenesis was carried out again, and the sucrose concentration in the medium was increased until to 381 g/L.





Supplement Figure S4 Comparison of cell morphology between mutant bacteria and non-mutant bacteria at sucrose concentration of 381g/L after 24 h.
(A) non-mutant *S. cerevisiae* (GJ2008); (B) the mutant bacterial library.

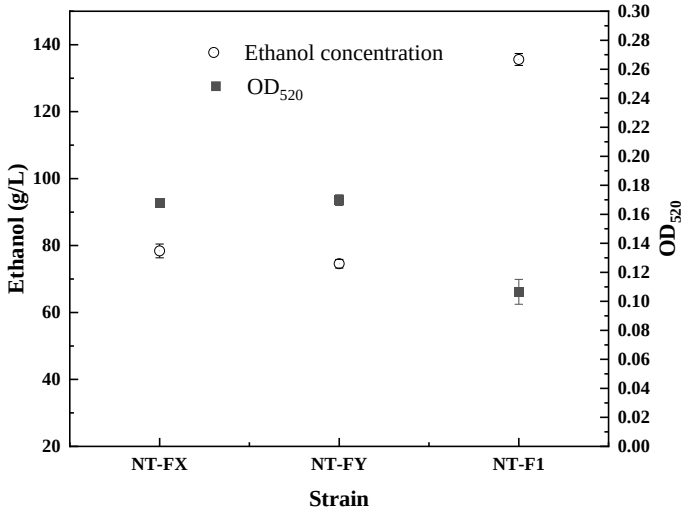


Supplement Table S1 Optimum strain fermentation and screening

name	Ethanol (g/L)
NT-F1	122.69±5.02
NT-F2	120.32±3.94
NT-F3	119.93±3.39
NT-F4	119.93±5.58
NT-F5	113.62±6.69
NT-F6	116.77±3.95

Those 6 strains with the lowest OD_{520} value were selected for shaking (286 g/L sucrose), and the dominant strain NT-F1 with the highest ethanol synthesis yield was obtained.

Supplement Figure S5 Ethanol production of the selected mutants with higher OD_{520} Two OD_{520} strains (NT-FX and NT-FY) with higher values were randomly selected for fermentation from the 380 strains.



Reference

1. Mehrotra, R.-N.; Hasan, T. Detection and spectrophotometric determination of pyruvic acid. Anal. Lett. **1986** , *19*, 1713-1724. doi: 10.1080/00032718608066497