

Supplementary material for

Identification and fermentation condition optimization of a high-yield
3-methylthiopropanol yeast

Table S1. Factors and levels of single factor design and their optimization conditions.

Factor	Level/type	Optimization condition
Glucose concentration (g/L)	10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60	40
Yeast extract concentration (g/L)	0, 0.4, 0.8, 1.2, 1.6 and 2.0	0.4
Surfactant type	Blank, glycerol, Tween 20, Tween 40, Tween 60, Tween 80, and Triton 100	Tween 80
Tween 80 concentration (g/L)	0, 2, 4, 8, 16, 32 and 64	2
L-methionine concentration (g/L)	0, 2, 4, 6, 8 and 10	4
L-methionine addition time (h)	0, 12, 24, 36, 48, 60 and 72	0
Temperature (°C)	20, 24, 28, 32, 36 and 40	32
Initial pH	3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5 and 7	5
Inoculum size (%)	0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4	0.8
Shaking speed (rpm)	0, 45, 90, 135, 180, 225 and 270	225
Liquid volume (mL)	25, 50, 75, 100 and 125	25
Fermentation time (h)	0, 12, 24, 36, 48, 60, 72, 84 and 96	60

Table S2. Physiological and biochemical characteristics of strain Y1402.

Test	Results		Test	Results	
	Sugars	Characteristics		Carbon sources	Characteristics
Sugar fermentation	L-Rhamnose	No acid, no gas, no growth	Carbon source assimilation	Ethanol	Good growth
	D-Galactose	Acid production, no gas, good growth		Glycerol	Growth
	D-Maltose	Acid production, no gas, good growth		D-Trehalose	No growth
	D-Xylose	No acid, no gas, no growth		D-Raffinose	No growth
	D-Arabinose	No acid, no gas, no growth		Inulin	Growth
	Glucose	Acid and gas production, good growth		Mannitol	No growth
	Lactose	No acid, no gas, no growth		D-Ribose	Good growth
	Sucrose	Acid production, no gas, good growth		D-Sorbitol	No growth
	Nitrogen sources	Characteristics		Nitrogen sources	Characteristics
Nitrogen source assimilation	Urea	Good growth	Nitrogen source assimilation	Potassium nitrate	Growth
	Ammonium sulfate	Good growth		L-Phenylalanine	Good growth

	Sodium nitrite	Growth		L-Lysine	Good growth
Hydrogen sulfide test	Negative		Indole test	Positive	
Citrate test	Positive		Methyl red test	Positive	
Urea test	Negative		Voges-Proskauer test	Negative	
Starch hydrolysis test	Positive		Gelatin liquefaction test	Negative	

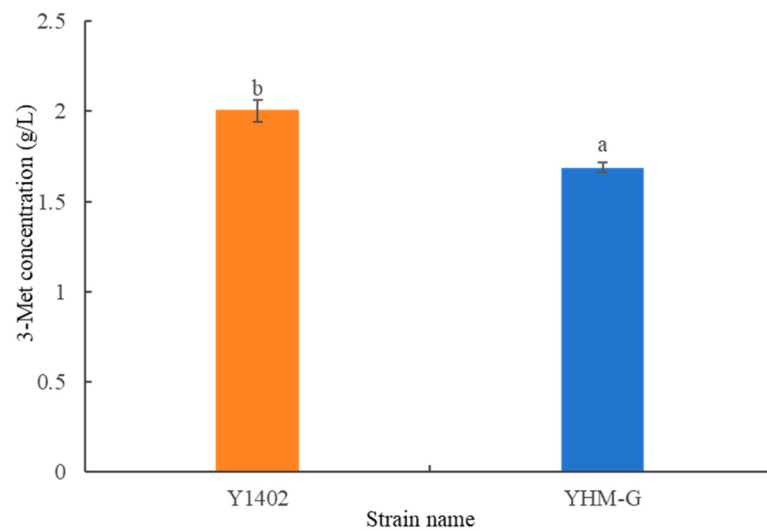


Figure S1. Comparison of 3-Met produced by yeast Y1402 and YHM-G.

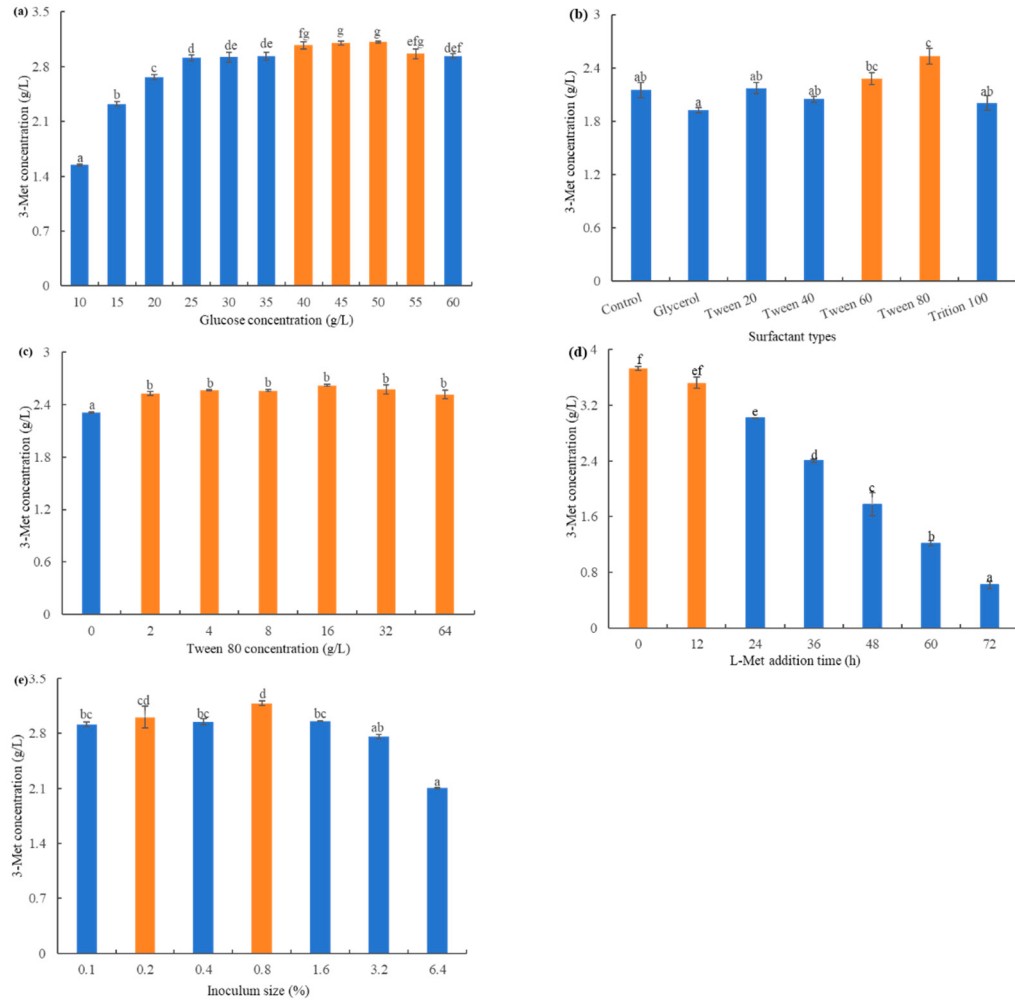


Figure S2. Effect of glucose concentration (10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 g/L) (a), surfactant types (control, glycerol, Tween 20, Tween 40, Tween 60, Tween 80 and Triton X-100) (b), Tween 80 concentration (0, 2, 4, 8, 16, 32 and 64 g/L) (c), L-Met addition time (0, 12, 24, 36, 48, 60 and 72 h) (d), inoculum size (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4%, v/v) (e) on 3-Met concentration by Y1402. Same letters in the column indicates that the data do not differ significantly at 5% probability by the Tukey test. The orange bar means the best conditions on 3-Met concentration.

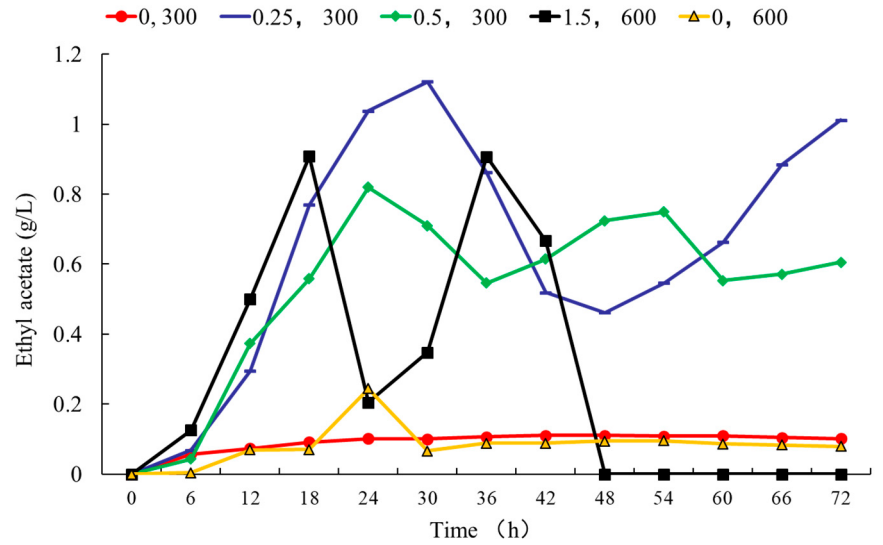


Figure S3. The yield of ethyl acetate at different conditions at *S. cerevisiae* Y3401 and *W. anomalus* Y3604 ratio of 1:1 by simultaneous mixed fermentation in a 5 L fermenter. Note: 0, 300: the aeration rate of the fermenter is 0 vvm and the agitation speed is 300 rpm; 0.25, 300: the aeration rate of the fermenter is 0.25 vvm and the agitation speed is 300 rpm; 0.5, 300: the aeration rate of the fermenter is 0.25 vvm and the agitation speed is 300 rpm; 1.5, 600: the aeration rate of the fermenter is 1.5 vvm and the agitation speed is 600 rpm; 0, 600: the aeration rate of the fermenter is 0 vvm and the agitation speed is 600 rpm.