Supplementary material for

Screening of yeasts isolated from *Baijiu* environments for producing 3-methylthiopropanol and optimizing production conditions

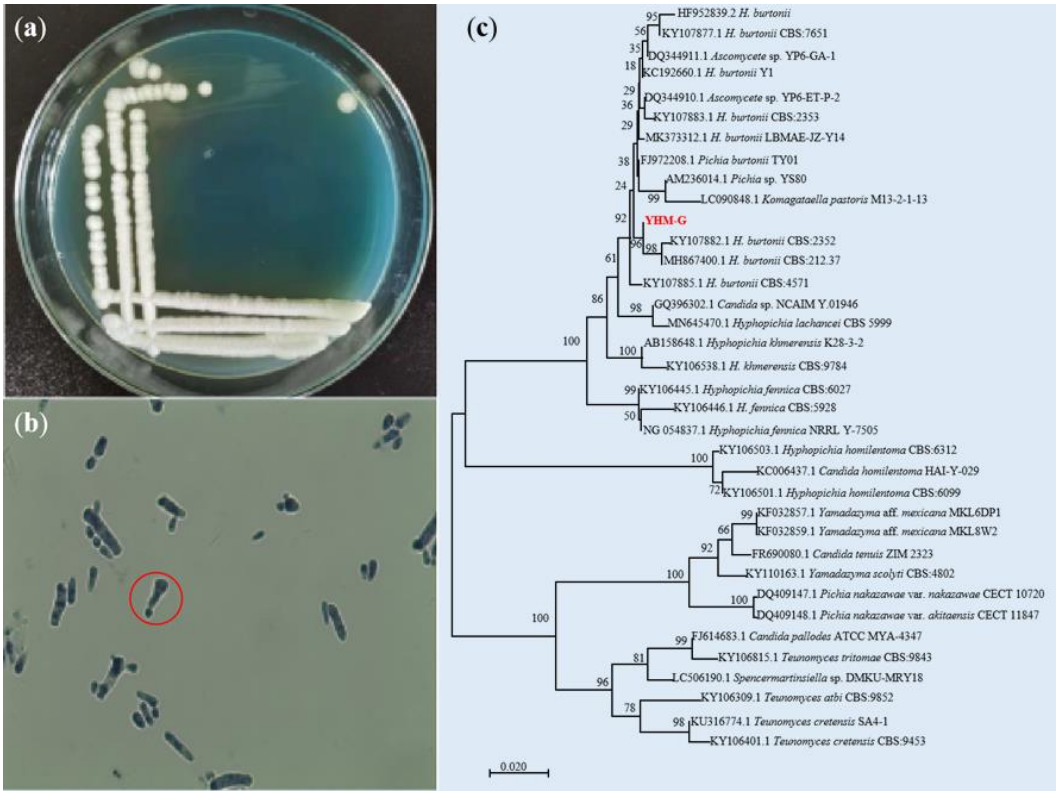
Supplementary Table S1. Factors and levels of single factor design and their optimization conditions.

Factor	Level/type	Optimization condition
Glucose concentration (g/L)	10, 20, 30, 40, 50, 60 and 70	**30-60**
Yeast extract concentration (g/L)	0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8	**2-2.8**
L-Met concentration (g/L)	0, 2, 4, 6, 8, 10, 12 and 14	**4**
Time point of L-Met addition (h)	0, 12, 24, 36, 48, 60 and 72	**24**
Initial pH	3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0	**4.0-5.5**
Temperature (℃)	20, 24, 28, 32, 36 and 40	**32**
Shaking speed (rpm)	0, 45, 90, 135, 180, 225 and 270	**135**
Loading volume (mL/250 mL)	25, 50, 75, 100 and 125	**75**
Inoculum size (%, v/v)	0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4	**0.2-3.2**
Surfactant types	Control, glycerol, Tween-20, Tween-40, Tween-60, Tween-80, Triton X-100 and Triton X-114	**Tween-20-80 and Triton X-100**
Tween-80 concentration (g/L)	0, 2, 4, 8, 16, 32 and 64	**2-64**
Time (h)	0, 12, 24, 36, 48, 60, 72, 84 and 96	**48-96**

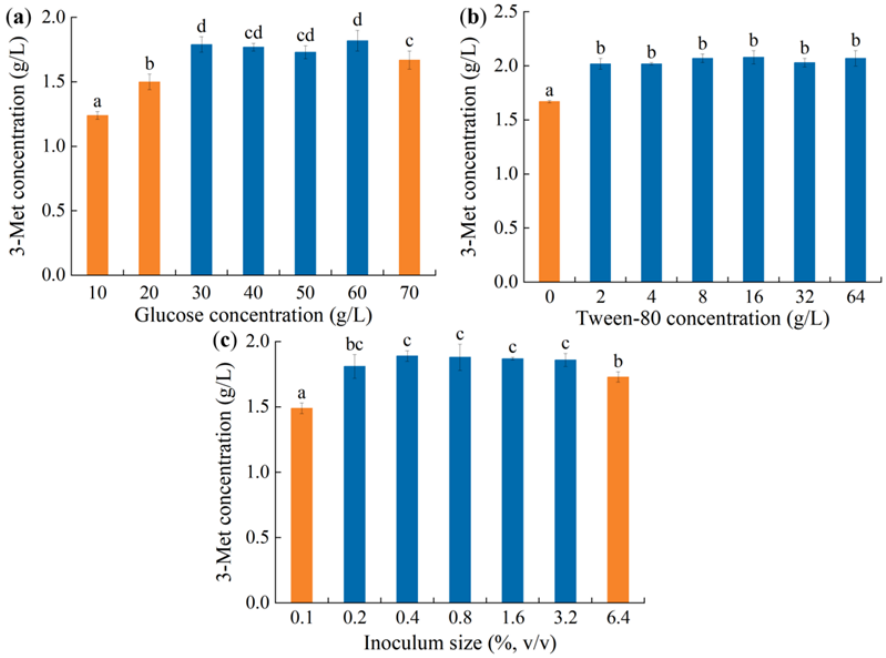
Supplementary Table S2. Physiological and biochemical characteristics of strain YHM-G.

Tests	Results	
Sugar fermentation tests	**Sugars**	**Characteristics of YHM-G**
	Glucose	Acid and gas production
	Maltose	
	D-Galactose	
	Sucrose	
	D-Maltose	Acid production, no gas
	D-Xylose	Gas production, no acid
	L-rhamnose monohydrate	
	D-Arabinose	No acid, no gas, but grow
	Lactose	
Carbon source assimilation tests	**Carbon sources**	**Characteristics of YHM-G**
	Ethanol	+
	Glycerol	
	Inulin	
	D-Raffinose	
	D-Trehalose	
	Mannose	−
	D-Ribose	
	D-Sorbose	
Nitrogen source assimilation tests	**Nitrogen sources**	**Characteristics of YHM-G**
	Urea	+
	Ammonium sulfate	
	Sodium nitrite	
	Potassium nitrate	
	L-Phenylalanine	
	L-Lysine	
Other tests	Indole test	+
	Methyl red test	
	Starch hydrolysis test	
	Citrate test	
	Urea test	
	Voges-Proskauer test	−
	Hydrogen sulfide test	
	Gelatin liquidized test	

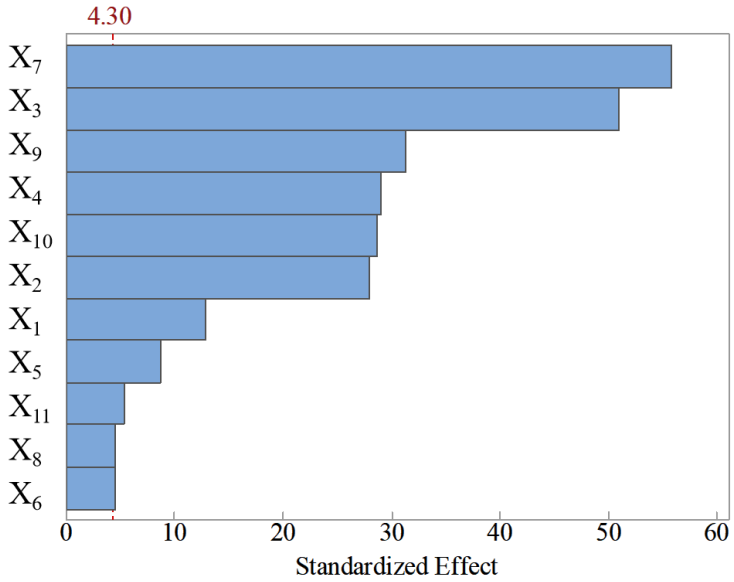
Note: "+", positive response; "−", negative response.



Supplementary Figure S1. Colony morphology on WL medium (**a**), cell morphology (10×100 times magnification) (**b**) and neighbor-joining phylogenetic tree based on ribosomal large subunit 26S rRNA gene (D1/D2 region) gene sequence and closest relative species (**c**) of strain YHM-G. Cell was stained by the methylene blue method. The asexual budding reproduction occurred at the ends of the cells was highlighted in the red circle.



Supplementary Figure S2. Effect of glucose concentration (10, 20, 30, 40, 50, 60 and 70 g/L) (a), T-ween-80 concentration (0, 2, 4, 8, 16, 32 and 64 g/L) (b), inoculum size (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4%, v/v) on 3-Met concentration. Same letters in the column indicates that the data do not differ significantly at 5% probability by the Tukey test.



Supplementary Figure S3. Standardized Pareto chart showing the effect of variables on 3-Met concentration. X1, shaking speed; X2, temperature; X3, glucose concentration; X4, initial pH; X5, loading volume; X6, inoculum size; X7, time point of L-Met addition; X8, culture time; X9, yeast extract concentration; X10, L-Met concentration; X11, Tween-80 concentration.