

Supplementary Materials for Optimisation of *Lactobacillus* fermentation conditions and its application in the fermentation of salt-free sauerkraut

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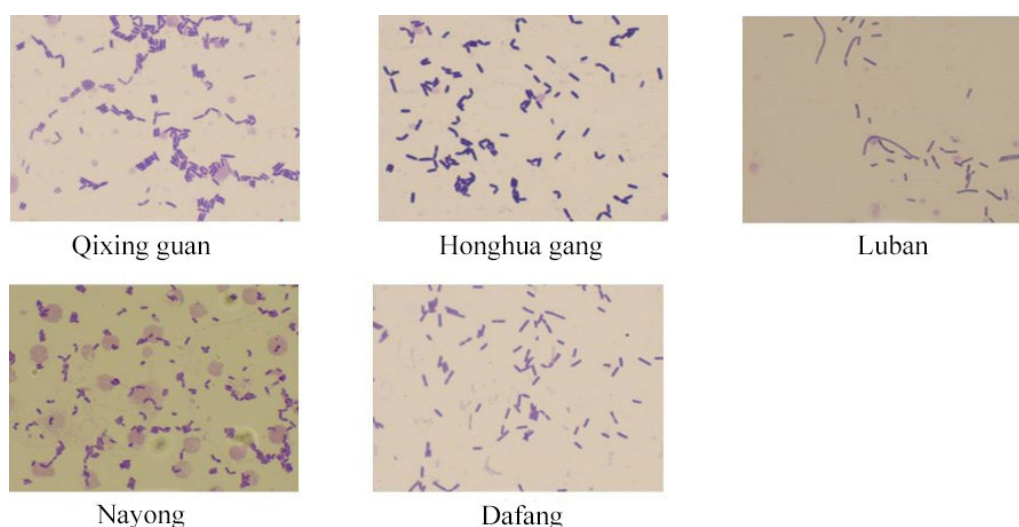


Figure S1 Gram staining and morphology of LAB isolated from the five locations indicated.

Plackett-Burman experimental design

The effect of culture time, starch/water ratio and inoculum on the yield of LAB cultivated in 100-mL Erlenmeyer flasks, we used single-factor experiments performed in triplicate, using the average LAB yield as the response. Fermentation time for each experiment was two days, and experiments were performed as described previously. A Plackett-Burman experimental design was used for initial screening of the medium and culture conditions for all three components. Each variable was assigned two levels (Table 1): low level (code -1) and high level (code 1). To examine the experimental design, two dummy variables (X4 and X11) were used and fitted into a first-order model.

$$Y = \alpha_0 + \sum \alpha_i X_i \quad (i = 1, 2, \dots, k) \quad (1)$$

where Y is the estimated target function, α_0 is the model intercept, α_i is the regression coefficient, and X_i is the coded independent factor.

Table S1 Plackett-Burman design to screen the variables

Variable	Factor	Low level (-1)	High level (1)
X_1	Culture time (h)	24	72
X_2	Starch/Water ratio (%)	1	2
X_3	Inoculum (%)	0.1	0.2
$X_4 - X_{11}$	Dummy variable	-1	1

● *Path of steepest ascent method*

Using the findings from the Plackett-Burman experimental design, examination of the optimal level for each chosen factor was performed using the steep ascent approach. Using the direction of the Plackett-Burman experimental value as the upwards inclination, the design of the steep ascent method was established. The adjustment in step size was validated based on the data from the Plackett-Burman experimental design. Whenever the variable effect was positive, higher values were chosen, and viceversa (Table 2).

Table S2 Steepest ascent experimental design

Experiment number	Culture time (h)	Starch/Water ratio (%)	Inoculum (%)
1	24	2.5	0.025
2	36	2.0	0.020
3	48	1.5	0.015
4	60	1.0	0.010
5	72	0.5	0.005

Table S3 Plackett-Burman design and results.

Run	Culture time (h)	Starch/water ratio (m/V)	Inoculum (V/V)	LAB yield (OD ₆₀₀)
1	1	-1	1	2.23
2	-1	1	1	1.09
3	1	1	1	1.97

4	-1	1	-1	1.42
5	1	-1	-1	2.55
6	-1	-1	1	1.49
7	1	1	-1	2.18
8	1	-1	1	1.79
9	-1	1	1	1.06
10	1	1	-1	2.09
11	-1	-1	-1	1.92
12	-1	-1	-1	2.01

Note: 1OD is equivalent to a LAB concentration of 1×10^8 cells/mL

Table S4 Statistical analysis of Plackett-Burman results.

Source	Sum of Squares	df	Mean Square	F value	p value	
Model	2.15	3	0.7166	28.30	0.0001	***
A-A (Culture time)	1.22	1	1.22	48.03	0.0001	***
B-B (Starch/Water ratio)	0.3960	1	0.3960	15.64	0.0042	**
C-C (Inoculum)	0.5376	1	0.5376	21.23	0.0017	***
Residual	0.2026	8	0.0253			
Lack of Fit	0.0972	4	0.0243	0.9228	0.5301	
Pure Error	0.1053	4	0.0263			
Cor Total	2.35	11				

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

Table S5 Design and results of path of steepest ascent method.

	Culture time (h)	Starch/Water ratio (%)	Inoculum (%)	LAB Yield (OD ₆₀₀)
1	24	2.5	0.025	1.88
2	36	2.0	0.020	2.23
3	48	1.5	0.015	2.84
4	60	1.0	0.010	2.16
5	72	0.5	0.005	1.63