



Supplementary

Purification and kinetic characterisation of an extracellular glutaminase from *Priestia aryabhatai* BS8W22 with deamidation activity on wheat gluten

Supplementary Figures

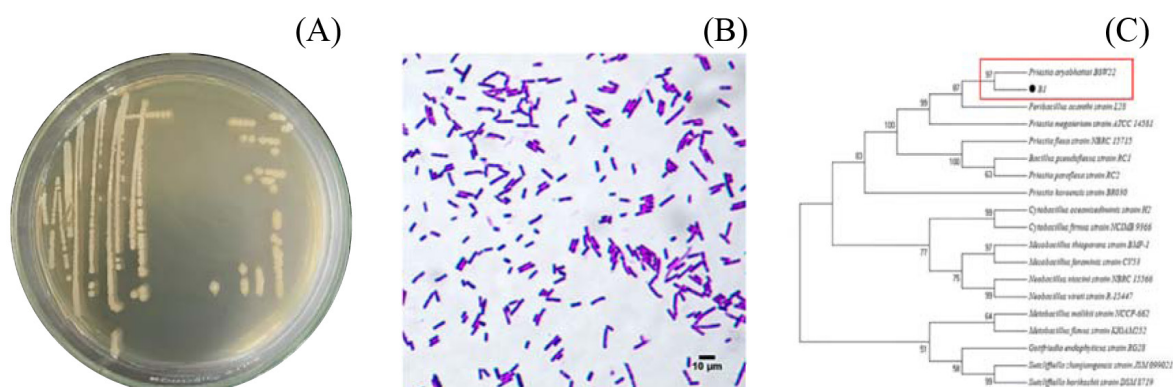


Fig. 1S Identification of glutaminase producing strain B1. (A) Growth of B1 on Nutrient agar media; (B) Gram-positive of strain B1; (C) Phylogenetic tree of isolate strain B1

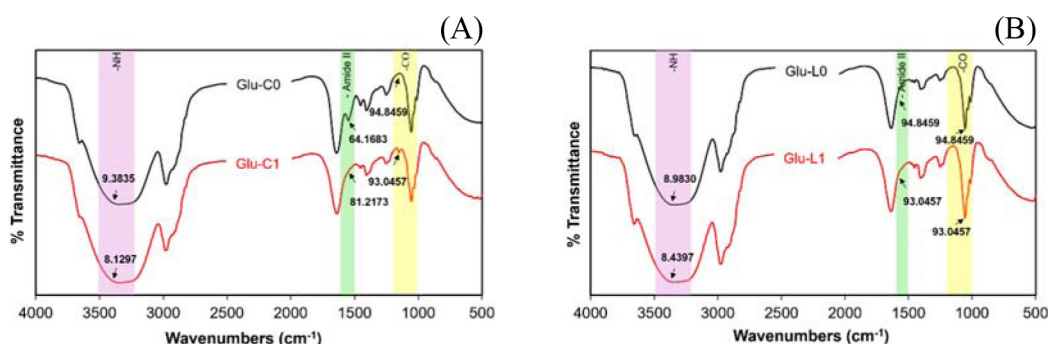


Fig. 2S Transmission spectra of two insoluble and soluble gluten samples after treating with glutaminase by FTIR method. (A): Transmission percentages of spectra ranges of 3300, 1550 and 1000 cm⁻¹ corresponding to three relative functional groups (NH, Amide II and CO) in insoluble gluten sample; (B) Transmission percentages of spectra ranges of 3300, 1550 and 1000 cm⁻¹ corresponding to three relative functional groups (NH, Amide II and CO) in soluble gluten sample.

Supplementary Tables

Table 1S Screening for influential factors for glutaminase production glutaminase production from strain B1

Enzyme activity (U/mL) under the influence of various factors						
Time incubation (day)						
0	1	2	3	4	5	6
5.33 ± 0.503 ^f	39.24 ± 0.960 ^c	55.90 ± 2.909 ^d	85.581 ± 1.466 ^c	132.50 ± 2.9 ⁶ 9a	108.28 ± 2.428 ^b	87.56 ± 3.3 ⁵ c
Temperature (°C)						
20	25	37				
25.22 ± 4.789 ^a	102.42 ± 0.799 ^b	132.50 ± 2.969 ^a				
pH						
6	7	8	9			
112.98 ± 1.911 ^c	139.74 ± 1.8 ¹ 9a	127.16 ± 0.894 ^b	71.86 ± 2.108 ^d			
Carbone source						
Glucose	Sucrose	Lactose	Starch	Fructose		
159.89 ± 0.69 ⁰ a	154.49 ± 4.126 ^b	148.54 ± 0.690 ^b	75.42 ± 1.162 ^d	95.239 ± 1.018 ^c		
Nitrogen sourcece						
Glutamin	Yeast extract	Tryptone	(NH ₄) ₂ SO ₄	NaNO ₃		
151.04 ± 6.23 ⁵ a	89.59± 6.235 ^d	120.32 ± 7.436 ^c	58.15 ± 4.842 ^b	59.09± 5.62 ³ d		
Glutamine concentration (%)						
0.5	1.0	1.5	2.0	2.5	3.0	
121.93± 0.201 ^d	136.55 ± 0.496 ^c	140.14 ± 0.612 ^c	161.57± 0.70 ⁴ a	132.83 ± 4.563 ^b	143.80 ± 4.388 ^b	

All data has presented as mean ± SD ($n=3$) statistical differences ($p < 0.05$) by Tukey's multiple comparisons test. The initial values provided a difference of survey factor of each period.

Table 2S Effect of temperature and pH on the biochemical properties of glutaminase

Effect of temperature and pH on the biochemical properties of glutaminase			
Temperature (°C)	Relative glutaminase activity (%)	pH	Relative glutaminase activity (%)
20	59.290 ± 5.483	3	37.224 ± 1.482
30	135.380 ± 3.405	4	60.842 ± 3.990
40	178.070 ± 5.705	5	98.760 ± 3.232
50	143.831 ± 7.137	6	147.855 ± 5.944
60	74.615 ± 2.063	7	185.683 ± 2.585
70	43.294 ± 2.526	8	158.654 ± 3.921
		9	126.259 ± 8.134
		10	67.875 ± 4.457
		11	48.156 ± 3.474
		12	31.167 ± 2.590

All data has presented as mean ± SD ($n=3$).

Table 3S Surveying factors influences to the degree of deamidation (%) of wheat gluten by glutaminase

% Deamidation degrees wheat gluten under the influence of various factors					
Time (hour)	Without glutaminase	With glutaminase	Mean difference (without-with)	<i>p</i> -value	Significance
0	1.25 ± 0.130	1.40 ± 0.135	-0.1557	>0.9999	ns
3	1.53 ± 0.119	7.24 ± 0.525	-5.703	0.0006	***
6	1.35 ± 0.097	17.45 ± 1.699	-16.10	<0.0001	****
9	1.47 ± 0.304	25.11 ± 1.162	-23.64	<0.0001	****
12	1.68 ± 0.098	44.06 ± 0.335	-42.38	<0.0001	****
18	1.61 ± 0.171	59.76 ± 3.342	-58.14	<0.0001	****
24	2.21 ± 0.173	73.89 ± 5.026	-71.68	<0.0001	****
36	2.34 ± 0.066	68.91 ± 1.049	-69.34	<0.0001	****
Temperature (°C)	Without glutaminase	With glutaminase	Mean difference (without-with)	<i>p</i> -value	Significance
30	2.07 ± 0.147	17.38 ± 1.099	-15.30	<0.0001	****
35	2.16 ± 0.475	24.90 ± 0.443	-22.74	<0.0001	****
40	3.08 ± 0.262	48.00 ± 2.942	-44.91	<0.0001	****
45	3.42 ± 0.109	60.54 ± 3.890	-57.12	<0.0001	****
50	2.88 ± 0.194	45.02 ± 2.222	-42.14	<0.0001	****
pH	Without glutaminase	With glutaminase	Mean difference (without-with)	<i>p</i> -value	Significance
4	2.62 ± 0.071	31.32 ± 2.520	-28.70	<0.0001	****
5	3.08 ± 0.250	54.87 ± 2.122	-51.79	<0.0001	****
6	2.21 ± 0.195	72.11 ± 5.667	-69.90	<0.0001	****
7	2.01 ± 1.076	67.18 ± 2.720	-65.17	<0.0001	****
8	2.48 ± 0.198	65.35 ± 4.838	-62.88	<0.0001	****
9	1.28 ± 0.183	55.31 ± 4.434	-54.03	<0.0001	****
10	1.35 ± 0.456	46.14 ± 4.042	-44.79	<0.0001	****
E/S	Without glutaminase	With glutaminase	Mean difference (without-with)	<i>p</i> -value	Significance
20	2.26 ± 0.328	30.03 ± 5.349	-27.8	<0.0001	****
30	2.29 ± 0.102	74.13 ± 5.031	-71.8	<0.0001	****
40	2.25 ± 0.200	66.04 ± 6.050	-63.8	<0.0001	****
50	2.59 ± 0.246	63.13 ± 7.513	-60.5	<0.0001	****

Data are presented as mean ± SD (*n*=3). Statistical differences between samples with and without glutaminase at each period were analyzed using two-way ANOVA followed by Sidak's multiple comparisons test. Abbreviations: ns, not significant; ****p* < 0.001; *****p* < 0.0001.