

Microbiome and Volatile Metabolic Profile of Acetic Acid Fermentation Using Multiple Starters for Traditional Grain Vinegar

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Supplementary files:

Table 1. Fermentation efficiency according to concentration of ethanol and initial pH

Table 2. Fermentation efficiency under multiple starters according to initial pH and fermentation period

Table 3. Volatile compounds according to fermentation period using *Acetobacter ascendens* GV-8 (A) and *A. ascendens* GV-12 (B) (Unit: Area%)

Table 4. The volatile compound according to fermentation period using *Acetobacter ascendens* GV-8 (A) and *Acetobacter pasteurianus* GV-22 (D) (Unit: Area%)

Table 5. The volatile compound according to fermentation period using *A. ascendens* GV-8 (A), *A. ascendens* GV-12 (B), *A. pasteurianus* GV-17 (C), and *A. pasteurianus* GV-22 (D) (Unit: Area%)

Table 6. Alpha diversity analysis under multiple starters and fermentation temperature

Table 1. Fermentation efficiency according to concentration of ethanol and initial pH.

Fermented condition	Strains	Efficiency (%) according to the fermentation period (days)				
		3	6	9	12	15
Concentration of EtOH	GV-5	14.72±2.61 ^{1,a}	35.12±7.36 ^a	50.61±5.68 ^{ab}	60.12±3.99 ^a	67.02±2 ^{ab}
	GV-8	13.96±1.38 ^a	32.67±4.3 ^a	53.53±5.06 ^{ab}	59.66±0.31 ^a	62.12±2.15 ^c
	GV-12	14.42±1.54 ^a	29.45±3.53 ^a	43.4±2.61 ^b	62.58±1.84 ^a	67.79±0.15 ^a
	GV-16	15.95±2.45 ^a	30.21±10.59 ^a	46.78±5.98 ^{ab}	59.36±6.6 ^a	65.34±3.38 ^{abc}
	GV-17	14.26±3.53 ^a	29.14±8.9 ^a	44.63±9.51 ^{ab}	54.14±8.13 ^a	66.26±2.3 ^{ab}
	GV-22	15.03±1.23 ^a	32.67±2.3 ^a	54.91±3.22 ^a	61.35±1.69 ^a	63.34±0.31 ^{bc}
	GV-5	1.34±1.06 ^b	7.38±2.11 ^b	18.12±4.6 ^b	27.32±2.59 ^b	40.55±3.36 ^c
	GV-8	0.96±0.77 ^b	6.9±2.3 ^b	17.16±3.45 ^b	26.36±2.69 ^b	37.96±4.79 ^c
	GV-12	4.51±0.68 ^a	15.34±2.3 ^a	27.51±5.66 ^{ab}	42.27±5.28 ^a	57.32±3.93 ^a
	GV-16	4.51±0.29 ^a	14.95±1.73 ^a	25.79±3.74 ^{ab}	32.59±1.34 ^b	46.01±2.97 ^{bc}
Initial pH	GV-17	5.66±1.63 ^a	19.27±5.85 ^a	32.11±10.16 ^a	40.84±8.25 ^a	54.26±10.36 ^{ab}
	GV-22	1.63±0.96 ^b	6.33±0.39 ^b	19.65±2.01 ^b	32.5±1.92 ^b	51.19±4.6 ^{ab}
	GV-5	0.51±0.17 ^{ab}	3.73±0.47 ^c	30.42±6.6 ^c	32.41±6.6 ^d	30.11±7.06 ^b
	GV-8	1.12±0.38 ^a	65.39±1.87 ^a	78.12±3.09 ^a	74.59±4.31 ^a	66.77±1.72 ^a
	GV-12	0.46±0.46 ^{ab}	51.38±9.83 ^b	65.8±10.9 ^{ab}	62.27±5.7 ^b	57.06±4.94 ^a
	GV-16	0.01±0.01 ^b	38.9±8.92 ^{bc}	55.78±6.02 ^b	58.84±1.04 ^{bc}	56.08±8.92 ^a
	GV-17	0.87±0.78 ^a	30.47±10.6 ^{cd}	53.93±10.3 ^b	53.63±2.26 ^c	51.94±13.51 ^a
	GV-22	0.31±0.4 ^{ab}	21.58±2.8 ^d	38.14±0.76 ^c	36.61±2.8 ^d	30.47±11.2 ^b
	GV-5	24.08±0.41 ^c	103.53±0.85 ^{ab}	107.67±0.15 ^c	106.75±3.45 ^b	103.68±0.85 ^a
	GV-8	45.65±2.62 ^a	105.32±21.78 ^{ab}	123.11±2.61 ^a	115.59±5.83 ^a	88.91±4.45 ^b
pH 4.0	GV-12	33.44±1.38 ^b	109.66±3.99 ^a	117.79±6.75 ^{ab}	115.03±7.67 ^{ab}	110.43±8.13 ^a
	GV-16	16.87±1.53 ^d	70.25±10.28 ^c	86.2±2.61 ^d	85.89±2.15 ^c	82.67±1.69 ^b
	GV-17	15.95±1.53 ^d	69.33±2.77 ^c	86.96±4.3 ^d	84.97±1.84 ^c	73.31±3.84 ^c
	GV-22	17.79±3.07 ^d	87.12±2.15 ^{bc}	113.04±1.38 ^{bc}	108.9±3.38 ^{ab}	107.52±5.37 ^a

¹ Values are presented as means ± standard deviation. Different letters in the same column show significant differences ($P < 0.05$) in Duncan's multiple range test.

Table 2. Fermentation efficiency under multiple starters according to initial pH and fermentation period.

Initial pH	Strains ¹	Efficiency (%) according to the fermentation period (days)								
		3	6	9	12	15	18	21	24	
pH 3.0	AB	0.64±0.21 ^{2,a}	3.14±1.18 ^b	10.58±0.49 ^b	31.11±2.45 ^b	40.33±0.7 ^b	45.37±0.94 ^{cde}	56.56±2.34 ^b	54.72±0.77 ^b	58.77±0.39 ^c
	AC	0.97±0.9 ^a	4.22±0.19 ^a	17.31±0.66 ^a	40.79±5.86 ^a	47.74±4.15 ^a	57.02±2.65 ^a	64.64±2.98 ^a	57.95±1.36 ^a	63.91±2.23 ^a
	AD	0.35±0.19 ^a	1.28±0.21 ^c	10.52±0.65 ^b	27.74±2.15 ^{bc}	35.61±0.43 ^{cd}	42.55±4.25 ^e	50.6±1.99 ^d	42.06±2.48 ^e	54.97±0.85 ^d
	BC	0.22±0.1 ^a	2.18±0.65 ^{bc}	8.21±0.37 ^c	23.89±0.37 ^c	32.49±1.02 ^d	47.37±2.62 ^{bed}	55.54±2.25 ^{bc}	51.55±0.55 ^c	61.81±0.7 ^b
	BD	0.97±0.99 ^a	3.1±0.28 ^b	7.1±0.21 ^d	25.18±2.82 ^c	34.03±2.03 ^{cd}	43.13±2.61 ^{de}	51.8±2.6 ^{cd}	47.62±1.01 ^d	55.06±0.11 ^d
	CD	0.66±0.34 ^a	2.04±0.38 ^{bc}	8.74±0.28 ^c	27.73±1.53 ^{bc}	40.03±0.43 ^b	50.84±1.92 ^b	63.27±1.31 ^a	58.29±0.21 ^a	59.82±0.65 ^{bc}
	ABCD	0.78±0.53 ^a	2.11±0.49 ^{bc}	8.52±0.28 ^c	28.44±2.03 ^{bc}	36.43±0.49 ^c	48.54±1.11 ^{bc}	63.6±3.78 ^a	58.01±0.64 ^a	61.14±1.61 ^b
pH 4.0	AB	1.3±0.28 ^{cd}	2.96±0.28 ^d	6.53±0.49 ^e	12.92±0.1 ^e	15.87±0.38 ^d	22.08±0.95 ^d	30.35±1.69 ^d	31.93±1.23 ^d	42.48±0.46 ^c
	AC	2.13±0.65 ^{ab}	3.05±0.28 ^d	6.78±0.36 ^e	13.56±0.53 ^{de}	14.91±0.21 ^e	21.31±1.59 ^d	25.46±1.84 ^e	29.29±1.54 ^{de}	38.34±4.14 ^c
	AD	2.66±0.21 ^a	5.48±0.28 ^a	14.33±0.38 ^a	26.81±0.28 ^a	31.98±0.46 ^a	46.98±2.62 ^a	59.05±2.92 ^a	58.28±0.92 ^a	72.55±3.53 ^a
	BC	1.58±0 ^{bc}	3.61±0.37 ^c	7.72±0.39 ^d	16.52±1.21 ^c	18.73±0.64 ^c	30.23±1.87 ^c	37.73±3.07 ^c	42.48±0.92 ^c	58.13±3.53 ^b
	BD	2.16±0.32 ^{ab}	3.95±0.28 ^c	11.26±0.28 ^b	22.63±0.37 ^b	25.22±0.49 ^b	32.35±1.5 ^{bc}	40.49±3.22 ^{bc}	47.09±0.46 ^b	61.2±2.15 ^b
	CD	0.69±0.32 ^d	1.37±0.28 ^e	6.65±0.1 ^e	14.64±0.57 ^d	15.5±0.65 ^{de}	22.64±2.17 ^d	26.69±2.46 ^{de}	28.83±2.92 ^e	38.19±0.15 ^c
	ABCD	1.33±0.42 ^{cd}	4.47±0.28 ^b	10.31±0.19 ^c	21.68±0.93 ^b	25.62±0.49 ^b	33.85±1.82 ^b	42.64±1.54 ^b	47.7±2.15 ^b	61.04±0.92 ^b

¹ Symbols: A, *Acetobacter ascendens* GV-8; B, *A. ascendens* GV-12; C, *A. pasteurianus* GV-17; D, *A. pasteurianus* GV-22.² Values are presented as means ± standard deviation. Different letters in the same column show significant differences ($P < 0.05$) in Duncan's multiple range test.

Table 3. Volatile compounds according to fermentation period using *Acetobacter ascendens* GV-8 (A) and *A. ascendens* GV-12 (B) (Unit: Area%)

Compounds	Period (days)	Temp. ¹				30°C			
		0	12	18	27	0	12	18	27
Acetic acid, ethyl ester		71.89±2.45 ^{2,b}	81.85±2.02 ^a	79.15±0.79 ^a	81.27±2.41 ^a	67.29±2.16 ^c	78.93±1.68 ^b	84.51±1.32 ^a	16.18±2.45 ^d
Ethanol		14.61±0.53 ^a	10.9±0.86 ^b	10.32±0.42 ^b	6.75±0.31 ^c	17.27±0.74 ^a	9.47±0.50 ^b	6.49±0.47 ^c	ND ³
2-Methyl-1-propanol		0.02±0.00 ^b	0.04±0.01 ^{ab}	0.03±0.02 ^b	0.06±0.02 ^a	0.01±0.00 ^c	0.04±0.00 ^{ab}	0.03±0.01 ^b	0.04±0.01 ^a
2-Octanone		0.02±0.00 ^c	0.06±0.01 ^b	0.05±0.02 ^b	0.08±0.01 ^a	ND	0.02±0.01 ^c	0.03±0.01 ^b	0.04±0.00 ^a
2-Methyl-1-butanol		0.02±0.00 ^b	0.03±0.00 ^b	0.02±0.00 ^b	0.05±0.02 ^a	ND	0.04±0.01 ^b	0.04±0.01 ^b	0.08±0.02 ^a
Octanal		0.07±0.01 ^a	0.08±0.02 ^a	0.09±0.01 ^a	0.07±0.01 ^a	0.07±0.01 ^{bc}	0.11±0.03 ^{ab}	0.15±0.03 ^a	0.06±0.01 ^c
2-Hexadecanol		0.07±0.00 ^b	0.17±0.03 ^a	0.18±0.05 ^a	0.17±0.04 ^a	0.16±0.05 ^a	0.13±0.05 ^a	0.16±0.04 ^a	0.15±0.05 ^a
6-Methyl-5-hepten-2-one		0.05±0.00 ^a	0.02±0.00 ^b	0.02±0.01 ^b	0.01±0.00 ^b	0.08±0.01 ^a	0.05±0.01 ^b	0.07±0.02 ^{ab}	0.08±0.02 ^{ab}
Acetic acid, 2-ethylhexyl ester		0.07±0.00 ^a	0.09±0.01 ^a	0.12±0.04 ^a	0.12±0.03 ^a	ND	0.05±0.02 ^a	0.06±0.02 ^a	0.07±0.03 ^a
Nonanal		0.17±0.00 ^b	0.17±0.03 ^b	0.23±0.04 ^a	0.18±0.02 ^{ab}	0.16±0.01 ^b	0.19±0.02 ^b	0.26±0.04 ^a	0.15±0.03 ^b
Acetic acid		1.08±0.02 ^c	3.78±0.02 ^a	1.28±0.22 ^c	2.47±0.10 ^b	0.69±0.02 ^d	2.11±0.06 ^c	2.98±0.10 ^b	6.30±0.09 ^a
2-Ethylhexanol		1.48±0.02 ^{bc}	1.35±0.03 ^c	1.65±0.22 ^{ab}	1.70±0.02 ^a	0.60±0.01 ^d	1.54±0.07 ^a	1.18±0.02 ^c	1.39±0.11 ^b
Decanal		0.38±0.02 ^a	0.20±0.02 ^b	0.22±0.03 ^b	0.27±0.08 ^b	0.40±0.02 ^b	0.47±0.05 ^b	0.68±0.12 ^a	0.41±0.03 ^b
2-Amino-5-methylbenzoic acid		0.55±0.04 ^c	0.64±0.06 ^{bc}	0.69±0.04 ^b	0.83±0.07 ^a	0.60±0.02 ^a	0.45±0.07 ^c	0.56±0.05 ^{ab}	0.49±0.02 ^{bc}
2-Hydroxy-Benzoic acid, methyl ester		1.38±0.02 ^d	3.85±0.08 ^c	5.70±0.02 ^a	5.18±0.30 ^b	2.37±0.09 ^d	2.70±0.21 ^c	3.30±0.09 ^a	3.05±0.09 ^b
2,4-Di-tert-butylphenol		2.34±0.05 ^b	3.32±0.18 ^a	3.43±0.34 ^a	3.25±0.12 ^a	1.44±0.05 ^c	2.29±0.31 ^b	2.69±0.10 ^a	1.67±0.03 ^c

¹ Fermentation temperature.

² Values are presented as means ± standard deviation. Different letters in the same row show significant differences ($P < 0.05$) in Duncan's multiple range test.

³ Not detected.

Table 4. Volatile compounds according to fermentation period using *Acetobacter ascendens* GV-8 (A) and *Acetobacter pasteurianus* GV-22 (D) (Unit: Area%).

Compounds	Temp. ¹		20°C				30°C			
	Period (days)	0	12	18	27	0	12	18	27	
Acetic acid, ethyl ester		123.43±1.26 ^{2,a}	108.39±1.57 ^c	118.44±1.39 ^b	87.30±0.66 ^d	70.28±1.11 ^c	96.86±3.01 ^a	91.62±1.10 ^b	1.42±0.12 ^d	
Ethanol		217.16±2.60 ^a	14.30±0.26 ^b	12.99±0.19 ^b	6.77±0.32 ^c	16.39±0.12 ^a	10.63±0.29 ^b	6.35±0.19 ^c	ND ³	
2-Methyl-1-propanol		0.23±0.05 ^a	0.05±0.01 ^b	0.05±0.01 ^b	0.12±0.05 ^b	ND	0.06±0.01 ^a	0.11±0.06 ^a	0.07±0.01 ^a	
2-Octanone		0.11±0.01 ^a	0.02±0.01 ^b	0.04±0.02 ^b	0.02±0.00 ^b	ND	0.04±0.02 ^a	0.02±0.01 ^{bc}	0.03±0.01 ^{ab}	
2-Methyl-1-butanol		0.29±0.05 ^a	0.06±0.01 ^b	0.07±0.02 ^b	0.08±0.02 ^b	ND	0.04±0.01 ^a	0.04±0.01 ^a	0.04±0.00 ^a	
Octanal		1.30±0.18 ^a	0.08±0.01 ^b	0.12±0.04 ^b	0.06±0.01 ^b	0.04±0.01 ^c	0.09±0.01 ^a	0.06±0.00 ^b	0.09±0.01 ^a	
2-Hexadecanol		0.63±0.07 ^a	0.06±0.02 ^c	0.08±0.01 ^c	0.18±0.08 ^b	0.14±0.06 ^a	0.15±0.05 ^a	0.15±0.04 ^a	0.19±0.10 ^a	
6-Methyl-5-hepten-2-one		0.16±0.07 ^a	0.04±0.01 ^b	0.09±0.01 ^{ab}	0.04±0.03 ^b	0.05±0.02 ^b	0.05±0.01 ^b	0.08±0.00 ^{ab}	0.13±0.07 ^a	
Acetic acid, 2-ethylhexyl ester		0.78±0.06 ^a	0.04±0.00 ^b	0.05±0.02 ^b	0.07±0.03 ^b	0.03±0.02 ^a	0.05±0.01 ^a	0.04±0.01 ^a	0.05±0.01 ^a	
Nonanal		3.20±0.06 ^a	0.31±0.05 ^b	0.25±0.05 ^{bc}	0.18±0.09 ^c	0.13±0.06 ^c	0.17±0.01 ^c	0.28±0.03 ^b	0.47±0.06 ^a	
Acetic acid		16.29±1.38 ^a	2.44±0.23 ^b	2.40±0.44 ^b	2.80±0.12 ^b	0.81±0.15 ^d	2.50±0.03 ^c	4.37±0.10 ^b	10.78±0.77 ^a	
2-Ethylhexanol		15.70±0.95 ^a	1.39±0.39 ^b	1.37±0.26 ^b	1.32±0.05 ^b	1.13±0.12 ^{bc}	1.10±0.07 ^c	1.30±0.07 ^b	1.56±0.11 ^a	
Decanal		6.15±0.03 ^a	0.55±0.18 ^b	0.48±0.09 ^b	0.21±0.07 ^c	0.28±0.08 ^b	0.50±0.15 ^a	0.32±0.06 ^{ab}	0.33±0.08 ^{ab}	
2-Amino-5-methylbenzoic acid		4.89±0.54 ^a	0.42±0.14 ^b	0.61±0.29 ^b	0.79±0.04 ^b	0.57±0.03 ^a	0.42±0.06 ^b	0.44±0.04 ^b	0.47±0.05 ^b	
2-Hydroxy-Benzoic acid, methyl ester		13.07±0.29 ^a	1.23±0.09 ^c	1.45±0.18 ^{bc}	1.76±0.04 ^b	3.01±0.05 ^c	3.47±0.11 ^b	3.56±0.02 ^{ab}	3.76±0.19 ^a	
2,4-Di-tert-butylphenol		18.93±0.68 ^a	1.83±0.07 ^b	2.49±0.19 ^b	2.52±0.37 ^b	2.76±0.07 ^{ab}	2.48±0.28 ^b	2.60±0.06 ^{ab}	3.05±0.39 ^a	

¹ Fermentation temperature.

² Values are presented as means ± standard deviation. Different letters in the same row show significant differences ($P < 0.05$) in Duncan's multiple range test.

³ Not detected.

Table 5. Volatile compounds according to fermentation period using *A. ascendens* GV-8 (A), *A. ascendens* GV-12 (B), *A. pasteurianus* GV-17 (C), and *A. pasteurianus* GV-22 (D) (Unit: Area%).

Compounds	Temp. ¹		20°C				30°C			
	Period (days)	0	12	18	27	0	12	18	27	
Acetic acid, ethyl ester		103.52±1.76 ^{2,a}	98.73±1.55 ^b	107.63±3.51 ^a	92.50±1.50 ^c	56.50±3.90 ^c	86.70±1.97 ^b	98.89±1.02 ^a	99.15±0.78 ^a	
Ethanol		18.6±0.46 ^a	11.17±0.55 ^b	11.68±0.28 ^b	6.14±0.11 ^c	18.93±0.82 ^a	17.23±1.77 ^{ab}	16.06±3.31 ^{ab}	12.63±2.79 ^b	
2-Methyl-1-propanol		0.03±0.01 ^a	0.06±0.03 ^a	0.03±0.00 ^a	0.06±0.01 ^a	ND ³	0.02±0.02 ^a	0.03±0.03 ^a	0.03±0.02 ^a	
2-Octanone		0.02±0.00 ^a	0.02±0.01 ^a	0.01±0.01 ^a	0.03±0.02 ^a	ND	ND	0.03±0.03 ^a	0.03±0.03 ^a	
2-Methyl-1-butanol		0.01±0.00 ^b	0.03±0.01 ^a	0.04±0.01 ^a	0.05±0.02 ^a	ND	ND	0.04±0.03 ^{ab}	0.07±0.01 ^a	
Octanal		0.11±0.06 ^a	0.05±0.01 ^a	0.04±0.00 ^a	0.06±0.01 ^a	0.07±0.02 ^b	0.17±0.10 ^{ab}	0.21±0.06 ^a	0.13±0.00 ^{ab}	
2-Hexadecanol		0.15±0.05 ^a	0.15±0.03 ^a	0.10±0.00 ^a	0.16±0.09 ^a	0.08±0.02 ^a	0.06±0.03 ^a	0.11±0.07 ^a	0.12±0.05 ^a	
6-Methyl-5-hepten-2-one		0.07±0.07 ^a	0.07±0.00 ^a	0.03±0.01 ^a	0.06±0.01 ^a	0.06±0.00 ^b	0.14±0.04 ^a	0.08±0.03 ^b	0.15±0.00 ^a	
Acetic acid, 2-ethylhexyl ester		0.12±0.06 ^a	0.08±0.02 ^{ab}	0.08±0.01 ^{ab}	0.05±0.01 ^b	0.06±0.01 ^a	0.10±0.02 ^a	0.10±0.07 ^a	0.07±0.01 ^a	
Nonanal		0.16±0.01 ^b	0.12±0.04 ^b	0.20±0.07 ^{ab}	0.25±0.03 ^a	0.19±0.02 ^b	0.39±0.08 ^a	0.51±0.07 ^a	0.38±0.09 ^a	
Acetic acid		1.56±0.12 ^b	1.53±0.10 ^b	1.87±0.10 ^b	4.70±1.65 ^a	1.10±0.14 ^c	1.46±0.13 ^b	1.55±0.12 ^b	2.18±0.14 ^a	
2-Ethylhexanol		1.39±0.03 ^a	1.69±0.25 ^a	1.62±0.05 ^a	1.39±0.26 ^a	1.41±0.07 ^a	1.74±0.19 ^a	1.44±0.22 ^a	1.64±0.24 ^a	
Decanal		0.32±0.08 ^{ab}	0.22±0.05 ^b	0.27±0.10 ^{ab}	0.39±0.03 ^a	0.29±0.08 ^b	0.59±0.09 ^a	0.67±0.22 ^a	0.51±0.07 ^{ab}	
2-Amino-5-methylbenzoic acid		0.61±0.05 ^a	0.62±0.06 ^a	0.51±0.07 ^a	0.70±0.25 ^a	0.41±0.07 ^a	0.46±0.11 ^a	0.49±0.08 ^a	0.44±0.12 ^a	
2-Hydroxy-Benzoic acid, methyl ester		2.54±0.35 ^a	2.53±0.25 ^a	1.49±0.03 ^b	2.36±0.54 ^a	1.44±0.12 ^b	1.75±0.20 ^{ab}	1.93±0.07 ^a	1.70±0.28 ^{ab}	
2,4-Di-tert-butylphenol		3.21±0.24 ^a	2.83±0.12 ^b	3.02±0.15 ^{ab}	1.77±0.18 ^c	2.54±0.12 ^b	3.26±0.28 ^a	2.91±0.59 ^{ab}	1.83±0.12 ^c	

¹ Fermentation temperature.

² Values are presented as means ± standard deviation. Different letters in the same row show significant differences ($P < 0.05$) in Duncan's multiple range test.

³ Not detected.

Table 6. Alpha diversity analysis under multiple starters and fermentation temperature.

Strain ¹	Temp.	Period (day)	Valid reads and OTUs		Species richness			Species evenness			Phylogenetic diversity	Good's coverage (%)
			Target reads	OTUs	ACE	CHAO	Jackknife	NPShannon	Shannon	Simpson		
AB	20°C	12	33,527	7	13	10	11	0	0	1	19	100
		27	30,341	9	14	12	14	0	0	1	44	100
	30°C	12	36,745	9	10	10	12	0	0	1	22	100
		27	33,253	5	11	7	8	0	0	1	10	100
AD	20°C	12	31,131	5	7	6	7	0	0	1	9	100
		27	28,716	12	18	15	18	0	0	1	28	100
	30°C	12	26,815	8	9	9	11	0	0	1	20	100
		27	29,326	9	37	20	20	0	0	1	40	100
ABCD	20°C	12	33,601	7	9	8	10	0	0	1	21	100
		27	30,470	5	11	7	8	0	0	1	14	100
	30°C	12	34,377	4	4	4	5	0	0	1	11	100
		27	26,846	5	11	7	8	0	0	1	19	100

¹ Symbols: A, *Acetobacter ascendens* GV-8; B, *A. ascendens* GV-12; C, *A. pasteurianus* GV-17; D, *A. pasteurianus* GV-22.