SUPPLEMENTAL FILE

Ratio of histamine-producing/non-histamine-producing subgroups of *Tetragenococcus halophilus* determines the histamine accumulation during spontaneous fermentation of soy sauce

Jinjin Ma, Yao Nie, Lijie Zhang*, Yan Xu*

Lab of Brewing Microbiology and Applied Enzymology, Key Laboratory of Industrial Biotechnology of Ministry of Education and School of Biotechnology, Jiangnan University, 1800 Lihu Avenue, Wuxi, Jiangsu 214122, China

Running title: Strain specificity related to histamine accumulation

*Corresponding author. Tel.: +86-510-85918201; Fax: +86-510-85918201.

E-mail address: yxu@jiangnan.edu.cn (Yan Xu); zhanglj@jiangnan.edu.cn (Lijie Zhang);

Supplemental files include:

Supplemental figures: Fig. S1-S7;

Supplemental tables: Table S1-S2;

Supplementary figures:

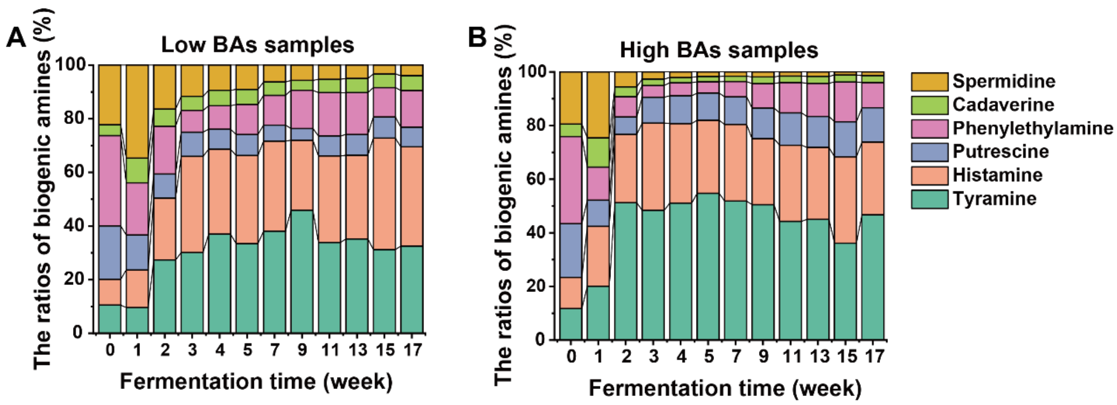

Fig. S1 The profiles of diverse BAs production in low-BAs soy sauce samples (A) and high-BAs soy sauce samples (B).

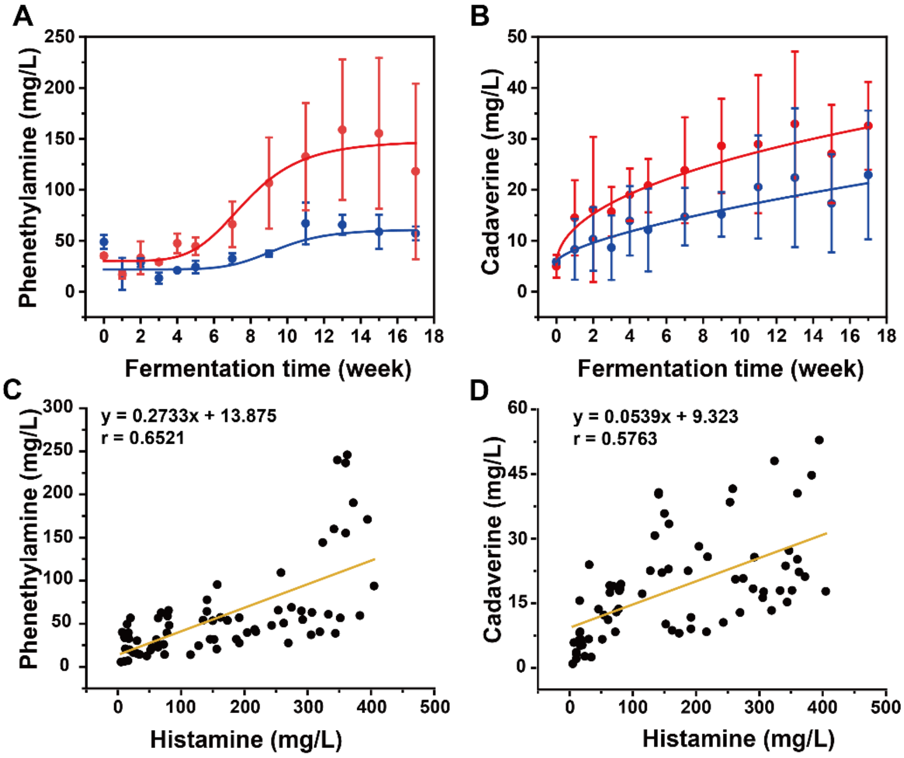

Fig. S2 Biogenic amines during the soy sauce fermentation. Dynamic of biogenic amines accumulation in high- (red line) and low- (blue line) BAs samples (A) Phenethylamine, (B) Cadaverine. Correlation analysis of phenethylamine (C) and cadaverine (D) with histamine. Data are indicated as the average of three samples ± standard deviation.

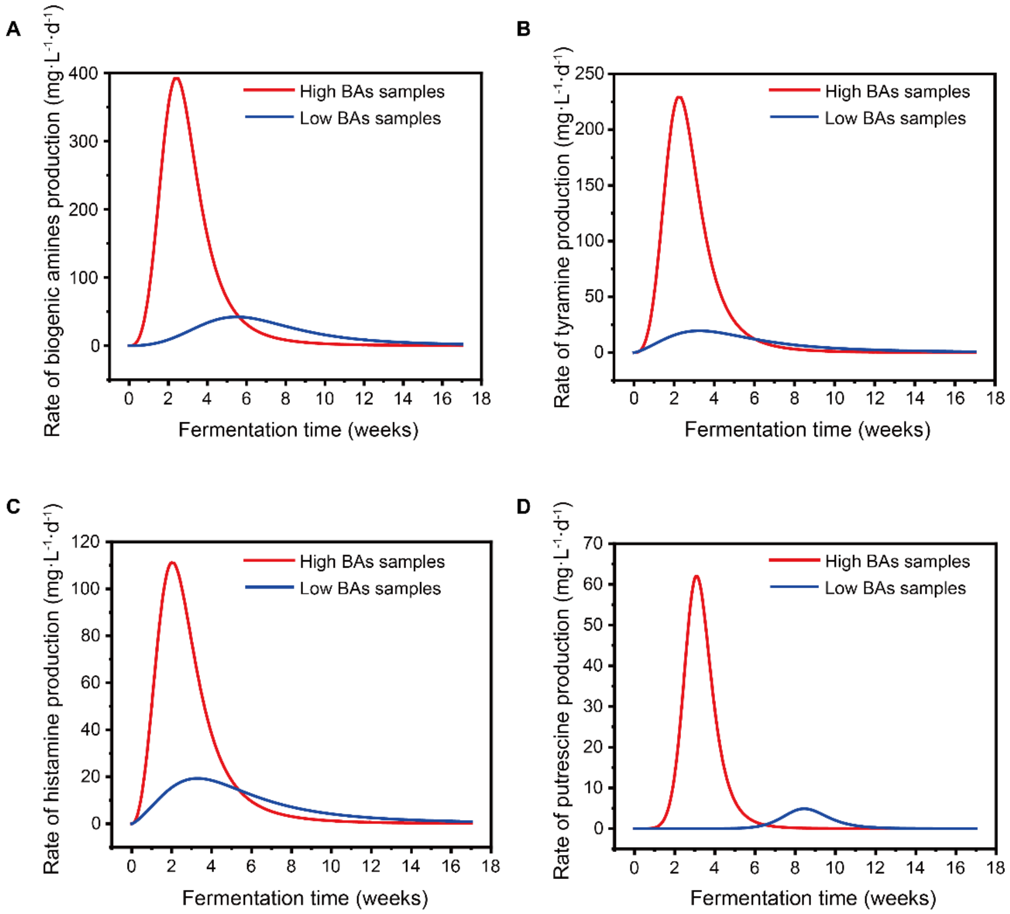

Fig. S3 BAs production rates during soy sauce fermentation. (A) The total of biogenic amines; (B) Tyramine; (C) Histamine; (D) Putrescine.

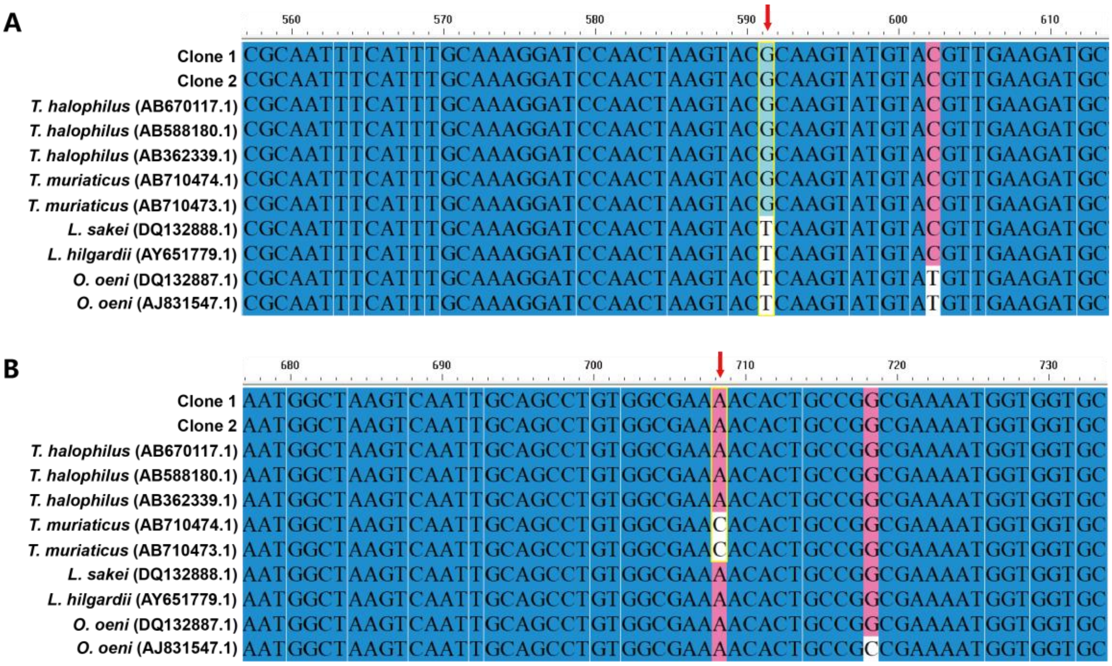

Fig. S4 Partial clone sequence alignment of *hdcA* gene clone library. The red arrows show the key differential bases of the different sequences. Note: Reference genomes from the same species retained only samples with sequence differences.

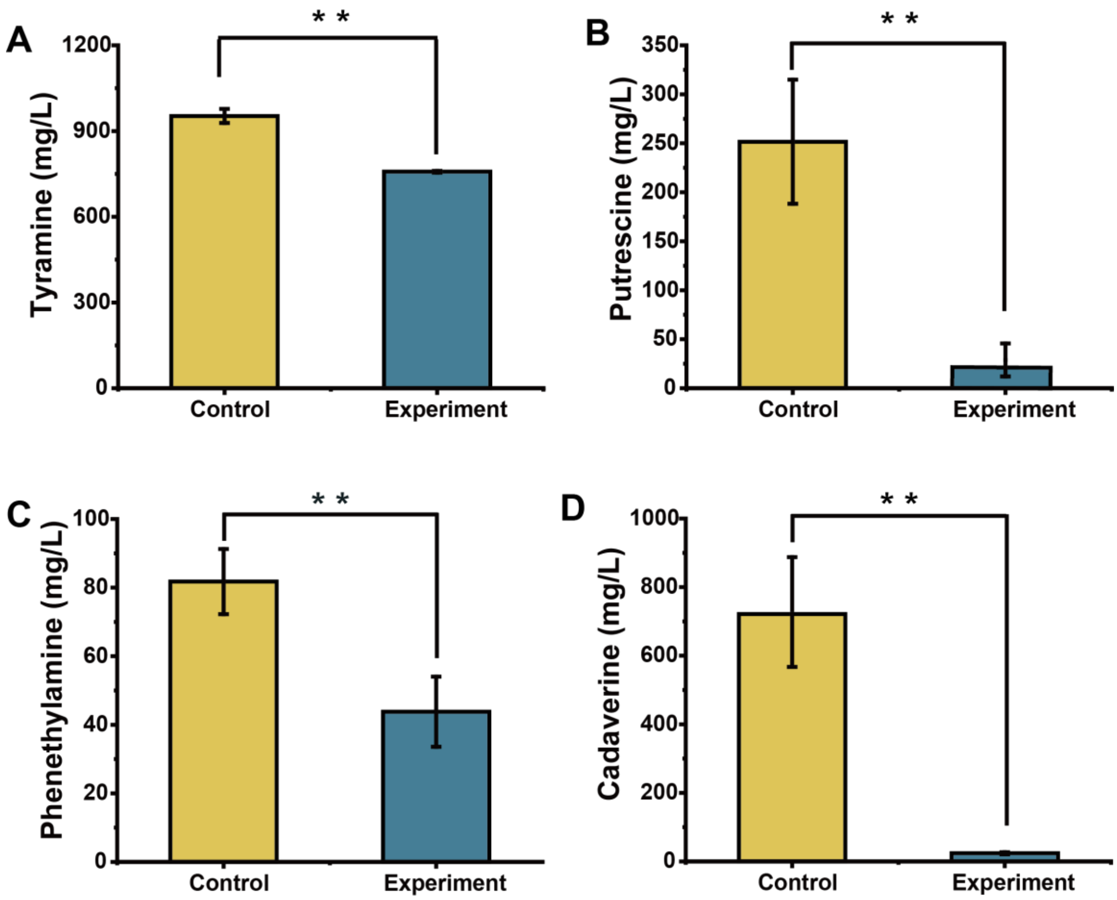

Fig. S5 Four BAs control by artificially modifying the ratio of histamine-producing to non-histamine-producing *T. halophilus* strains during soy sauce fermentation. (A) Tyramine; (B) Putrescine; (C) Phenethylamine; (D) Cadaverine. ANOVA: ** means $p < 0.01$

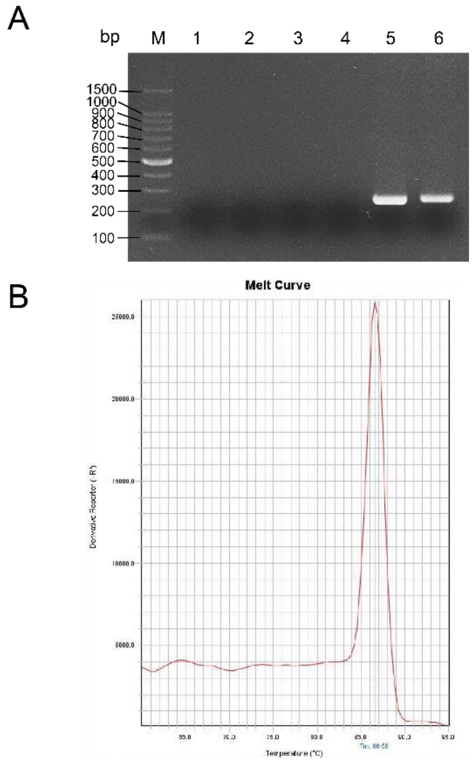

Fig. S6 PCR and qPCR were used to verify the specificity of the primers TetRT-F1 and TetRT-R1, which was used for the quantitative of *T. halophilus*. A: 16 S rRNA amplification of 6 kinds of dominant soy sauce bacteria by using primers TetRT-F1 and TetRT-R1. M. marker; 1. *Weissella paramesenteroides*, 2. *Weissella cibaria*, 3. *Weissella viridescens*, 4. *Weissella confuse*, 5. Histamine-producing *T. halophilus*, 6. Non-histamine-producing *T. halophilus*. B: Melt curve of qPCR.

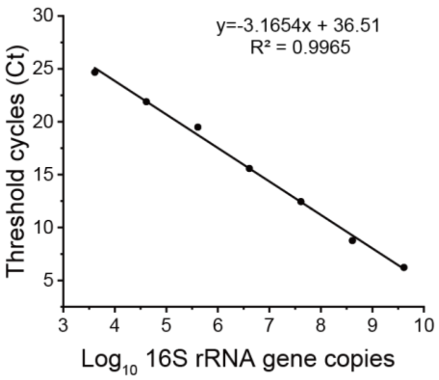

Fig. S7 Real-time quantitative PCR (qPCR) analysis of 10-fold serial dilutions of control plasmid DNA. Cycle threshold values (Ct) are plotted against the calculated plasmid copy number. The equation and R2 value of the regression line are indicated.

Supplementary tables:

Table 1 Maximum histamine production capacity of the reported histamine-producing microorganisms.

Histamine-producing Microorganisms	Maximum production of histamine (mg/L)	Culture Conditions	References
Morganella morganii	6000	TSB medium	(1)
Tetragenococcus muriaticus	4870	MRSH medium	(2)
Photobacterium damselae subsp. *Damselae*	3670	TSB medium	(1)
Klebsiella pneumoniae	3416.43	HDB medium	(3)
Photobacterium phosphoreum	3140	TSB medium	(1)
Tetragenococcus halophilus	3000	HB medium	(4)
Morganella psychrotolerans	3000	Canned tuna (fish)	(5)
Klebsiella oxytoca	2231	Broth	(6)
Staphylococcus epidermidis	1945.9	HEB medium	(7)
Photobacterium kishitanii	1545	LSW-70 broth	(8)
Staphylococcus capitis	1260	TSB medium	(9)
Klebsiella aerogenes	1000	Histidine broth	(10)
Streptococcus thermophilus	540	LM17 medium	(11)

Lentilactobacillus saerimneri	522.41	MRSH medium	(12)
Lentilactobacillus parabuchneri	470.16	MRSH medium	(13)
Lentilactobacillus buchneri	452.38	MRSH medium	(12)
Lentilactobacillus hilgardii	235	H-MDBmod medium	(14)
Oenococcus oeni	99	H-MDBmod medium	(14)
Clostridium perfringens	41.1	Decarboxylating broth	(15)

Table S2 Histamine production of *T. halophilus* in MRS medium containing 1% histidine and soy sauce simulated fermentation system

NO.	Strain	source	*hdcA* gene	MRS+1% histidine (mg/L)	Simulated soy sauce fermentation (mg/L)
1	*T. halophilus* H178	High-BAs samples	+	1287.48	555.85
2	*T. halophilus* H128	High-BAs samples	+	1286.32	609.61
3	*T. halophilus* H168	High-BAs samples	+	1241.23	655.97
4	*T. halophilus* L64	Low BAs samples	+	1221.43	635.27
5	*T. halophilus* H181	High-BAs samples	+	1220.83	620.39
6	*T. halophilus* H189	High-BAs samples	+	1209.31	617.33
7	*T. halophilus* H124	High-BAs samples	+	1207.35	599.12
8	*T. halophilus* H42	High-BAs samples	+	1202.8	520.63
9	*T. halophilus* H29	High-BAs samples	+	1200.25	515.77
10	*T. halophilus* H125	High-BAs samples	+	1184.64	559.59
11	*T. halophilus* L100	Low BAs samples	+	1181.65	684.25
12	*T. halophilus* H159	High-BAs samples	+	1170.62	633.9
13	*T. halophilus* H156	High-BAs samples	+	1168.66	558.12
14	*T. halophilus* L45	Low BAs samples	+	1164.55	655.06
15	*T. halophilus* L137	Low BAs samples	+	1153.3	540.84
16	*T. halophilus* L106	Low BAs samples	+	1151.65	560.61
17	*T. halophilus* L134	Low BAs samples	+	1151.61	650.03

18	*T. halophilus* L192	Low BAs samples	+	1146.98	512.07
19	*T. halophilus* H45	High-BAs samples	+	1145.96	655.45
20	*T. halophilus* L40	Low BAs samples	+	1125.15	557.12
21	*T. halophilus* H121	High-BAs samples	+	1116.27	583.21
22	*T. halophilus* H131	High-BAs samples	+	1113.86	633.19
23	*T. halophilus* H52	High-BAs samples	+	1110.21	596.89
24	*T. halophilus* L143	Low BAs samples	+	1108.21	593.93
25	*T. halophilus* H32	High-BAs samples	+	1104.75	670.82
26	*T. halophilus* L31	Low BAs samples	+	1103.76	634.41
27	*T. halophilus* H180	High-BAs samples	+	1091.05	622.49
28	*T. halophilus* L15	Low BAs samples	+	1088.45	508.68
29	*T. halophilus* H99	High-BAs samples	+	1085.82	611.1
30	*T. halophilus* L123	Low BAs samples	+	1081.43	563.37
31	*T. halophilus* H115	High-BAs samples	+	1074.06	664.31
32	*T. halophilus* H103	High-BAs samples	+	1061.32	654.11
33	*T. halophilus* H111	High-BAs samples	+	1037.37	521.32
34	*T. halophilus* H114	High-BAs samples	+	1034.16	594.84
35	*T. halophilus* H48	High-BAs samples	+	1023.55	504.65
36	*T. halophilus* H47	High-BAs samples	+	1008.36	508.14
37	*T. halophilus* L205	Low BAs samples	+	898.45	612.5

Note: Data for non-histamine-producing strains were not shown.

REFERENCES

1. Kanki M, Yoda T, Ishibashi M, Tsukamoto T. 2004. *Photobacterium phosphoreum* caused a histamine fish poisoning incident. Int J Food Microbiol 92:79-87.
2. Kobayashi T, Wang X, Shigeta N, Taguchi C, Ishii K, Shozen K-i, Harada Y, Imada C, Terahara T, Shinagawa A. 2016. Distribution of histamine-producing lactic acid bacteria in canned salted anchovies and their histamine production behavior. Ann Microbiol 66:1277-1284.
3. Özoğul F. 2004. Production of biogenic amines by *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei* using a rapid HPLC method. Eur Food Res Technol 219:465-469.
4. Satomi M, Furushita M, Oikawa H, Yoshikawa-Takahashi M, Yano Y. 2008. Analysis of a 30 kbp plasmid encoding histidine decarboxylase gene in *Tetragenococcus halophilus* isolated from fish sauce. Int J Food Microbiol 126:202-209.
5. Wang D, Yamaki S, Kawai Y, Yamazaki K. 2020. Histamine production behaviors of a psychrotolerant histamine-producer, *Morganella psychrotolerans*, in various environmental conditions. Curr Microbiol 77:460-467.
6. Lopez-Sabater EI, Rodriguez-Jerez JJ, Roig-Sagues AX, Teresa Mora-Ventura M. 1994. Bacteriological quality of tuna fish (Thunnus thynnus) destined for canning: effect of tuna handling on presence of histidine decarboxylase bacteria and histamine level. J Food Prot 57:318-323.
7. Hernández-Herrero MM, Roig-Sagués AX, Rodríguez-Jerez JJ, Mora-Ventura MT. 1999. Halotolerant and halophilic histamine-forming bacteria isolated during the ripening of salted anchovies (Engraulis encrasicholus). J Food Prot 62:509-514.
8. Bjornsdottir-Butler K, Abraham A, Harper A, Dunlap P, Benner Jr R. 2018. Biogenic amine production by and phylogenetic analysis of 23

photobacterium species. J Food Prot 81:1264-1274.

9. Kung HF, Lee YH, Teng DF, Hsieh PC, Wei CI, Tsai YH. 2006. Histamine formation by histamine-forming bacteria and yeast in mustard pickle products in Taiwan. Food Chem 99:579-585.

10. Torido Y, Ohshima C, Takahashi H, Miya S, Iwakawa A, Kuda T, Kimura B. 2014. Distribution of psychrophilic and mesophilic histamine-producing bacteria in retailed fish in Japan. Food Control 46:338-342.

11. Rossi F, Gardini F, Rizzotti L, La Gioia F, Tabanelli G, Torriani S. 2011. Quantitative analysis of histidine decarboxylase gene (hdcA) transcription and histamine production by *Streptococcus thermophilus* PRI60 under conditions relevant to cheese making. Appl Environ Microbiol 77:2817-2822.

12. Sumner SS, Speckhard MW, Somers EB, Taylor SL. 1985. Isolation of histamine-producing *Lactobacillus buchneri* from Swiss cheese implicated in a food poisoning outbreak. Appl Environ Microbiol 50:1094-1096.

13. Diaz M, del Rio B, Sanchez-Llana E, Ladero V, Redruello B, Fernández M, Martin MC, Alvarez MA. 2018. *Lactobacillus parabuchneri* produces histamine in refrigerated cheese at a temperature-dependent rate. Int J Food Sci Technol 53:2342-2348.

14. Landete JM, Ferrer S, Pardo I. 2010. Which lactic acid bacteria are responsible for histamine production in wine? J Appl Microbiol 99:580-586.

15. Moon JS, Cho SK, Choi HY, Kim JE, Kim S-Y, Cho K-J, Han NS. 2010. Isolation and characterization of biogenic amine-producing bacteria in fermented soybean pastes. J Microbiol 48:257-261.