KOREA
We think the Unthinkable

KOREA UNIVERSITY
1905

생활한 기상이 뜨거운 열정과 손잡는 곳, 세계무대와 통하는 민족지성의 심장
UNIVERSITY

August 12, 2015

4th International Conference and Exhibition on
Food Processing & Technology
London, UK

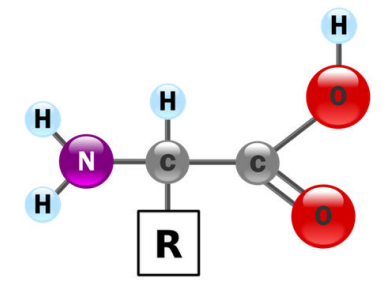
Occurrence, Impact and Biocontrol of Biogenic Amines in Fermented Soybean Foods

Professor Jae-Hyung Mah

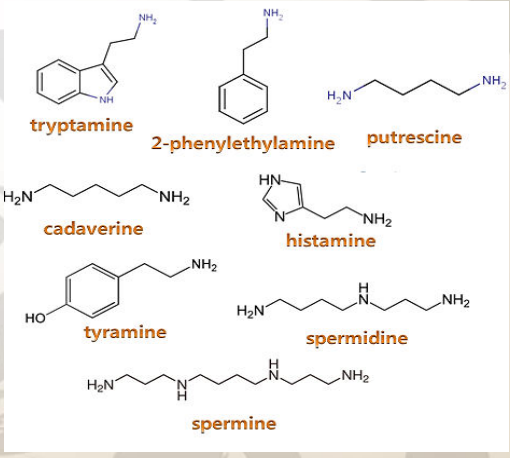
Department of Food and Biotechnology
Korea University

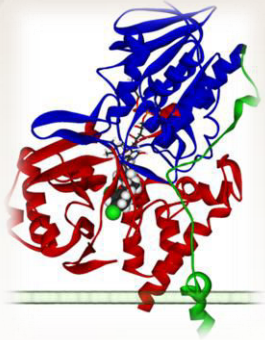
Biogenic amines

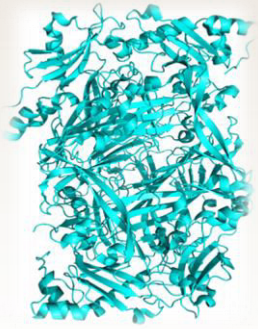
Biogenic amines are mainly produced by the growth of decarboxylase-positive **microorganisms**



Microbial decarboxylation







High dose of amines

Detoxication system

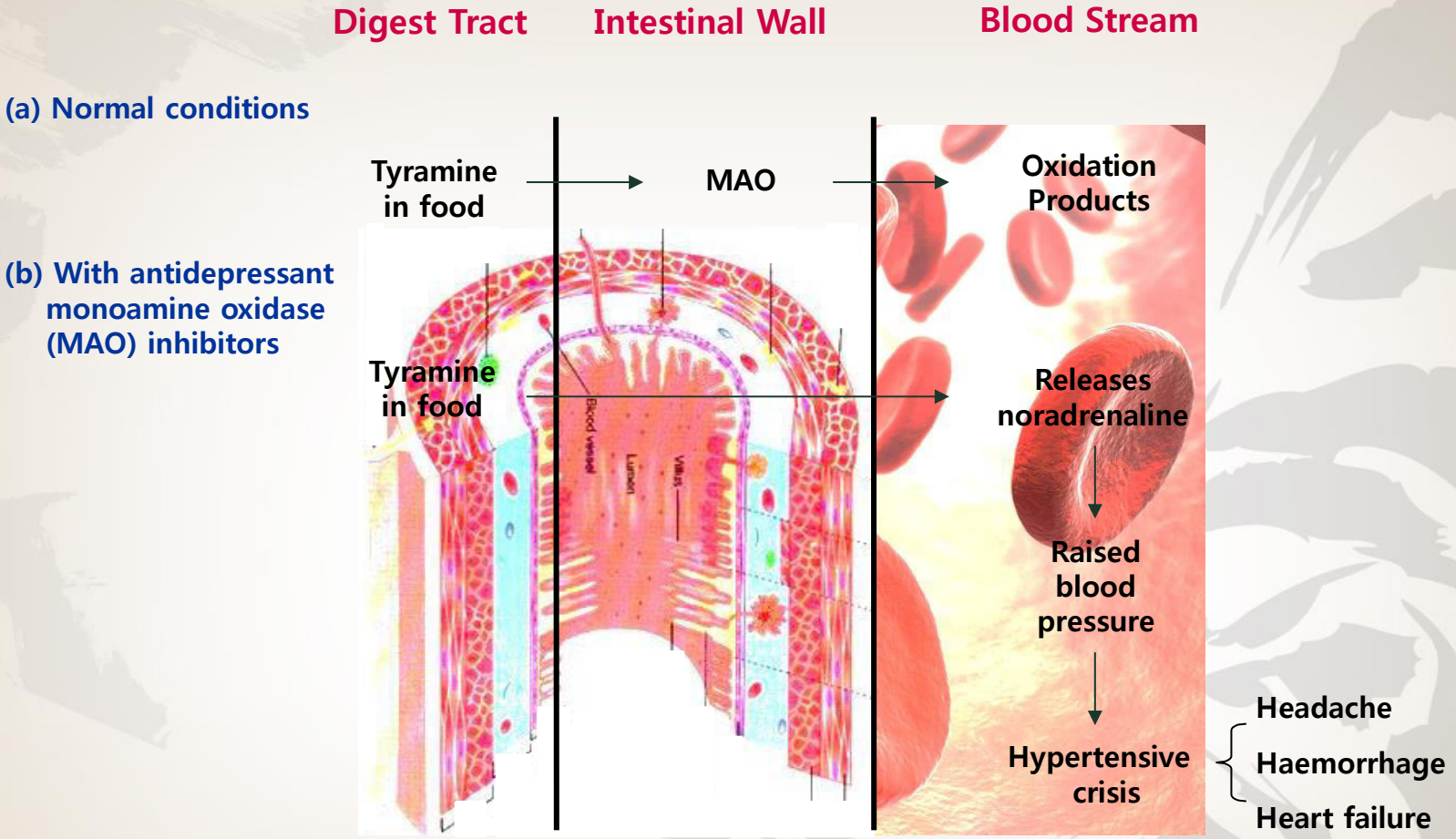
Specific inhibitors

Guidelines

Tyramine : 100-800 mg/kg
Histamine : 100 mg/kg
ß-phenylethylamine : 30 mg/kg

Monoamine oxidase
Diamine oxidase
Polyamine oxidase

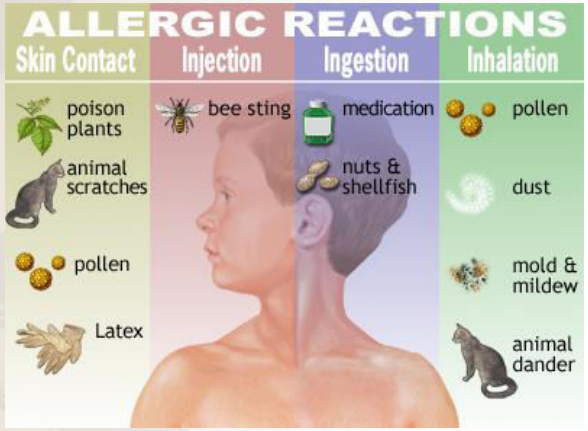
Pargyline,
Phenelzine,
Brofaromine,
Isocarboxazide,
Tranylcypromine



The mechanism of detoxication and inhibition of monoamine oxidase against the tyramine in food

Allergic reaction

IgE + mastcell → mastcell degranulation → release mediators (e. g. histamine)



indirect → vegetative nervous system

direct → lungs / skin / intestine

Non allergic reaction → Food intolerance

Biogenic amines
e. g. histamine



대한민국을 이끌어온 자랑스럽고 위대한 역사, 눈부신 고대의 새로운 백년도 지켜가겠습니다.

Biogenic amines in foods and their physiological and toxicological effects

Biogenic amines	Precursor	Physiological effects	Toxicological effects
Histamine	Histidine	Neurotransmitter, local hormone, gastric acid secretion, cell growth and differentiation, regulation of circadian rhythm, body temperature, food intake, learning and memory, immune response, allergic reactions	Headaches, sweating, burning nasal secretion, facial flushing, right red rashes, dizziness, itching rashes, oedema (eyelids), urticaria, difficulty in swallowing, diarrhea, respiratory distress, bronchospasm, increased cardiac output, tachycardia, extrasystoles, blood pressure disorders
Tyramine	Tyrosine	Neurotransmitter, peripheral vasoconstriction, increase cardiac output, increase respiration, elevate blood glucose, release of norepinephrine	Headaches, migraine, neurological disorders, nausea, vomiting, respiratory disorders, hypertension
Putrescine and Cadaverine	Ornithine and lysine	Regulation of gene expression, maturation of intestine, cell growth and differentiation	Increased cardiac output, tachycardia, hypotension, carcinogenic effects

Alvarez *et al.*, 2014. The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. Food science and technology. 39, 146-155.
(Ladero, Calles-Enriquez *et al.*, 2010; Ladero, Fernandez *et al.*, 2010; Ladero, Martienz *et al.*, 2010)

Tolerance levels of histamine in foods

▷ The regulation for canned albacore, skipjack, yellowfin tuna, mahimahi (FDA, 1982)
- 20 mg% : "decomposed" as an indication of prior mishandling
- **50 mg%** : "danger to health" as an indication of a potential health hazard

▷ The guideline for histamine content of fish (Shalaby, 1996)
- < 5 mg% : safe for consumption
- 5~20 mg% : possibly toxic
- 20~100 mg% : probably toxic
- **> 100 mg%** : toxic and unsafe for consumption



▷ The regulation for maximum allowable levels (EU, 1991)
- fresh fish (n=9; c=2; m=100 ppm; M=200 ppm)
- enzymatically ripened fish products (n=9; c=2; m=200 ppm; M=400 ppm)

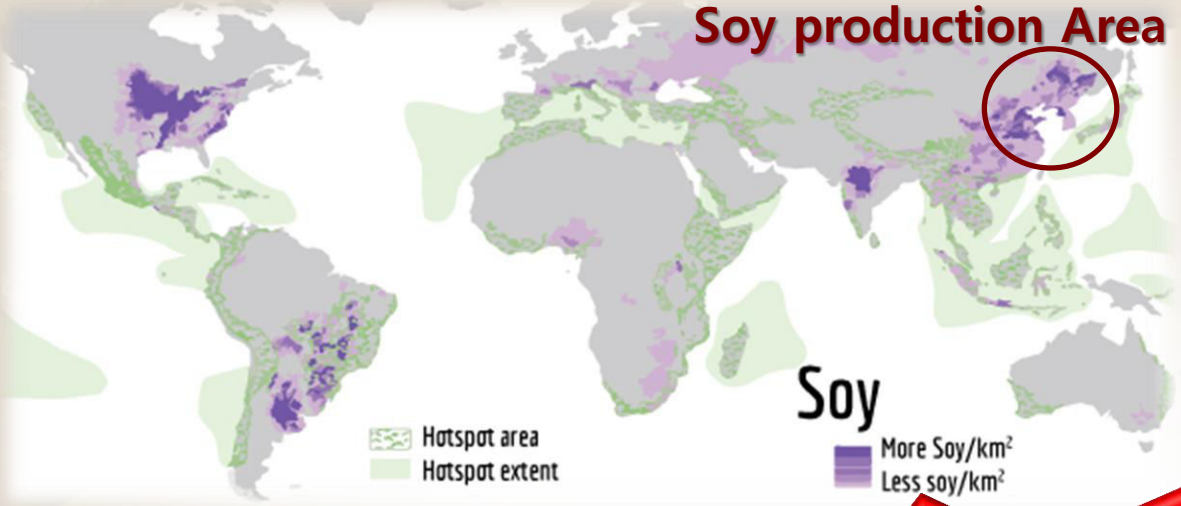
▷ The permissible limit of wine : 10 mg/L (Switzerland)

Studies on the tolerance levels of biogenic amines in foods

Food	Maximum amounts (mg/kg)						Ref
	His	Cad	Put	Tyr	β-Phe	Total	
General	-	-	-	-	-	40[a]	Ayres *et al.*, 1980
	100	**-**	**-**	**100-800**	**30**	**-**	**Brink *et al.*, 1990**
	-	-	-	-	3	-	Sandler *et al.*, 1974
	-	-	-	6	-	-	Blackwell, 1965
Fish	-	-	-	-	-	300[b]	Spanjer and Van Roode, 1991
Canned tuna	-	0.5	-	-	-	-	Sims *et al.*, 1992
Cheese	-	-	-	-	-	900[c]	Spanjer and Van Roode, 1991
Sauerkraut	10	25	50	20	5	-	Kuensh *et al.*, 1989

[a] As total BA per meal
[b] Sum of his + cad + put
[c] Sum of his + cad + put + tyr

Soy production Area



Fermented soybeans?







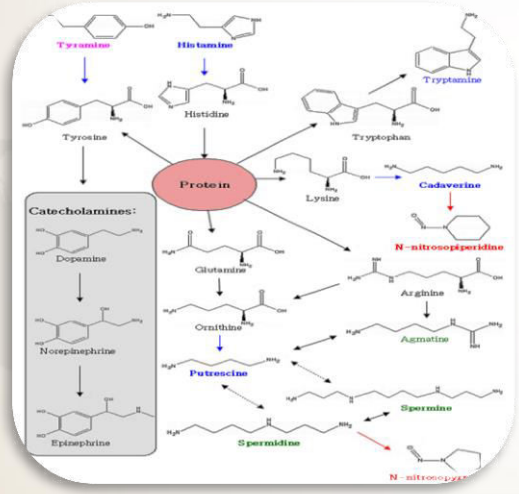




High protein foods



Microorganisms



Biogenic amines



Toxicological effects



Carcinogen precursors

Fermented foods



Doenjang, Cheonggukjang, Gochujang





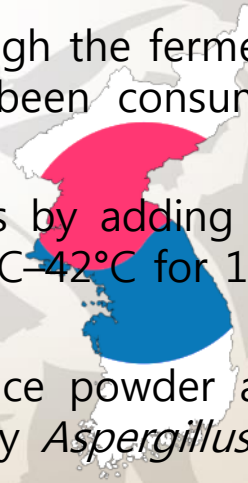


Korean traditional fermented soybean pastes

Doenjang is a traditional soybean paste, produced from *meju* through the fermentation of soybeans by naturally occurring bacteria and fungi and has been consumed for centuries as a rich protein source and flavoring ingredient in Korea

Cheonggukjang is made through fermentation of boiled soybeans by adding *Bacillus subtilis*, which is usually in the air or in *jip* (dried rice plants), at 40°C–42°C for 1–3 days without adding salt

Gochujang is produced by mixing *meju* powder with glutinous rice powder and red pepper powder, and then the mixture is fermented by using mainly *Aspergillus oryzae* and *Bacillus subtilis*



Natto



Japanese fermented soybean product made from boiled soybeans fermented for 15–24 h with *B. subtilis* var. *natto* (previously called *B. natto*) that produces a strong flavor and sticky texture (Ohta, 1986)

The product is generally served as it is with mustard and/or soy sauce and often used as a topping for cooked rice

Miso



Japanese fermented soybean paste made from soybeans and salt (and sometimes cereal grains) through a two-step fermentation with molds, yeasts and bacteria (Fukushima, 1981; Wang & Hesseltine, 1979)



Doubanjiang



Chinese traditional fermented soybean product produced through the fermentation of soybeans, broad beans, chili peppers, salt and spices by naturally occurring bacteria (Yamaura, 2004, Whitaker, 1978)



Tempeh

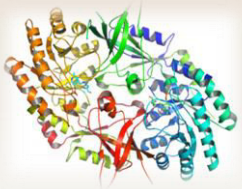


Indonesian traditional soybean product made by a natural culturing and controlled fermented process that binds soybeans into a cake from, similar to a very firm vegetarian burger patty



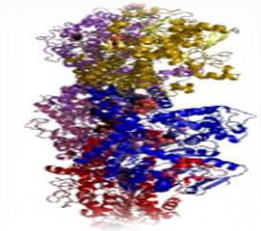


Bacillus subtilis



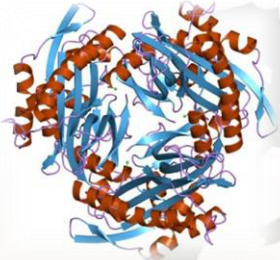
Ornithine decarboxylase

Putrescine



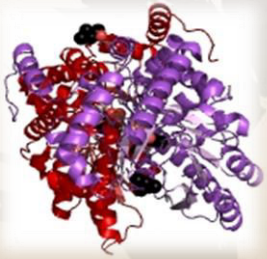
Lysine decarboxylase

Cadaverine



Histidine decarboxylase

Histamine



Tyrosine decarboxylase

Tyramine

Contents

1. Levels of biogenic amines in fermented soybean foods

2. Biocontrol technology for reduction of biogenic amines in foods

3 Molecular studies on biogenic amine producers in fermented soybean foods











Levels of biogenic amines in fermented soybean foods



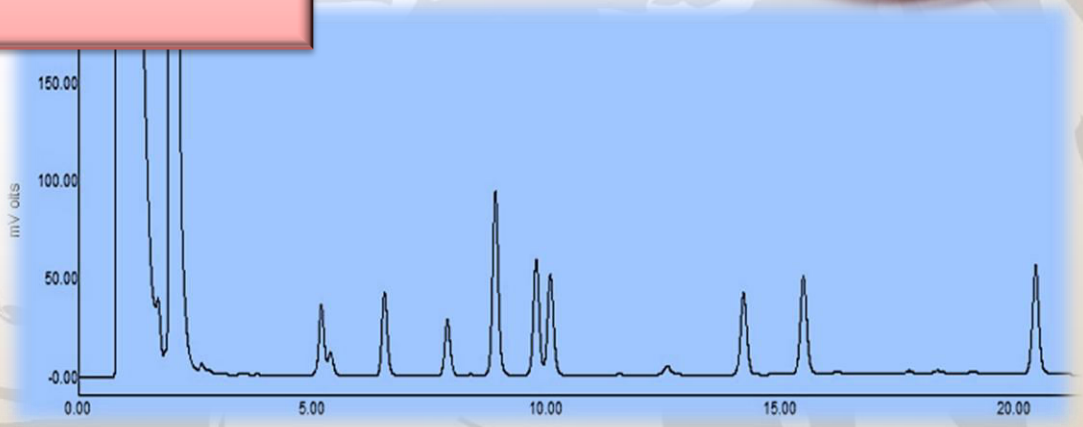


Table 1. The contents of biogenic amines in *Tofu* samples.

Tofu	Biogenic amines (mg/kg)							
	Trp[1)]	Phe	Put	Cad	His	Tyr	Spd	Spm
1	2.96±0.46[3)]	1.55±2.19	3.68±0.36	0.52±0.73	4.91±0.46	ND	28.37±0.88	9.90±0.05
2	1.06±1.49	ND[2)]	1.90±0.53	1.71±0.31	4.06±0.86	ND	23.49±1.49	7.87±0.56
3	1.88±0.12	ND	4.73±1.05	1.07±0.31	4.80±0.37	ND	28.09±0.47	7.19±0.38
4	6.10±0.43	ND	1.27±1.80	0.93±1.32	3.98±1.32	ND	19.43±0.66	4.75±0.42
5	5.48±2.23	1.89±2.67	1.14±1.61	1.25±1.77	4.38±0.69	ND	18.69±1.01	5.94±0.73
6	3.31±0.30	3.60±0.12	2.20±0.48	1.62±0.49	4.76±0.12	ND	19.94±0.78	4.67±0.15
7	8.10±1.03	2.91±0.10	0.99±1.40	ND	4.01±0.05	ND	18.89±0.71	4.08±0.40
8	9.46±2.93	5.36±0.25	1.64±0.44	0.80±1.13	4.15±0.32	ND	17.41±0.53	4.05±0.77
9	4.37±0.76	3.61±0.22	1.35±0.94	1.38±0.55	4.11±0.06	ND	19.70±0.07	4.78±0.14
10	7.35±0.12	4.65±0.56	1.69±0.70	2.12±0.08	4.59±0.19	ND	21.08±0.73	6.36±0.27
11	3.53±0.89	0.71±1.00	3.08±0.73	1.12±1.58	3.52±0.05	ND	29.11±1.12	5.95±0.88
12	4.45±1.18	1.75±2.48	4.03±0.32	2.21±0.28	4.41±0.67	ND	26.08±0.83	5.29±0.59
13	8.95±3.78	4.58±0.46	2.53±0.96	2.45±0.34	4.89±0.57	ND	27.58±1.31	9.09±0.10
14	6.22±1.99	4.49±1.21	4.65±0.01	3.35±0.03	4.81±0.22	ND	32.96±1.16	8.37±0.49
15	6.19±2.87	4.06±1.47	5.01±0.75	2.82±0.08	4.55±0.01	ND	22.37±1.42	17.04±0.94
16	7.51±1.24	4.76±0.01	2.80±0.44	2.19±0.21	4.67±0.09	ND	18.06±0.26	5.82±0.11
17	2.59±1.42	1.53±2.17	3.71±0.07	2.62±0.25	5.76±0.32	ND	35.30±3.64	19.15±3.44
18	3.80±0.72	ND	2.28±0.17	0.52±0.73	4.80±1.55	ND	26.34±0.38	8.48±0.01
19	2.76±0.47	ND	0.00	0.75±1.06	5.15±0.35	ND	14.97±1.20	4.83±0.75
Average	5.06±2.46	2.39±1.95	2.56±1.43	1.55±0.90	4.54±0.51	0.00	23.57±5.63	7.56±4.11

[1)] Trp: Tryptamine, Phe: β-phenylethylamine, Put: Putrescine, Cad: Cadaverine, His: Histamine, Tyr: Tyramine, Spd: Spermidine, Spm: Spermine.
[2)] ND: Not detected (amine level is less than 0.1 mg/kg).
[3)] Mean ± standard deviation.

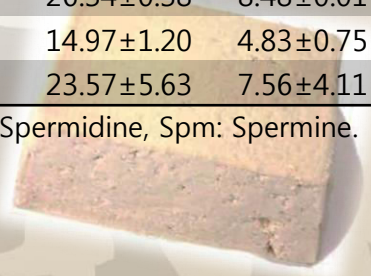


Table 2. The contents of biogenic amines in *Natto* samples.

Source	*Natto*	Biogenic amines (mg/kg)							
		Typ[1]	Phe	Put	Cad	His	Tyr	Spd	Spm
Japanese	1	14.20 ± 20.10[3]	21.40 ±30.30	ND	ND	ND	ND	297.70 ± 34.90	20.50 ± 29.00
	2	36.70 ± 1.50	ND	18.10 ± 1.70	33.50 ± 0.70	ND	ND	398.50 ± 29.10	52.20 ± 4.50
	3	30.00 ± 1.40	ND	12.20 ± 0.50	19.50 ± 9.90	ND	ND	344.50 ± 4.30	31.90 ± 8.20
	4	33.50 ± 1.00	13.60 ± 19.30	ND	9.50 ± 13.40	ND	ND	313.40 ± 20.20	55.40 ± 12.20
	5	38.20 ± 5.80	48.10 ± 13.40	ND	1.10 ± 1.50	ND	ND	411.30 ± 104.20	54.80 ± 12.50
	6	30.30 ± 18.00	36.70 ± 0.70	ND	18.50 ± 1.70	ND	ND	309.30 ± 4.20	33.70 ± 2.10
	7	12.70 ± 7.20	13.20 ± 18.60	ND	10.90 ± 15.40	ND	ND	321.00 ± 32.50	18.80 ± 26.50
	8	5.10 ± 7.20	ND	15.00 ± 6.00	28.40 ± 14.00	ND	ND	410.20 ± 144.20	49.90 ± 0.30
	9	ND[2]	ND	43.10 ± 0.40	12.80 ± 0.10	ND	ND	473.40 ± 7.50	61.40 ± 5.90
	10	11.60 ± 16.40	11.10 ± 15.80	27.70 ± 1.40	ND	ND	ND	333.40 ± 21.50	63.20 ± 7.00
	11	24.70 ± 6.90	40.80 ± 3.70	12.60 ± 6.00	36.80 ± 19.70	ND	300.20 ± 8.80	478.10 ± 194.20	61.20 ± 16.90
	12	ND	38.00 ± 5.60	ND	5.10 ± 7.20	ND	ND	270.00 ± 22.10	43.60 ± 9.40
	13	37.30 ± 0.50	18.40 ± 26.10	ND	19.00 ± 8340	ND	ND	370.90 ± 0.60	50.00 ± 6.20
	14	ND	29.10 ± 6.20	17.80 ± 1.60	ND	ND	ND	292.00 ± 12.10	35.90 ± 4.50
	15	31.30 ± 5.00	44.60 ± 13.50	14.70 ± 3.40	20.80 ± 6.50	6.10 ± 1.40	155.20 ± 219.40	410.30 ± 97.60	66.50 ± 11.70
	16	40.70 ± 0.80	42.10 ± 5.60	ND	ND	ND	ND	321.90 ± 2.90	28.90 ± 12.90
	17	11.10 ± 15.70	50.30 ± 1.50	ND	ND	ND	ND	305.60 ± 11.30	34.50 ± 7.70
Korean	18	45.80 ± 5.00	20.80 ± 29.40	ND	ND	12.30 ± 17.40	ND	246.50 ± 15.50	27.10 ± 6.40
	19	ND	ND	ND	ND	ND	290.50 ± 6.20	281.30 ± 27.50	39.10 ± 4.50
	20	ND	51.50 ± 1.10	ND	ND	34.40 ± 0.40	243.00 ± 82.40	265.00 ± 5.20	80.10 ± 6.50
	21	ND	ND	6.40 ± 9.10	ND	1.80 ± 2.50	ND	279.50 ±10.30	46.40 ± 5.10
Average		19.20 ± 16.41	22.84 ± 19.12	7.98 ± 11.66	10.28 ± 12.21	2.60 ± 7.85	47.09 ± 102.73	339.70 ± 67.17	45.48 ± 16.21

[1] Trp: tryptamine, Phe: β-phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine, Tyr: tyramine, Spd: spermidine, Spm: spermine.
[2] ND: Not detected (amine level is less than 0.1 mg/kg).
[3] Mean ± standard deviation.

Table 3. The contents of biogenic amines in *Miso* samples.

Miso samples	Biogenic amines (mg/kg)							
	Trp[1)]	Phe	Put	Cad	His	Tyr	Spd	Spm
1	5.99 ± 0.84[3)]	5.89 ± 1.03	5.31 ± 0.35	0.14 ± 0.20	ND	ND	ND	ND
2	1.87 ± 2.64	4.83 ± 6.83	4.35 ± 1.00	ND	24.42 ± 2.84	66.66 ± 4.88	ND	1.26 ± 0.15
3	3.59 ± 0.90	7.43 ± 0.73	4.55 ± 0.38	ND	3.07 ± 0.57	4.91 ± 0.44	ND	1.21 ± 0.10
4	2.84 ± 4.02	2.38 ± 3.37	2.69 ± 0.80	ND	3.18 ± 0.67	4.37 ± 0.12	ND	1.31 ± 0.03
5	3.56 ± 0.28	9.13 ± 1.93	8.74 ± 1.12	0.12 ± 0.17	ND	ND	11.98 ± 0.02	0.77 ± 1.08
6	1.73 ± 2.44	8.00 ± 0.23	6.96 ± 0.12	0.90 ± 0.04	ND	ND	9.88 ± 0.59	ND
7	9.71 ± 0.15	4.78 ± 0.95	5.72 ± 0.04	ND	ND	ND	9.12 ± 0.38	ND
8	ND[2)]	5.86 ± 1.70	6.97 ± 0.12	1.31 ± 0.00	ND	ND	4.48 ± 2.41	0.65 ± 0.92
9	1.92 ± 2.71	4.64 ± 6.56	9.89 ± 1.58	0.69 ± 0.67	0.69 ± 0.98	ND	ND	1.84 ± 0.36
10	ND	4.46 ± 6.31	4.74 ± 5.88	0.48 ± 0.67	0.77 ± 1.08	ND	11.66 ± 3.07	0.18 ± 0.25
11	6.41 ± 0.09	7.34 ± 0.14	8.29 ± 0.43	0.85 ± 0.14	0.81 ± 1.15	ND	1.49 ± 2.10	1.07 ± 0.64
12	2.91 ± 4.12	4.06 ± 5.75	3.61 ± 4.23	0.34 ± 0.49	ND	ND	13.19 ± 4.34	2.79 ± 0.32
13	2.69 ± 0.23	5.94 ± 0.82	10.34 ± 0.71	0.20 ± 0.02	ND	ND	28.31 ± 3.08	2.85 ± 0.22
14	1.96 ± 0.26	4.76 ± 0.33	10.50 ± 0.34	0.20 ± 0.01	ND	ND	27.84 ± 2.08	0.80 ± 0.19
15	2.03 ± 0.00	5.85 ± 0.00	2.83 ± 0.00	ND	ND	ND	ND	1.05 ± 0.00
16	2.66 ± 0.94	5.09 ± 0.71	14.09 ± 3.79	0.17 ± 0.23	ND	ND	ND	1.84 ± 0.44
17	5.61 ± 1.39	8.92 ± 0.55	12.82 ± 1.71	ND	12.63 ± 2.01	20.53 ± 3.97	ND	1.35 ± 0.21
18	5.00 ± 0.59	5.94 ± 0.43	6.76 ± 3.00	ND	1.82 ± 0.24	5.79 ± 0.80	6.47 ± 1.37	ND
19	4.79 ± 3.54	5.13 ± 0.55	11.89 ± 0.50	ND	0.68 ± 0.08	ND	ND	0.71 ± 0.02
20	3.19 ± 0.05	3.91 ± 1.97	4.85 ± 0.08	ND	0.26 ± 0.11	ND	ND	1.11 ± 0.52
21	2.90 ± 0.02	6.35 ± 0.17	4.39 ± 0.37	ND	0.85 ± 0.09	ND	2.59 ± 3.67	1.86 ± 0.01
22	3.52 ± 0.02	11.76 ± 0.09	11.52 ± 0.27	ND	9.67 ± 0.24	14.89 ± 0.29	15.81 ± 0.29	1.86 ± 0.01
Average	3.40 ± 2.20	6.02 ± 2.08	7.36 ± 3.41	0.25 ± 0.37	2.67 ± 5.85	5.33 ± 14.70	6.49 ± 8.74	1.11 ± 0.83

[1)] Trp: Tryptamine, Phe: β-phenylethylamine, Put: Putrescine, Cad: Cadaverine, His: Histamine, Tyr: Tyramine, Spd: Spermidine, Spm: Spermine.
[2)] ND: Not detected (amine level is less than 0.1 mg/kg).
[3)] Mean ± standard deviation.

Table 4. The contents of biogenic amines in *Doenjang* and *Gochujang* samples.

Samples		Biogenic amines (mg/kg)								
		Trp[1)]	Phe	Put	Cad	His	Tyr	Spd	Spm	Ref
Doenjang	1	16.6±4.2	30.8±9.9	54.4±2.2	10.9±1.3	82.2±3.6	90.8±11.1	15.6±1.3	NT	Kim *et al.*, 2011
	2	13.5±1.9	23.7±2.2	69.3±7.1	12.9±1.8	133.5±11.3	46.4±8.2	23.6±3.9	NT	
	3	45.9±5.1	65.0±6.9	168.2±11.0	10.9±1.0	382.4±8.0	1,190.7±12.4	ND	NT	
	4	21.2±1.1	18.5±2.4	47.0±3.1	ND	149.8±4.6	102.9±8.3	21.1±3.2	NT	
	5	19.2±0.6	8.6±0.5	150.3±3.5	ND	201.1±1.3	120.9±5.7	20.6±1.5	NT	
	6	15.6±0.9	3.3±0.4	46.7±1.5	5.4±0.7	241.8±7.9	82.9±3.9	24.7±2.3	NT	
	7	17.2±1.8	4.0±0.1	54.4±2.8	5.2±3.0	71.1±0.8	192.3±2.6	22.0±1.5	NT	
Gochujang	1	8.1±2.0	9.3±2.2	36.4±1.0	14.1±2.1	59.0±4.0	116.5±4.2	ND	NT	
	2	ND	9.8±2.9	23.5±1.2	18.1±4.0	17.8±1.5	23.4±1.9	ND	NT	
	3	ND	4.5±0.6	20.5±2.1	ND	13.8±1.6	13.4±3.1	14.5±2.9	NT	
	4	ND	24.8±0.2	13.5±2.1	5.5±1.3	2.2±0.7	2.9±1.0	1.5±0.4	NT	
	5	ND	1.5±0.3	10.4±2.8	2.1±0.3	3.3±0.3	60.2±8.8	11.7±1.2	NT	
	6	ND	3.8±0.1	15.0±0.6	0.5±0.1	5.4±0.2	126.8±14.3	3.3±0.2	NT	
	7	ND	1.8±0.2	16.8±0.1	2.8±0.0	5.4±0.3	3.8±0.4	6.9±0.9	NT	

[1)] Trp: Tryptamine, Phe: β-phenylethylamine, Put: Putrescine, Cad: Cadaverine, His: Histamine, Tyr: Tyramine, Spd: Spermidine, Spm: Spermine



Table 5. The contents of biogenic amines in *Doubanjiang* samples.

Doubanjiang	Biogenic amines (mg/kg)							
	Trp[1)]	Phe	Put	Cad	His	Tyr	Spd	Spm
A	1.71±0.33[3)]	4.62±0.73	2.08±0.67	0.17±0.24	ND	1.17±0.27	ND	1.69±0.06
B	3.39±0.65	4.05±0.37	4.95±0.44	0.12±0.17	ND	25.75±2.53	ND	0.43±0.18
C	3.06±0.23	12.39±8.00	81.34±0.88	ND	ND	2.61±0.07	ND	1.53±0.30
D	2.28±0.18	1.43±0.27	1.15±0.19	ND	ND	7.83±1.10	ND	ND
E	62.43±9.81	185.61±0.70	129.17±18.70	ND	ND	2.14±0.22	0.18±0.26	0.42±0.03
F	3.01±0.49	4.13±1.82	1.87±0.35	ND	ND	5.61±0.93	ND	0.67±0.25
G	ND[2)]	6.65±0.22	1.42±0.01	ND	ND	ND	0.09±0.13	0.47±0.51
Average	10.84±22.78	31.27±68.14	31.71±52.12	0.04±0.07	ND	6.44±8.92	0.04±0.07	0.74±0.63

[1)] Trp: tryptamine, Phe: β-phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine, Tyr: tyramine, Spd: spermidine, Spm: spermine.
[2)] ND: Not detected (amine level is less than 0.1 mg/kg).
[3)] Mean ± standard deviation.

6 times higher than toxic dose of this amine (30 mg/kg)



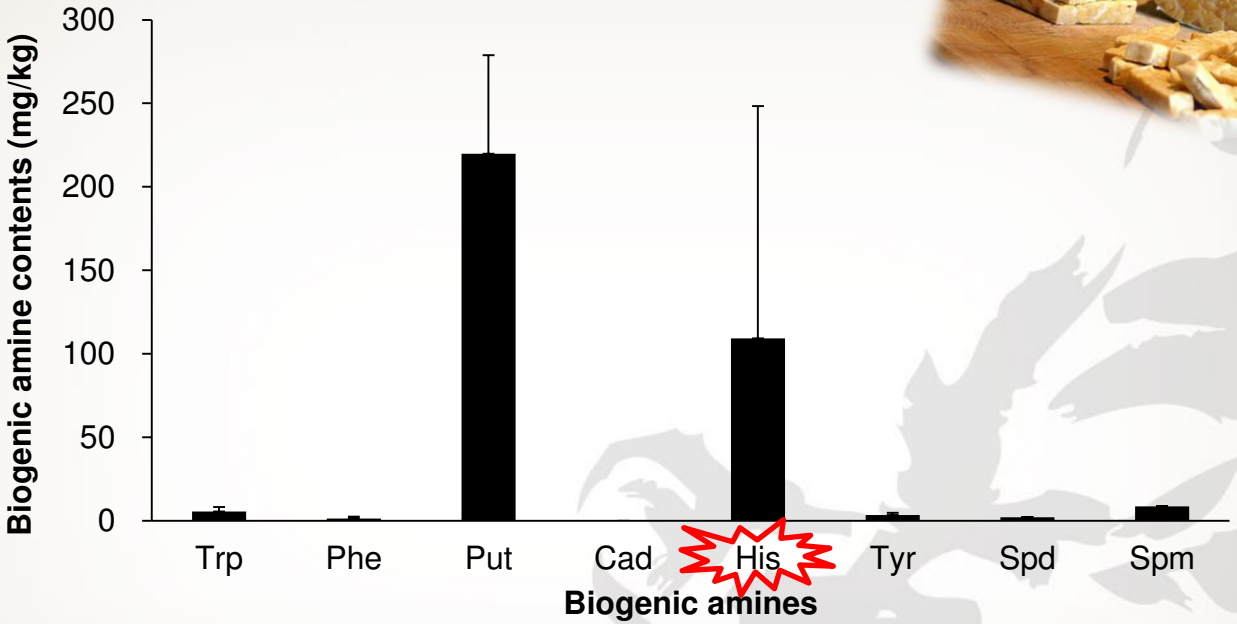


Fig. 1. The contents of biogenic amines in *Tempeh* samples.

Trp: tryptamine, Phe: β-phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine, Tyr: tyramine, Spd: spermidine, Spm: spermine.

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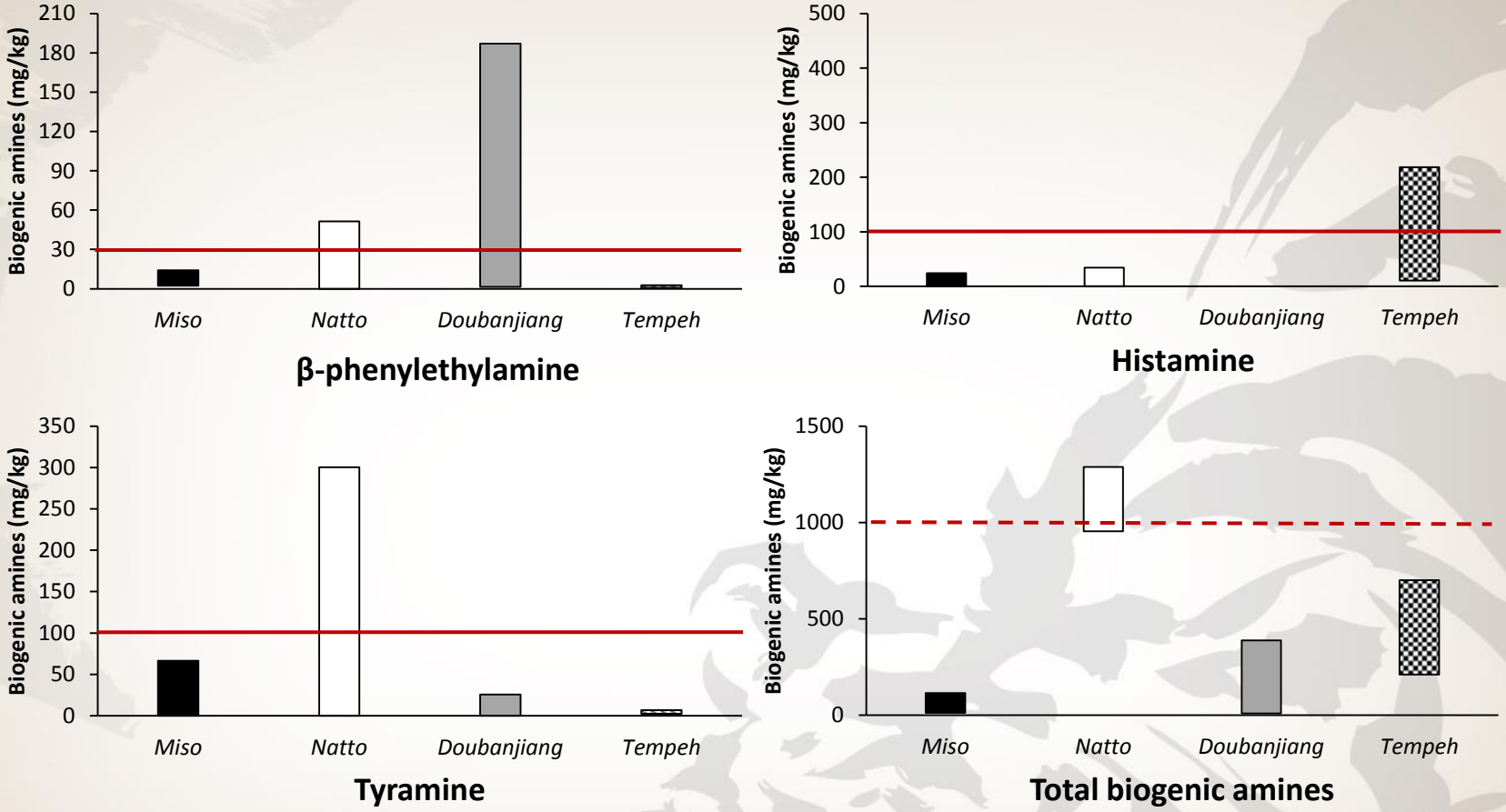


Fig. 2. Guideline suggested versus experimental data of biogenic amine contents in different fermented soybean foods.

Red line indicates Brink's guideline (1994) of each biogenic amine. Upper and lower lines of each bar indicate minimum and maximum content of each biogenic amine in the food indicated, respectively.

Table 6. Detection frequency of fermented soybean products containing excess amounts of biogenic amines from retail markets in Korea.

Samples	His > 100 mg/kg	Tyr > 100 mg/kg	Phe > 30 mg/kg	Ref
Tofu	0[1)]/19[2)]	0/19	0/19	Byun et al., 2013
Doenjang	**5/7**	**4/7**	**2/7**	Kim et al., 2011
Gochujang	0/7	**2/7**	0/7	Kim et al., 2011
Natto	0/21	**4/21**	**7/21**	Kim et al., 2012
Miso	0/22	0/22	0/22	Byun et al., 2012
Doubanjiang	0/7	0/7	**1/7**	Byun et al., 2013

[1)] Number of products containing hazardous levels of biogenic amines.
[2)] Total number of fermented soy bean products analyzed.

Table 7. The contents of biogenic amines in fermentation foods of Asia.

Raw material	Samples	N[1]	Biogenic amine content (mg/kg)								ref.
			Trp[2]	Phe	Put	Cad	His	Tyr	Spd	Spm	
Fermented soybean foods	**Gochujang**	5	17.9-36.6	0.7-9.1	2.5-3.2	ND-1.1	0.6-1.3	2.1-4.9	1.6-3.4	1.4-1.8	Cho *et al.*, 2006
		7	**ND-8.1**	**1.5-24.8**	**10.4-36.4**	**ND-18.1**	**2.2-59.0**	**2.9-126.8**	**ND-14.5**	**NT**	**Kim *et al.*, 2011**
		7	**13.5-45.9**	**3.3-65.0**	**47.0-168.8**	**ND-12.9**	**71.1-382.4**	**46.4-1190.7**	**ND-24.7**	**NT**	**Kim *et al.*, 2011**
	Doenjang	14	6.1-234.1	ND-529.2	9.9-1453.7	0.3-65.4	1.5-952.0	3.4-1430.7	4.2-23.4	ND-10.2	Cho *et al.*, 2006
		10	ND-449.8	ND-544.0	28.8-1076.6	2.7-144.1	1.4-329.2	12.5-967.6	ND-30.3	ND-9.8	Lee *et al.*, 2009
		23	ND-2808.1	ND-8704.6	ND-4292.3	ND-3235.5	ND-2794.8	ND-6616.1	ND-8804.0	ND-9729.5	Fd Chem Tx, 2010
		102	NT	NT	NT	NT	ND-755.40	ND-1913.51	NT	NT	Ko *et al.*, 2012
	Chungkukjang	10	6.7-76.9	ND-32.6	4.7-148.5	4.6-70.6	0.2-70.3	4.2-483.1	39.6-67.7	7.2-23.1	Han *et al.*, 2007
		7	6.7-236.4	ND-40.8	4.7-121.3	2.1-20.2	1.3-54.3	0.7-483.1	39.6-59.2	7.1-14.7	Cho *et al.*, 2006
	Natto	39	ND-301.0	ND	ND-27.0	ND-42.0	ND-457.0	ND-45.0	ND-124.0	ND-71.0	Tsai *et al.*, 2007
		21	**ND-45.80**	**ND-51.50**	**ND-43.10**	**ND-36.80**	**ND-34.40**	**ND-300.20**	**246.50-478.10**	**18.80-80.10**	**Kim *et al.*, 2012**
	Miso	40	ND-762	ND	ND-12	ND-201	ND-221	ND-49	ND	ND-216	Kung *et al.*, 2007
		8	ND-1.3	ND-42.0	ND-12.9	ND-8.7	ND	ND-41.9	ND-35.7	ND	Shukla *et al.*, 2011
		22	**ND-9.71**	**2.38-11.76**	**2.69-14.09**	**ND-1.31**	**ND-24.42**	**ND-66.66**	**ND-28.31**	**ND-2.85**	**Byun *et al.*, 2012**
	Chunjang	5	21.6-23.7	0.7-8.1	16.4-23.2	2.8-3.2	0.8-1.1	2.0-95.3	9.5-21.9	1.3-3.1	Cho *et al.*, 2006
		4	**19.57-28.67**	**ND-6.79**	**3.26-28.59**	**ND-2.04**	**1.85-272.55**	**19.78-131.27**	**0.24-11.63**	**ND-1.49**	**Bai *et al.*, 2013**
		4	13.3-19.9	2.2-11.8	9.2-11.7	1.7-6.6	11.6-22.4	29.7-54.6	1.4-12.8	ND-2.9	Cho *et al.*, 2006
	Sufu	22	ND-81	ND	ND-45	ND-371	ND-158	ND-65	ND	ND-82	Kung *et al.*, 2007
		38	ND-104.1	ND-36.3	0.5-316.9	0.6-85.8	ND-196.9	ND-446.6	ND-4.0	ND-6.9	Guan *et al.*, 2013
	Soy sauce	23	NT	ND-190	ND-775	ND-170	0.2-126	ND-5250	NT	NT	Stute *et al.*, 2002
		40	NT	NT	NT	ND-550	ND-592	ND-673	ND-486	ND-145	Yongmei *et al*, 2009
	Douchi	26	ND-440	ND-239	ND-596	ND-191	ND-808	ND-529	ND-719	ND-242	Tsai *et al.*, 2007
	Doubanjiang	**7**	**ND-62.4**	**1.43-185.61**	**1.15-129.17**	**ND-0.17**	**ND**	**ND-25.75**	**ND-0.18**	**ND-1.69**	**Byun *et al.*, 2013**
	Tofu	**19**	**1.06-9.46**	**ND-5.36**	**ND-4.73**	**ND-3.35**	**3.52-5.76**	**ND**	**14.97-35.30**	**4.05-19.15**	**Byun *et al.*, 2013**
	Tempeh	2	ND-<5	NT	475-3200	<5-225	<5-100	500-575	NT	NT	Nout *et al.*, 1993

[1] Number of samples examined.

[2] Trp, tryptamine; Phe, β-phenylethylamine; Put, Putrescine; Cad, cadaverine; His, histamine; Tyr, tyramine; Spd, spermidine; Spm, spermine.

[3] The range from minimun-maximum.

[4] ND: Not detected (amine level is less than 0.1 mg/kg, NT: Not tested.

		Natto	*Miso*	*Doubanjiang*
				
Problematic BAs		**Phe, Tyr, Polyamines**	**His, Tyr**	**Phe**
Factors	Physicochemical contribution	X	X	O (salt conc)
	Microbial contribution (TPC)	X	X	△
	Ratio of ingredient in raw materials	-	O	-
	Bacterial activity	**O**	**O**	**O (period)**

Biocontrol technology for reduction of biogenic amines in foods

대한민국을 이끌어온 자랑스럽고 위대한 역사, 눈부신 고대의 새로운 백년도 지켜가겠습니다.

Table 8. Microorganisms producing biogenic amines in fermented soybean food.

Biogenic amines	Bacteria	Reference
Decarboxylase activity (common foods)	*Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Photobacterium*, *Lactobacillus*, *Pediococcus* and *Streptococcus*	Rice *et al.*, 1976; Brink *et al.*, 1990; Huis in't Veld *et al.*, 1990
Amine-producing LAB	*Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus bulgaricus, Lactobacillus curuatus, Lactobacillus carnis, Lactobacillus dicergens* and *Lactobacillus hilgardii*	Maijala *et al.*, 1993
Tyrosine decarboxylase (from *Miso*)	*Enterococcus faecium, Lactobacillus bulgaricus*	Ibe *et al.*, 1992
Histidine decarboxylase (from *Miso*)	*Lactobacillus* species and *Lactobacillus sanfrancisco*	Ibe *et al.*, 1992
Histamine producers (from *Natto*)	*Lactobacillus* species	Tsai *et al.*, 2007
Histamine and tyramine producing bacteria from traditional soybean pastes	*Clostridium* and *Pseudomonas* strains	Moon *et al.*, 2010

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Table 9. Environmental factors influencing biogenic amine production in fermented foods.

Factors	Range	Reduction in the formation of biogenic amines	Reference
pH	3.0-6.0	Optimal for bacteria to produce decarboxylase	Santos, 1996
	4.0-5.5	Amino acid decarboxylase activity	Teodorovi, 1994
	3.0-6.0	Increasing the decarboxylase activity	Kim *et al.*, 2003
Salt content	0-6%	Reduction of amine production by *L. bulgaricus*	Chander *et al.*, 1989
	0.5-10%	Inhibition of histamine formation by *Staphylococcus capitis* and *S. epidermidis*	Hernandez-Herrero *et al.*, 1999
	3.5%	Partial inhibition of *L. bucheri* forming histamine	Maijala *et al.*, 1995
	3.5-5.5%	Inhibition of histamine production	Chin and Koehler, 1986
	20%	Inhibition of histamine formation and its producer	Hernandez-Herrero *et al.*, 1999
	20%	14% of Cad, 5.84% of His, 49.33% of Tyr, 28.81% of Spd[1)]	Mah and Hwang, 2009
Temperature	4°C	Lowering BA amounts	Bover-Cid *et al.*, 2000
	25°C	*Carnobacterium divergens* produced more tyramine	Masson *et al.*, 1999
	Higher temperature	Increased amine concentration	Bover-Cid *et al.*, 2000

[1)]Cad; cadaverine, His; histamine, Tyr; tyramine, Spd, spermidine.

Table 10. Studies on the effect of amine-negative starter cultures on biogenic amine reduction during the manufacture of fermented sausages.

Products	Starter culture	% of reduction	Reference
Fuet	commercial *M. carnosus* + *L. plantarum* **(Texel, France)**	25% of TI, 61% of CA, 25% of PU[1)]	Hernández-Jover *et al.*, 1997
	commercial *M. carnosus* + *P. pentosaceus* **(Texel, France)**	34% of TI, 50% of CA, 56% of PU	
Fuet	*Staphylococcus carnosus* LTH2102	25% of TY, 23% of CA, 17% of PU	Bover-Cid *et al.*, 1999
	S. xylosus CTC3037	69% of TY, 66% of CA, no effect on PU	
	S. xylosus CTC3050	69% of TY, 17% of CA, 28% of PU	
Fuet	*L. sakei* CTC494 + *S. carnosus* LTH2102	90% of TY, 87% of CA, 37% of PU	Bover-Cid *et al.*, 2000
	L. sakei CTC494 + *S. xylosus* CTC3037	87% of TY, 87% of CA, 37% of PU	
	L. sakei CTC494 + *S. xylosus* CTC3050	90% of TY, 87% of CA, 37% of PU	
Fuet	*L. sakei* CTC494 (high quality raw material)	87% of TY, 38% of HI, 41% of CA, 67% of PU	Bover-Cid *et al.*, 2001
Fuet	*L. sakei* CTC6626 + *S. xylosus* CTC6013	19% of TY, 46% of PU	Latorre-Moratalla *et al.*, 2010
	L. sakei CTC494 + *S. xylosus* CTC6013	45% of TY, 50% of PU	
Salchichón	*P. acidilactici* MS200 + *S. vitulus* RS34*	38% of TY, 74% of HI, 77% of CA, no effect on PU	Casquete *et al.*, 2011
		70% of TY, 82% of HI, 64% of CA, 89% of PU	
	P. acidilactici MS198 + *S. vitulus* RS34*	65% of CA, no effect on HI, TY, PU	
		58% of HI, 71% of CA, 72% of PU, no effect on TY	
Chorizo	*L. sakei* K29	98% of TY, 100% of CA, 98% of PU	González-Fernández *et al.*, 2003
	Pediococcus sp. P22	92% of TY, 67% of CA, 93% of PU	
	Pediococcus sp. P208 (Rhodia Food, France)	81% of TY, 100% of CA, 89% of PU	
Chorizo	*L. sakei* CTC6469 + *L. sakei* CTC6626 + *S. xylosus* CTC6013 + *S. xylosus* CTC6169	76% of TY, 97% of CA, 90% of PU	Garriga *et al.*, 2005

* Starter was tested in different technological conditions., which appeared the different% of reduction for each technological condition.
1) TI; tyramine, CA; cadaverine, PU; putrescine, HI; histamine, TY, tryptamine

Products	Starter culture	% of reduction	Reference
Xouriço	*L. sakei*	17% of TY, 75% of CA. No effect on PU	Latorre-Moratalla *et al.*, 2010
	S. equorum	45% of CA. No effect on TY and PU	
	L. sakei + *S. equorum*	15% of TY, 89% of CA. No effect on PU	
French fermented	*L. sakei* + *S. succinus* + *S. equorum*	87% of TY, 35% of CA, 38% of PU	Talon *et al.*, 2008
Greek fermented sausage	*L. sakei* (+ 0.5% of satureja tymbra extract oil)	62% of TY, 71% of HI, 100% of PU	Latorre-Moratalla *et al.*, 2010
Greek fermented sausage	*L. sakei* 4413	13% of TY, 72% of PU. No effects on CA	Bakaetal. 2011
	L. sakei 8426	25% of CA. No effect on TY and PU	
	L. plantarum 7423	26% of CA. No effect on TY and PU	
	L. curvatus 8427	9% of TY, 29% on PU. No effect on CA	
Finnish fermented sausage	commercial *P. pentosaceus* + *S. carnosus* **(Rudolf Müller and Co, Germany)***	41% of TY, 28% of HI, 86% of CA. No effect on PU	Maijala *et al.*, 1995
		79% of TY, 62% of HI, 70% of CA. No effect on PU	
		67% of TY, 77% of HI. No effect on PU and CA	
Turkish Soudjoucks	commercial *L. sakei* + *P. pentosaceous* + *S. carnosus* + *S. xylosus* **(Bioback-K, Germany)**	100% of PU. No effect on TY	Ayhan *et al.*, 1999
Turkish Sucuk	commercial *L. sakei* + *S. carnosus* **(CHR-HANSEN, Germany)**	88% of TY, 54% of CA, 63% of PU. No effecton HI	Gençcelep *et al.*, 2007
	commercial *P. acidilactici* + *L. curvatus* + *S. xylosus* **(CHR-HANSEN, Germany)**	86% of TY, 27% of HI, 62% of CA, 60% of PU	
Turkish fermented sausage	commercial *L. sakei* + *S. xylosus* **(CHR-HANSEN, Germany)**	54% of TY, 62% of PU	Gücükoglu and Küplülü. 2010
	commercial *L. plantarum* + *S. carnosus* **(CHR-HANSEN, Germany)**	52% of TY, 61% of PU	
	L. curvatus + *S. carnosus* + *S. xylosus* **(CHR-HANSEN, Germany)**	55% of TY, 63% of PU	
Chinese fermented sausage	*P. pentosaceus* + *S. xylosus*	66% of TY, 49% of CA, 30% of PU. No effect on HI	Lu *et al.*, 2010
	L. farciminis + *S. saprophyticus*	83% of TY, 99% of HI, 99% of CA, 66% of PU	
Thai fermented sausage	*L. plantarum* BCC9546 **(BIOTEC, Thailand)**	94% of TY, 75% of CA, 97% of PU	Tosukhowong *et al.*, 2011
	L. plantarum BCC9546 + *L. brevis* BCC26756 **(BIOTEC, Thailand)**	37% of TY, 75% of CA, 99% of PU	

Latorre-Moratalla *et al.*, 2012. Control of biogenic amines in fermented sausages: role of starter cultures. Frontiers in Microbiology. 3, 169-178.

Table 11. Studies on biogenic amine degradation by food microorganisms.

Matrix	Bacteria	Reduction in the formation of biogenic amines	Reference
Fermented anchovy	***B. coagulans, St. xylosus* and *Kocuria rhizophila***	**3.27-34.31% of His, 0.75-4.36% of Tyr[1)]**	**Mah and Hwang, 2009**
In vitro	*L. plantarum, L. sakei, L. pentosus, Pediococcus acidilactici, Rhodococcus* sp., *Arthrobacter sp., Micrococcus* sp., *Brevibacterium linens, Geotrichum candidum*	0.00-87.12% of His, 0.00-5.20% of Tyr	Leuschner *et al.*, 1998
Munster cheese	*B. linens*	55-70% of His and Tyr	Leuschner and Hammes, 1998
Ensiled fish slurry	*L. sakei*	98.00-100.00% of His	Dapkevicius *et al.*, 2000
In vitro	*B. amyloliquefaciens, St. carnosus*	29.10% and 59.90% of His	Zaman *et al.*, 2010
In vitro	*B. subtilis, St. intermedius*	30.40% of Put, 28.90% of Cad	Zaman *et al.*, 2010
Fish sauce fermentation	*B. amyloliquefaciens, St. carnosus*	27.70% and 15.40% of His	Zaman *et al.*, 2011
In vitro	*St. xylosus*	53.00-100.00% of His	Martuscelli *et al.*, 2000
In vitro	*L. casei, L. plantarum*	60.00-98.00% of Tyr	Fadda *et al.*, 2001
Cabrales cheese model	*L. casei*	14.92-56.52% of His, 12.76-47.90% of Tyr	Herrero-Fresno *et al.*, 2012
Culture media	*L. casei, L. hilgardii, P. parvulus, Oenococcus oeni, L. plantarum, P. pentosaceus*	10.00-54.00% of His, 12.00-55.00% of Tyr, 16.00-69.00% of Put	Garcıa-Ruiz *et al.*, 2011
Wine	*L. casei*	54.00% of His, 55.00% of Tyr, 65.00% of Put	Garcıa-Ruiz *et al.*, 2011
In vitro	*L. plantarum*	0.00-22.12% of Tyr, 3.47-31.09% of Put, 2.21-8.63% of His, 0.00-6.68% of Cad	Capozzi *et al.*, 2012
In vitro/ Commercial wines	*Penicillium citrinum, Alternaria* sp., *Phoma* sp., *Ulocladium chartarum, Epicoccum nigrum*	0.00-100.00% of His, 20.08-100.00% of Tyr, 22.80-100.00% of Put	Cueva *et al.*, 2012

[1)]Put; putrescine, Cad; cadaverine, His; histamine, Tyr; tyramine.

Alvarez *et al.*, 2014. The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. Food science and technology. 39, 146-155.

Molecular studies on biogenic amine producers in fermented soybean foods

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Table 12. List of the decarboxylase genes in *Bacillus subtilis* available on the NCBI BLAST.

Name	Bacteria name	No. of amino acids	Locus	GenBank	Size
Ornithine decarboxylase	37.0% identity over 119 aa to the *E. coli* ornithine decarboxylase antizyme; **putative** [*Bacillus subtilis* subsp. *subtilis* str. 168]	331	AAA96638	AAA96638.1	1052 bp
Lysine decarboxylase	*Bacillus subtilis*	490	AAC24937	AAC24937.1	1442 bp

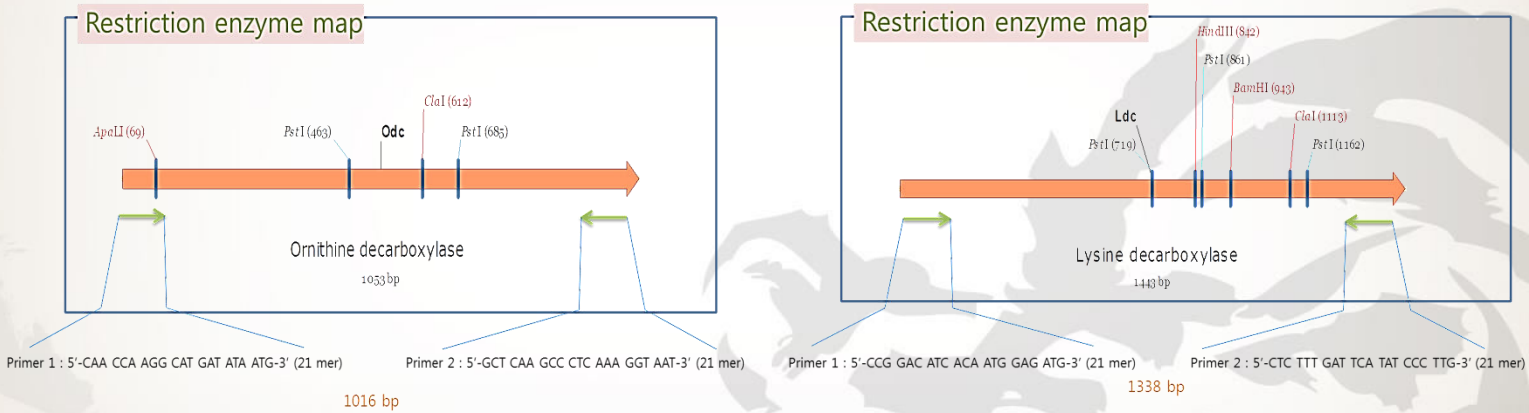


Fig. 3. Gene maps of *odc* and *ldc* in *Bacillus subtilis*.

Assignment !

Hdc & Tdc ?

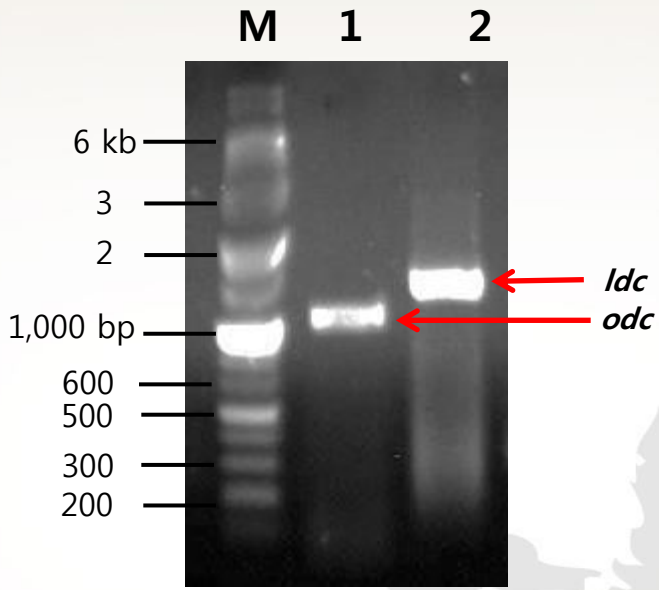


Fig. 4. PCR amplification of the 2 amino acid decarboxylase genes in *Bacillus subtilis* KCTC 1021 by using primers 4Od-det-fw and 4Od-det-rv giving an amplicon of 1016 bp (1), primers 4Ld-det-fw and 4Ld-det-rv giving an amplicon of 1338 bp (2).

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Strains from a culture collection

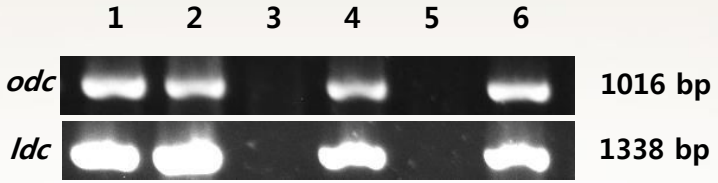


Fig. 5. PCR based detection of *odc* and *ldc* genes in different *B. subtilis* strains.
1, *Bacillus subtilis* subsp. *subtilis* (type strain, KCTC 3135); 2, *B. subtilis* (from kimchi, KCTC 13112) 3, *B. subtilis* (from **tea leaves**, KCTC 13021); 4, *B. subtilis* (from soil or fermented soy bean, KCTC 3014); 5, *B. subtilis* (from **tea leaves**, KCTC 13022); 6, *B. subtilis* subsp. *subtilis* (from fermented soy bean, Maejoo, Sunchang, Korea, KCTC 13241).

Spontaneously mutated?

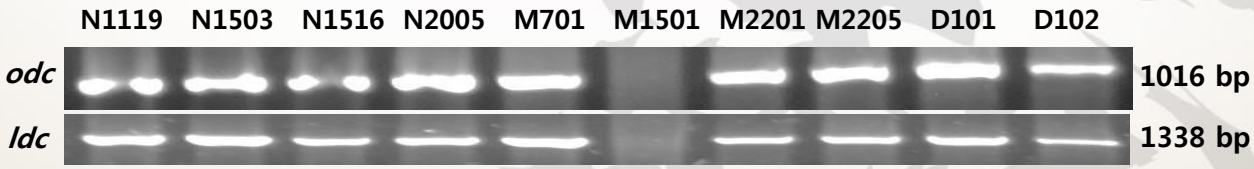


Fig. 6. PCR based detection of *odc* and *ldc* genes in *B. subtilis* strains isolated from fermented soybean products.

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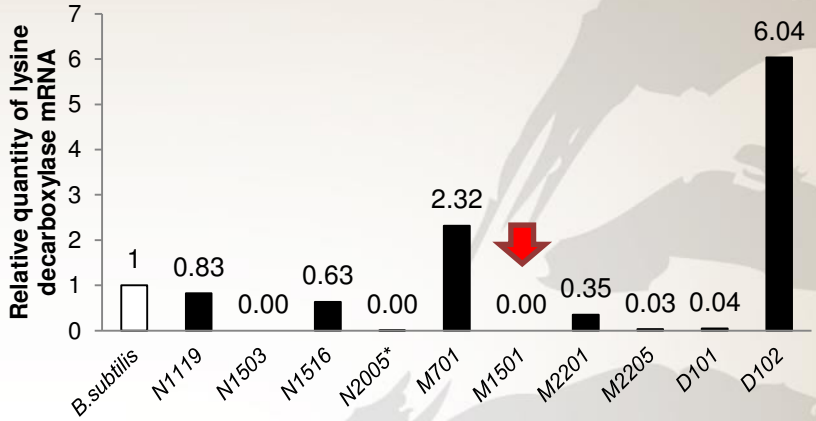


Fig. 7. Quantitative analysis of expression levels of *odc* and *ldc* genes in *B. subtilis* strains isolated from fermented soybean products.

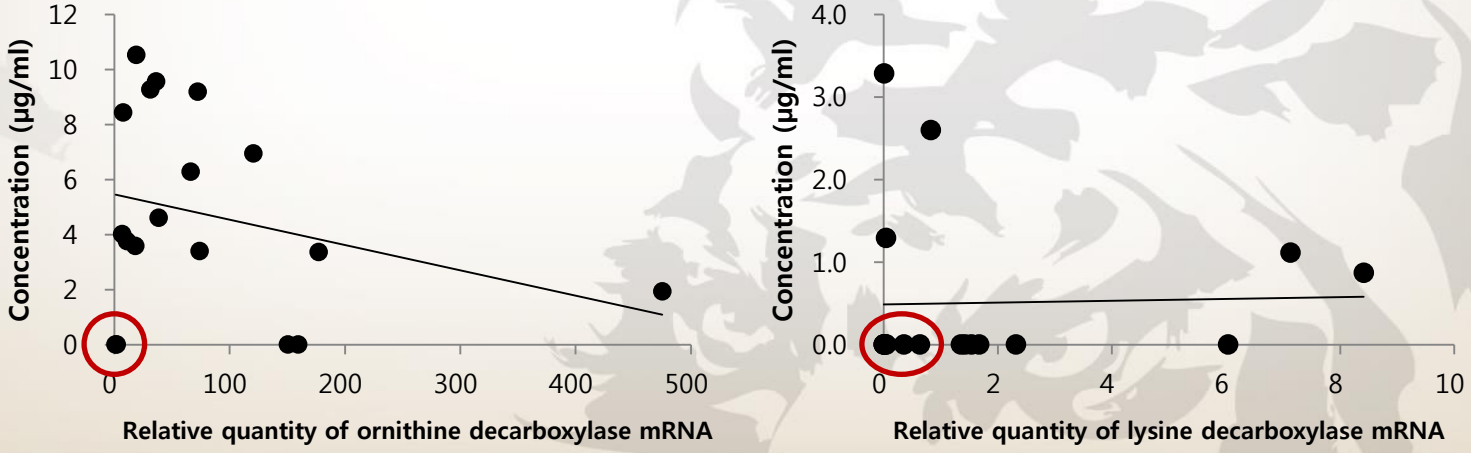


Fig. 8. Screening of starter culture candidates through HPLC and RT-PCR analyses.

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International Journal of Food Microbiology

Genetically modified starter and protective cultures

R. Geisen*, W.H. Holzapfel

Federal Research Centre for Nutrition, Institute of Hygi...

Genetically designed cultures needs to be considered to ensure the healthy functions as well as safety of fermented foods in the future !

...ective culture improvement rely on advances ... microorganisms used for food production, gene technological ... been well developed. By recombinant DNA technology, 'tailor-made' starter and protective cultures may be constructed so as to combine technically desirable features. A single strain which normally would fail to accomplish a given 'task' may now be improved so as to meet a set of requirements necessary for a specific production or preservation process (e.g. wholesomeness, no off-flavour production, overproduction of bacteriocins or particular enzymes). In addition, undesirable properties (e.g. mycotoxin or antibiotic production by cheese moulds) may be eliminated by techniques such as 'gene disruption'.

Keywords: Lactic acid bacteria (LAB); Yeasts; Fungi; Genetic improvement; Transformation systems; Heterologous gene expression

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KNOCKOUT Strategy



Application of *odc* and *ldc* gene knockout mutants for reducing biogenic amine formation

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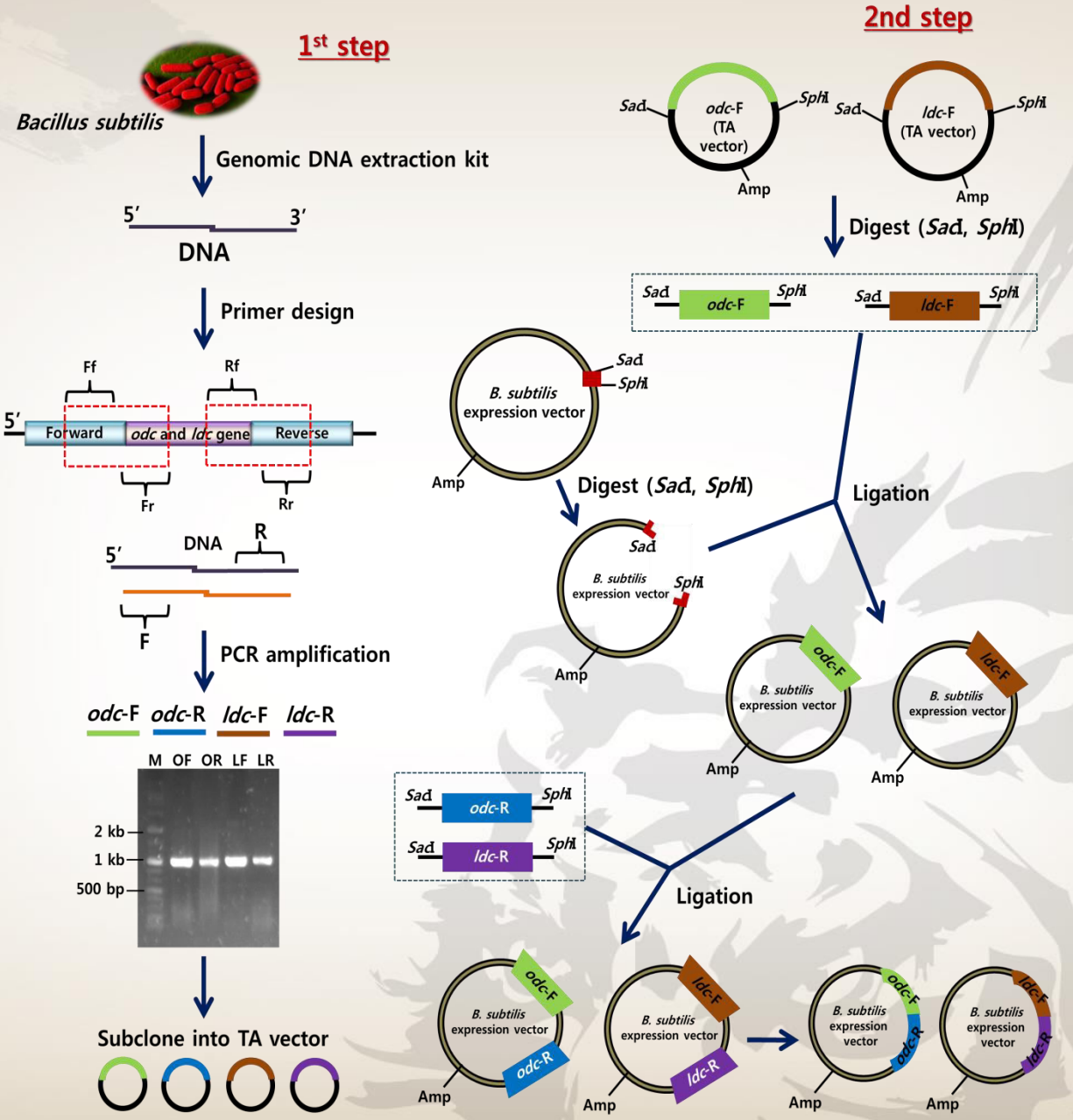


Fig. 9. Scheme of double-crossover homologous recombination.

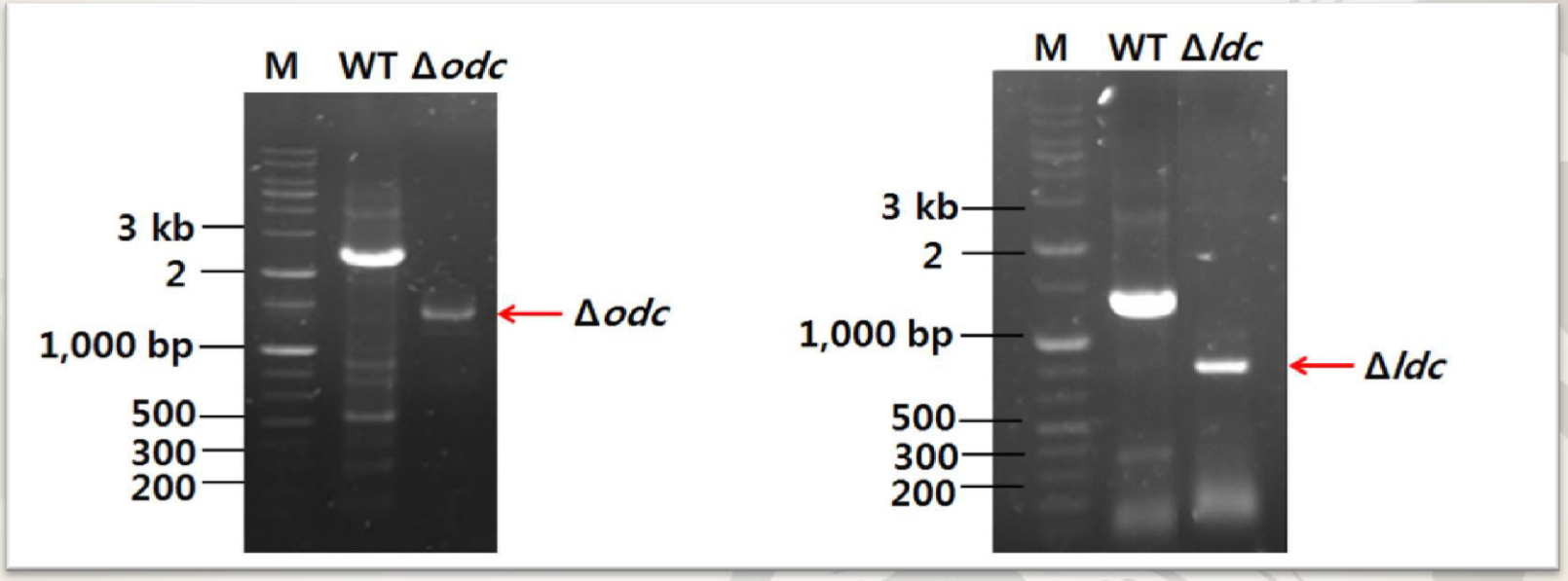


Fig. 10. PCR amplification of *odc* and *ldc* genes in WT and K/O strains of *B. subtilis.*

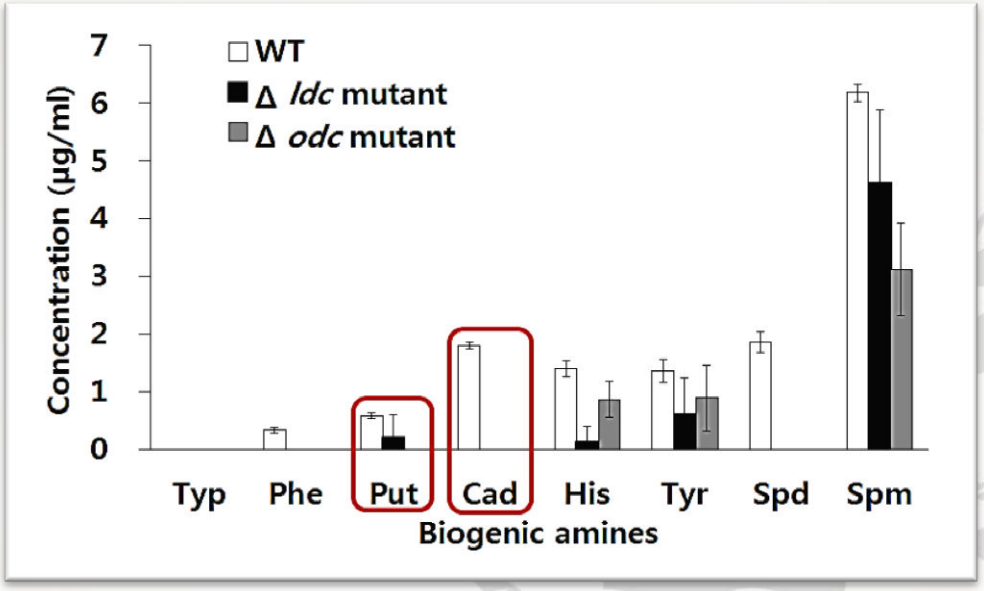


Fig. 11. Comparison of biogenic amine contents in the cultures of WT and K/O strains of *B. subtilis*.
Trp: tryptamine, Phe: ß-phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine, Tyr: tyramine, Spd: spermidine, Spm: spermine.

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Summary I

- The amounts of biogenic amines in most samples of fermented soybean products are within the **safe levels** for human consumption.
  - ✓ Some samples had β-phenylethylamine or tyramine contents **greater than toxic dose** of each amine although no samples showed total amounts of biogenic amines higher than harmful level.

- The variability of biogenic amine levels in the fermented soybean products are attributable to:
  - ✓ The ratio of ingredients used in raw material,
  - ✓ Microbial and/or physicochemical contribution, and
  - ✓ Conditions and periods of the entire food supply chain.

Summary II

- **Understanding of biogenic amine occurrence** in fermented foods would probably provide direction and framework for developing strategies to reduce biogenic amine formation chemically, biologically and physically.

- **Biocontrol strategies** have been vigorously developed for reducing biogenic amine formation in common fermented foods, mainly focusing on microbial intervention
  - ✓ The use of **starter culture** would be a successful way to reduce biogenic amine formation in fermented soybean foods.
  - ✓ It causes less adverse organoleptic and unhealthy alterations than that of additives.

Summary III

- The abundance and diversity of biogenic amines in fermented soybean foods are closely related to the **expression levels of decarboxylase genes** in *B. subtilis*.
  - ✓ ***Bacillus* strains** belonging to the same species **have different levels of expression** of *odc* and *ldc* genes and production of putrescine and cadaverine **depending on the types of fermented soybean products** from which they were isolated.

- The formation of **overall biogenic amines** as well as putrescine and cadaverine can be successfully **reduced by using the Δ*odc* and Δ*ldc* mutants**.
  - ✓ The use of **genetically modified starter and protective cultures** needs to be considered to ensure the safety of fermented foods in the near future.

Key Publications
on Biogenic Amines in fermented soybean foods

- Mah, J. -H. *. 2015. **Fermented soybean foods**: Significance of biogenic amines. Austin Journal of Nutrition & Food Sciences. 3(1), 1058.
- Bai, X., B. Y. Byun and J. -H. Mah*. 2013. Formation and destruction of biogenic amines in **Chunjang** (a black soybean paste) and **Jajang** (a black soybean sauce). Food Chemistry, 141(2), 1026-1031.
- Byun, B. Y., X. Bai and J. -H. Mah*. 2013. Occurrence of biogenic amines in **Doubanjiang** and **Tofu**. Food Science and Biotechnology, 22(1), 55-62.
- Byun, B. Y. and J. -H. Mah*. 2012. Occurrence of biogenic amines in **Miso**, Japanese traditional fermented soybean paste. Journal of Food Science, 77(12), T216-T223.
- Oh, S. -J., J. -H. Mah, J. -H. Kim, Y. -W. Kim and H. -J. Hwang. 2012. Reduction of tyramine by addition of Schizandra chinensis Baillon in **Cheonggukjang**. Journal of Medicinal Food, 15(12), 1109-1115.
- Kim, B., B. Y. Byun and J. -H. Mah*. 2012. Biogenic amine formation and bacterial contribution in **Natto** products. Food Chemistry, 135(3), 2005-2011.
- Kim, T. -K., J. -I. Lee, J. -H. Kim, J. -H. Mah, H. -J. Hwang and Y. -W. Kim. 2011. Comparison of ELISA and HPLC methods for the determination of biogenic amines in commercial **Doenjang** and **Gochujang**. Food Science and Biotechnology, 20(6), 1747-1750.

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Hyeock Youn, MS student
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Huiling Xu, MS student
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Thank you











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