Appendix A

1. Media

MRS (De Man Rogosa Sharpe) broth (Atlas, 1946)

Composition per liter:

glucose	18.5	g
Pancreatic digest of gelatin	10	g
Beef extract	8.0	g
Yeast extract	4.0	g
Sodium acetate	3.0	g
K_2HPO_4	2	g
ammonium citrate	2	g
$MgSO_4.7H_2O$	0.2	g
$MnSO_4.4H_2O$	0.05	g
Polysorbate 80	1.0	g
Distilled water	1	L

pH 6.2±0.2 at 25°C

Preparation of Medium: Add component to distilled/deionized water and bring volume to 1.0 L. Mix thoroughly. Distribute into tubes or flask. Autoclave for 15 min at 15 psi pressure-121°C.

MRS (De Man Rogosa Sharpe) agar (Atlas, 1946) **and**

MRS (De Man Rogosa Sharpe) agar + $CaCO_3$

Composition per liter:

glucose	18.5	g
Agar	13.5	g

Pancreatic digest of gelatin	10	g
Beef extract	8.0	g
Yeast extract	4.0	g
Sodium acetate	3.0	g
K_2HPO_4	2	g
ammonium citrate	2	g
$MgSO_4.7H_2O$	0.2	g
$MnSO_4.4H_2O$	0.05	g
Polysorbate 80	1.0	g
$CaCo_3$	5	g
Distilled water	1.0	L

pH 6.2±0.2 at 25°C

Preparation of Medium: MRS agar, Add component to distilled/deionized water and bring volume to 1.0 L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flask. Autoclave for 15 min at 15 psi pressure-121°C. Pore into sterile Petri dishes or leave in tube and MRS agar + $CaCO_3$ method preparation of medium same as MRS agar but add $CaCO_3$ 5 g to distilled/demonized water and bring volume to 1.0 L. Mix thoroughly.

Nutrient Broth, Nutrient agar

Composition per liter:

Beef extract	3	g
Peptone	5	g
Agar	15	g
Distilled water	1.0	L

pH 7±0.2 at 25°C

Preparation of Medium: Add component to distilled/deionized water and bring volume to 1.0 L. Mix thoroughly. Distribute into tubes or flask. Autoclave for 15 min at 15 psi pressure-121°C. While, Nutrient agar, Add Agar 15 g and bring volume to 1.0 L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flask. Autoclave for 15 min at 15 psi pressure -121°C. Pore into sterile Petri dishes or leave in tube.

2. <u>Chamical preparation</u>

2.1 <u>Buffer solution for plasmid extraction</u> (Anderson and Mckay, 1983)

2.1.1 Buffer I

50 mM Tris base
1mM EDTA base
6.7% Sucrose

Adjust pH 8.0 with glacial acetic acid.

2.1.2 Buffer II

50 mM Tris base
0.25 mM Na_2-EDTA base

Adjust pH 8.0 with NaOH 10 M.

2.1.3 Buffer for SDS

50 mM Tris base
20 mM Na_2-EDTA base

Adjust pH 8.0 with glacial acetic acid. Autoclave for 15 min at 15 psi pressure-121°C and add Sodium dodecyl sulphate(SDS) 20%.

2.1.4 Buffer for lysozyme

2.5 mM Tris base

Adjust pH 8.0 with glacial acetic acid. Autoclave for 15 min at 15 psi pressure-121°C and add lysozyme 20 mg/ml.

2.1.5 Buffer Tris HCL

2.0 M Tris HCl

Adjust pH 7.0 with NaOH 10 M

2.1.6 NaCl solution

5.0 M NaCl

Buffer solution of 2.1.1-2.1.6 were autoclave for 15 min at 15 psi pressure-121°C.

2.1.7 NaOH solution

3.0 N NaOH

NaOH solution (new prepare for use).

2.1.8 Phenol solution

Prepare phenol saturate in NaCl 3% keep at 20 °C in the dark.

2.1.9 Choloroform: Iso-amylalcohol

Mix Choloroform and Iso-amylalcohol in ratio 24:1 keep at room temparature.

2.2 <u>Buffer solution for electrophoresis</u> (Sambrook *et al*, 1989)

2.2.1 50X TAE (Tris-acetate)

Tris base	242.0 g
Glacial acetic acid	57.0 ml
0.5 M Na, EDTA (pH 8.0)	100.0 ml

Bring volume to 1000 and autoclave for 15 min at 15 psi pressure -121°C.

Appendix B

Appendix 1 Sixty-six isolates of presumptive LAB isolated from fermented meat products

Number	Isolates	origin	Source
1	FP1-01/2	Nham(pork)	Pa-Yao (Mea-Poum market)
2	FP1-01/5	Nham(pork)	Pa-Yao (Mea-Poum market)
3	FP3-01/4	Nham(pork)	Pa-Yao (Mea-Poum market)
4	FM2-01/1	Plaa-ra	Lum-Phun (Barn Pann market)
5	FF2-01/4	Plaa-som	Lum-Phun (Barn Pann market)
6	FF2-01/8	Plaa-som	Lum-Phun (Barn Pann market)
7	FF2-02/3	Plaa-som	Lum-Phun (Barn Pann market)
8	FF2-02/7	Plaa-som	Lum-Phun (Barn Pann market)
9	FF6-01/4	Plaa-som	Lum-Phun (Woug Tran market)
10	FF3-01/1	Plaa-som	Lum-Phun (Woug Tran market)
11	FF3-01/2	Plaa-som	Lum-Phun (Woug Tran market)
12	FF3-01/3	Plaa-som	Lum-Phun (Voug Tran market)
13	FF3-01/4	Plaa-som	Lum-Phun (Voug Tran market)
14	FF3-01/5	Plaa-som	Lum-Phun (Voug Tran market)
15	FF3-01/6	Plaa-som	Lum-Phun (Voug Tran market)
16	FF3-01/10	Plaa-som	Lum-Phun (Voug Tran market)
17	FF4-01/1	Plaa-som	Lum-Phun (Barn Pann market)
18	FF4-01/2	Plaa-som	Lum-Phun (Barn Pann market)
19	FF4-01/5	Plaa-som	Lum-Phun (Barn Pann market)
20	FF4-01/8	Plaa-som	Lum-Phun (Barn Pann market)
21	FF4-01/4	Plaa-som	Lum-Phun (Barn Pann market)
22	FB5-01/1	Nham(Meat)	Hang-Dong market
23	FB5-01/2	Nham(Meat)	Hang-Dong market
24	FB5-01/3	Nham(Meat)	Hang-Dong market

Appendix 1 Sixty-six isolates of presumptive LAB isolated from fermented meat products (continue)

Number	Isolates	origin	Source
25	FB5-01/4	Nham(Meat)	Hang-Dong market
26	FB5-01/5	Nham(Meat)	Hang-Dong market
27	FF5-01/1	Plaa-som	Hang-Dong market
28	FF5-01/2	Plaa-som	Hang-Dong market
29	FF5-01/3	Plaa-som	Hang-Dong market
30	FF5-01/4	Plaa-som	Hang-Dong market
31	FF5-01/5	Plaa-som	Hang-Dong market
32	FF5-01/6	Plaa-som	Hang-Dong market
33	FF5-01/7	Plaa-som	Hang-Dong market
34	FF5-01/8	Plaa-som	Hang-Dong market
35	FF6-01/2	Plaa-som	Mae-Hea market
36	FP5-01/1	Nham(pork)	Hang-Dong market
37	FP5-01/2	Nham(pork)	Hang-Dong market
38	FP5-01/3	Nham(pork)	Hang-Dong market
39	FP5-01/4	Nham(pork)	Hang-Dong market
40	FP5-01/5	Nham(pork)	Mae-Hea market
41	FF6-01/1	Plaa-jom	Mae-Hea market
42	FP5-01/6	Nham(pork)	Mae-Hea market
43	FA6-01/1	Plaa-jom	Mae-Hea market
44	FA6-01/2	Plaa-jom	Mae-Hea market
45	FA6-01/3	Plaa-jom	Mae-Hea market
46	FA6-01/4	Plaa-jom	Mae-Hea market
47	FE6-01/1	fermented fish egg	Mae-Hea market
48	FE6-01/2	fermented fish egg	Mae-Hea market
49	FE6-01/3	fermented fish egg	Mae-Hea market
50	FE6-01/4	fermented fish egg	Mae-Hea market
51	FE6-01/5	fermented fish egg	Mae-Hea market

Appendix 1 Sixty-six isolates of presumptive LAB isolated from fermented meat products (continue)

Number	Isolates	origin	Source
52	FE6-01/6	fermented fish egg	Mae-Hea market
53	FE7-01/1	fermented fish egg	Burirum
54	FE7-01/2	fermented fish egg	Burirum
55	FE7-01/3	fermented fish egg	Burirum
56	FE7-01/4	fermented fish egg	Burirum
57	FE7-01/5	fermented fish egg	Burirum
58	FE7-01/7	fermented fish egg	Burirum
59	FE7-01/8	fermented fish egg	Burirum
60	FE7-01/9	fermented fish egg	Burirum
61	FS7-01/1	Mum	Chaiyapoom
62	FS7-01/3	Mum	Chaiyapoom
63	FS7-01/4	Mum	Chaiyapoom
64	FS7-01/5	Mum	Chaiyapoom
65	FS7-01/6	Mum	Chaiyapoom
66	FS7-01/8	Mum	Chaiyapoom

Appendix 2 Number of lactic acid bacteria after incubation for 24 h at 37°C at pH 2, 3 and 4

Isolates	pH	Control	Viable bacteria (cfu/ml)	Viable bacteria (log cfu/ml)
FF2-02/3	2	2.80×10^{9}	2.95×10^{5}	5.5
	3	2.80×10^{9}	1.83×10^{6}	8.26
	4	2.80×10^{9}	2.86×10^{8}	8.45
FF4-01/5	2	2.02×10^{9}	1.14×10^{4}	4.1
	3	2.02×10^{9}	1.15×10^{6}	6.06
	4	2.02×10^{9}	1.01×10^{7}	7.0
FP5-01/4	2	2.30×10^{9}	5.01×10^{3}	3.7
	3	2.30×10^{9}	3.98×10^{3}	3.6
	4	2.30×10^{9}	9.6×10^{6}	7.0

Apendix 3 Number of lactic acid bacteria after incubation in 0.15 and 0.30% bile salts (bs) in MRS broth at 37°C

Isolates	Time(h)	Colony (cfu/ml)			Colony (log cfu/ml)		
		Control	0.15%	0.30%	Control	0.15%	0.30%
FF2-02/3	0	2.83×10^{9}	1.50×10^{8}	5.6×10^{4}	9.5	8.2	4.7
	0.5	2.83×10^{9}	1.80×10^{8}	31×10^{5}	9.5	8.3	5.5
	1.0	2.83×10^{9}	1.95×10^{8}	6.6×10^{5}	9.5	8.3	5.8
	2.5	2.83×10^{9}	2.01×10^{8}	5.9×10^{4}	9.5	8.3	4.7
	4.0	2.83×10^{9}	1.98×10^{9}	3.2×10^{3}	9.5	9.2	3.5
FF4-01/5	0	2.08×10^{8}	8.60×10^{7}	1.85×10^{8}	8.4	7.9	8.3
	0.5	2.08×10^{8}	1.20×10^{8}	8.5×10^{7}	8.4	8.1	7.9
	1.0	2.08×10^{8}	1.80×10^{8}	3.0×10^{7}	8.4	8.3	7.9
	2.5	2.08×10^{8}	1.00×10^{8}	1.80×10^{7}	8.4	8.0	7.3
	4.0	2.08×10^{8}	8.5×10^{7}	1.10×10^{7}	8.4	7.9	7.0
FP5-01/4	0	2.09×10^{8}	5.8×10^{7}	1.01×10^{5}	8.5	7.8	6.0
	0.5	2.09×10^{8}	8.0×10^{7}	5.8×10^{5}	8.5	7.9	5.8
	1.0	2.09×10^{8}	3.0×10^{7}	8.5×10^{5}	8.5	7.5	5.9
	2.5	2.09×10^{8}	1.85×10^{7}	1.80×10^{6}	8.5	7.3	6.3
	4.0	2.09×10^{8}	1.02×10^{7}	8.9×10^{4}	8.5	7.0	4.9

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