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APPENDIX

APPENDIX I

Bacterial Culture Media and Buffers

Medium **Component per liter**

1. **MRS Agar** for isolating and propagating Lactobacilli (de Man et al., 1960)

Oxoid peptone	10	g
Meat extract	10	g
Yeast extract	5	g
K_2HPO_4	2	g
Diammonium citrate	2	g
Glucose	20	
Tween 80	1	ml
Na acetate	5	g
$MgSO_4$. $7H_2O$	0.58	g
$MnSO_2$. $4H_2O$	0.25	g

For 1 liter of medium, dissolve 15 g of agar in 1 liter of distilled water by steaming, add all the above ingredients, and adjust pH to 6.2–6.4. Sterilize at 121°C for 15 min

2. **LB** for *E.coli* and derivatives

Tryptone	10	g
Yeast extract	5	g
NaCl	10	g

For 1 liter of medium, dissolve 15 g of agar in 1 liter of distilled water by steaming, add all the above ingredients, and adjust pH to 6.2–6.4. Sterilize at 121°C for 15 min
Antibiotics can be added to after autoclave

Antibiotics stock solution

Kanamycin stock solution: 25 mg/ml in H_2O, sterile filter, store in aliquots at –20°C

Ampicillin stock solution: 100 mg/ml in H_2O, sterile filter, store in aliquots at–20°C

IPTG (1 M): 238 mg/ml in H2O, sterile filter, store in aliquots at–20°C

Commonly Used Buffers and Solutions

0.5 M EDTA, pH 8.0

Ethylenediaminetetraacetic acid (EDTA)·2H2O 186.1 g

NaOH ~20 g

Adjust pH to 8.0 with NaOH

EDTA will not go into solution until the pH is about 8.0

1 M Tris·Cl

Tris base 121.1 g

Adjust to desired pH with HCl

TE, pH 7.4

1 M Tris·Cl, pH 7.4 10 ml

0.5 M EDTA, pH 8.0 2 ml

10% SDS

Sodium dodecyl sulfate (SDS) 100 g

Adjust pH to 7.2 with HCl

There is no need to sterilize 10% SDS

Agarose Gel Electrophoresis Buffers for Analysis of DNA

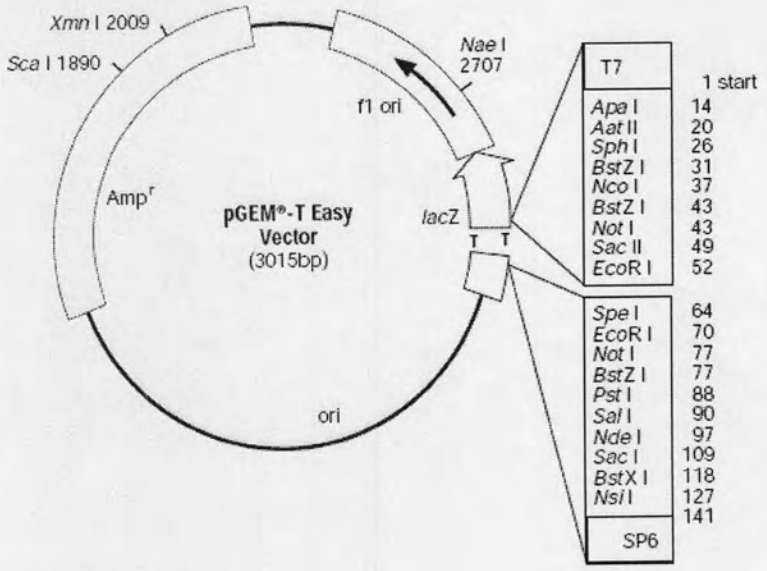
Buffer	Composition of working solution	Stock solution components per liter
TAE	**1x** 40 mM Tris·acetate 1 mM EDTA	**50x** Tris base 242 g Glacial acetic acid 57.1 ml 0.5 M EDTA, pH 8.0 100 ml
TBE	**0.5x** 45 mM Tris·borate 1 mM EDTA	**5x** Tris base 54 g Boric acid 27.5 g 0.5 M EDTA, pH 8.0 20 ml

Gel loading buffer	Components per 10 ml
6x 0.25% bromophenol blue	Bromophenol blue 25 mg
0.25% xylene cyanol FF	Xylene cyanol FF 25 mg
40% (w/v) sucrose*	Sucrose *4 g

30% glycerol can be used instead of sucrose

APPENDIX II

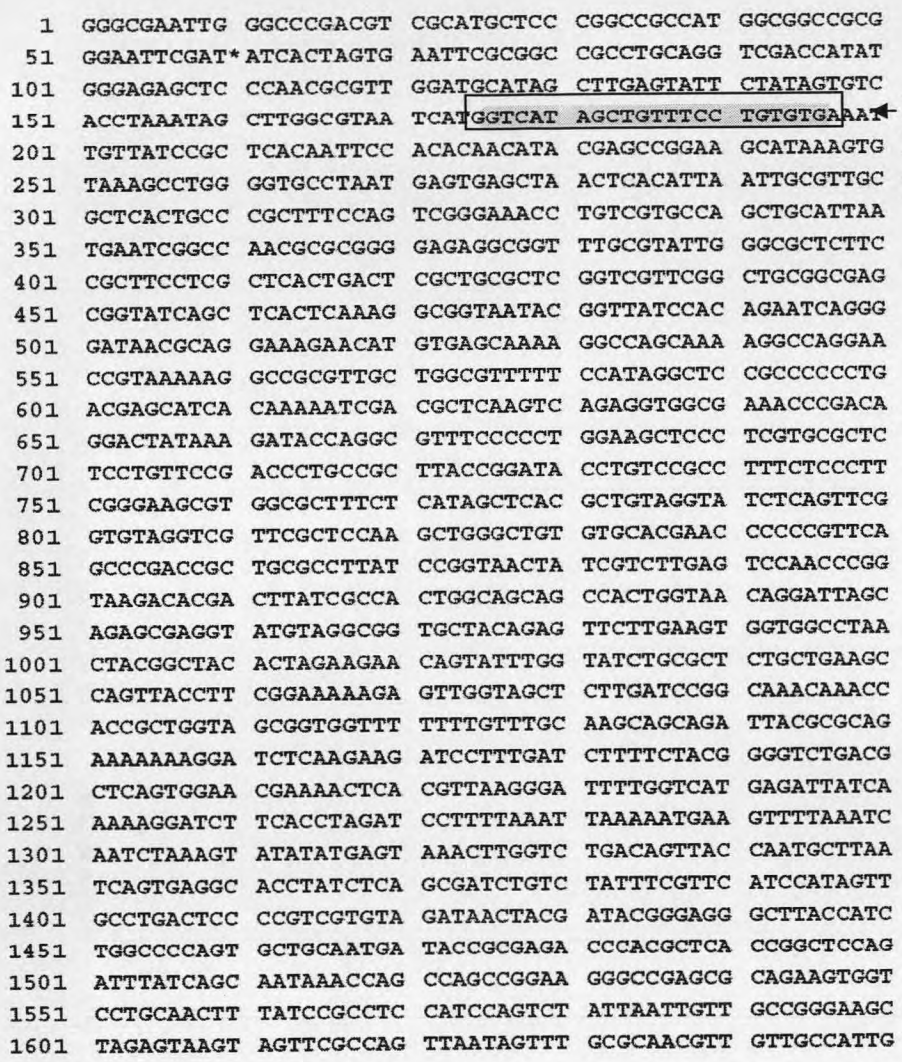
Map and sequence of plasmid vector used in this study



pGEM®-T Easy Vector circle map and sequence reference points.

pGEM®-T Easy Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–128
SP6 RNA polymerase promoter (–17 to +3)	139–158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176–197
lacZ start codon	180
lac operator	200–216
β-lactamase coding region	1337–2197
phage f1 region	2380–2835
lac operon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA polymerase promoter (–17 to +3)	2999–3



pGEM®-T Easy Vector Sequence the boxed are the annealing positions for sequencing primers M13

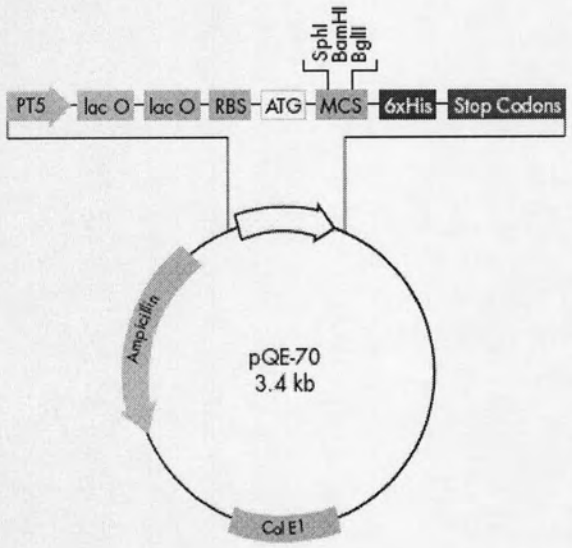
Restriction enzymes that cut the pGEM®-T Easy vector between 1 and 5 times

Enzyme	# of Sites	Location
***Aat* II**	1	20
***Acc* I**	1	91
***Acy* I**	2	17, 1947
Afl III	2	114, 517
***Alw26* I**	2	1471, 2247
***Alw44* I**	2	831, 2077
*Alw*N I	1	933
***Apa* I**	1	14
*Asp*H I	4	109, 835, 1996, 2081
***Ava* II**	2	1548, 1770
***Ban* I**	3	261, 1358, 2641
***Ban* II**	3	14, 109, 2679
***Bbu* I**	1	26
***Bgl* I**	4	39, 42, 1530, 2848
Bsa I	1	1471
*Bsa*A I	1	2604
*Bsa*H I	2	17, 1947
*Bsa*J I	5	37, 46, 256, 677, 2951
*Bsp*120 I	1	10
*Bsp*H I	2	1237, 2245
*Bsp*M I	1	77
*Bss*S I	2	690, 2074
***Bst*O I**	5	257, 545, 666, 679, 2952
***Bst*X I**	1	118
***Bst*Z I**	3	31, 43, 77
*Cfr*10 I	2	1490, 2705
***Dde* I**	4	792, 1201, 1367, 1907
***Dra* I**	3	1276, 1295, 1987
Dra III	1	2604
Drd I	2	625, 2559
Dsa I	2	37, 46
Eag I	3	31, 43, 77
Ear I	3	401, 2205, 2893
***Ecl*HK I**	1	1410
***Eco52* I**	3	31, 43, 77
***Eco*ICR I**	1	107
EcoR I	2	52, 70
***Eco*R V**	1	60 (see above)

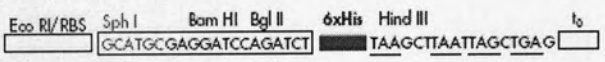
Enzyme	# of Sites	Location
***Fok* I**	5	134, 1376, 1557, 1844, 2931
Fsp I	2	1632, 2855
***Hae* II**	4	395, 765, 2755, 2763
Hga I	4	628, 1206, 1936, 2821
***Hinc* II**	1	92
Hind II	1	92
***Hsp92* I**	2	17, 1947
Mae I	5	65, 1012, 1265, 1600, 2755
***Mlu* I**	1	114
***Nae* I**	1	2707
***Nci* I**	4	30, 897, 1593, 1944
***Nco* I**	1	37
***Nde* I**	1	97
***Ngo*M IV**	1	2705
***Not* I**	2	43, 77
***Nsi* I**	1	127
Nsp I	2	26, 521
*Ppu*10 I	1	123
***Pst* I**	1	88
***Pvu* I**	2	1780, 2876
***Pvu* II**	2	341, 2905
***Rsa* I**	1	1890
***Sac* I**	1	109
***Sac* II**	1	49
***Sal* I**	1	90
***Sca* I**	1	1890
***Sin* I**	2	1548, 1770
***Spe* I**	1	64
***Sph* I**	1	26
*Sse*8387 I	1	88
***Ssp* I**	2	2214, 2396
***Sty* I**	1	37
***Taq* I**	5	56, 91, 617, 2061, 2637
Tfi I	2	352, 492
***Vsp* I**	3	288, 347, 1582
***Xmn* I**	1	2009

pQE-70 Vector

Positions of elements in bases	
Vector size (bp)	3426
Start of numbering at *Xho*I (CTCGAG)	1–6
T5 promoter/lac operator element	7–87
T5 transcription start	61
6xHis-tag coding sequence	133–150
Multiple cloning site	113–132
Lambda t_0 transcriptional termination region	173–267
rrnB T1 transcriptional termination region	1029–1127
ColE1 origin of replication	1603
β-lactamase coding sequence	3221–2361



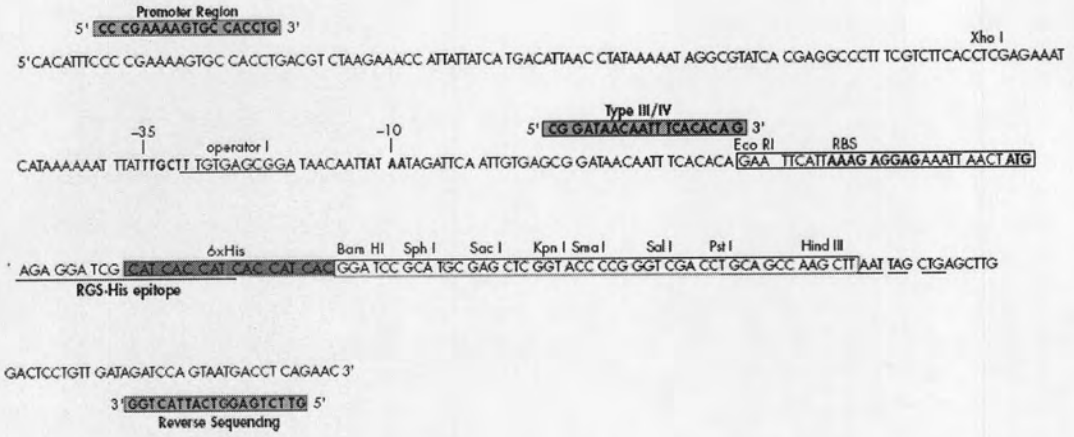
pQE-70



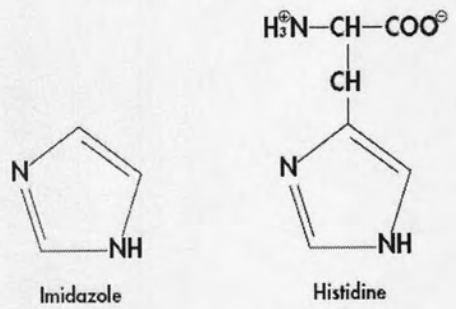
pQE-70 expression vector circle map and their multiple cloning sites

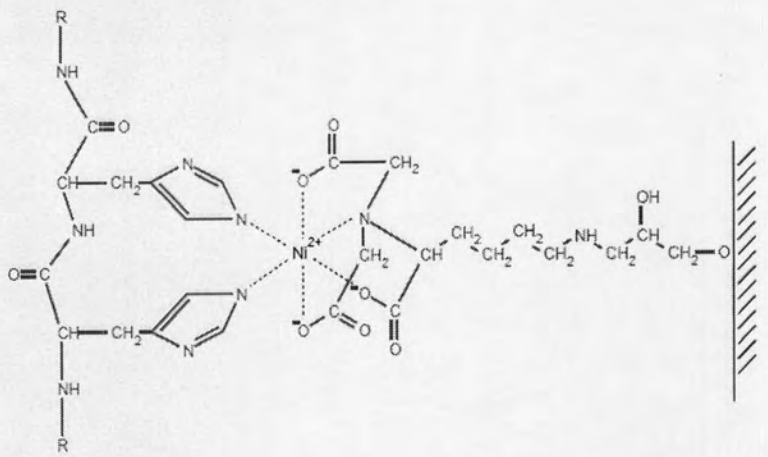
pQE vectors are designed for cloning and expression of 6xHis-tagged proteins.

Sequencing primers for pQE vectors



Chemical structures of histidine and imidazole used for in purification of His-tagged expressed proteins and Interaction between neighboring residues in the 6xHis tag and Ni-NTA matrix. Imidazole is used as an eluting reagent.





Symbols for Nucleic Acids and incompletely specified bases in nucleic acid sequences

Symbol	Meaning	Origin of designation
G	G	Guanine
A	A	Adenine
T	T	Thymine
C	C	Cytosine
R	G or A	puRine
Y	T or C	pYrimidine
M	A or C	aMino
K	G or T	Keto
S	G or C	Strong interaction (3 H bonds)
W	A or T	Weak interaction (2 H bonds)
H	A or C or T	not-G, H follows G in the alphabet
B	G or T or C	not-A, B follows A
V	G or C or A	not-T (not-U), V follows U
D	G or A or T	not-C, D follows C
N	G or A or T or C	aNy

Source: **IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN)**

(http://www.chem.qmul.ac.uk/iupac/AminoAcid/)

Nomenclature and Symbolism for Amino Acids and Peptides

One-letter symbol	Three-letter symbol	Amino acid
A	Ala	alanine
B	Asx	aspartic acid or asparagine
C	Cys	cysteine
D	Asp	aspartic acid
E	Glu	glutamic acid
F	Phe	phenylalanine
G	Gly	glycine
H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Leu	leucine
M	Met	methionine
N	Asn	asparagine
P	Pro	proline
Q	Gln	glutamine
R	Arg	arginine
S	Ser	serine
T	Thr	threonine
U*	Sec	selenocysteine
V	Val	valine
W	Trp	tryptophan
X**	Xaa	unknown or 'other' amino acid
Y	Tyr	tyrosine
Z	Glx	glutamic acid or glutamine (or substances such as 4-carboxyglutamic acid and 5-oxoproline that yield glutamic acid on acid hydrolysis of peptides)

The symbols are listed, in alphabetical order of amino-acid names

Source: **IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN)**

(http://www.chem.qmul.ac.uk/iupac/AminoAcid/)

Standard Codon Usage Table

Codon Usage Table*

First Position	Second Position: U	Second Position: C	Second Position: A	Second Position: G	Third Position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop (och)	UGA Stop (opal)	A
	UUG Leu	UCG Ser	UAG Stop (amb)	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val (Met)	GCG Ala	GAG Glu	GGG Gly	G

* Bases are given as ribonucleotides. GUG usually codes for valine, but it can code for methionine to initiate an mRNA chain. Stop (och) refers to the ochre termination triplet and Stop (amb) refers to the amber.

Copied from Invitrogen Support

Common Conversions of Nucleic Acids and Proteins
Weight Conversions
1 μg=10-6 g
1 ng=10-9 g
1 pg=10-12 g
1 fg=10-15 g

Spectrophotometric Conversions
1 A_{260} unit of double-stranded DNA = 50 μg/ml
1 A_{260} unit of single-stranded DNA = 33 μg/ml
1 A_{260} unit of single-stranded RNA = 40 μg/ml

DNA Molar Conversions
1 μg of 1,000 bp DNA = 1.52 pmole (3.03 pmoles of ends)
1 pmole of 1,000 bp DNA = 0.66 μg

Protein Molar Conversions
100 pmoles of 100,000 dalton protein = 10 μg
100 pmoles of 50,000 dalton protein = 5 μg
100 pmoles of 10,000 dalton protein = 1 μg

Protein/DNA Conversions
1 kb of DNA = 333 amino acids of coding capacity
= 3.7 x 104 dalton protein
10,000 dalton protein = 270 bp DNA
50,000 dalton protein = 1.35 kb DNA
100,000 dalton protein = 2.7 kb DNA

Formulas

Picomole Ends per Microgram of Double-Stranded Linear DNA
(2 x 106)/(660 x Number of Bases) = pmole ends/μg

Exact Molecular Weight of an Oligonucleotide
[(A x 312.2) + (G x 328.2) + (C x 288.2) + (T x 303.2) - 61.0]
= MW (g/mol) of specific oligonucleotide

Protein Molar Conversions
Micrograms Protein
= Protein Size (kDa) x pmole protein x 10^9 μg/g x mole/10^{12} pmol

APPENDIX III

1. Sequence identity matrix of 16S rRNA gene of the strain *Lactobacillus* sp. nov. L13 compared with selected lactobacilli.

Sequence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. **L13**	100																				
2. ***L.parabrevis***	**98.5**	100																			
3. ***L.hammesii***	98.9	**98.8**	100																		
4. *L.frumenti*	90.1	89.8	90.2	100																	
5. *L.panis*	89.8	89.5	89.8	97.7	100																
6. *L.pontis*	90.1	89.8	90.3	97.6	97.1	100															
7. *L.reuteri*	88.8	88.6	88.9	95.0	94.1	94.7	100														
8. *L.fermentum*	89.4	89.3	89.6	92.1	91.8	91.9	91.1	100													
9. *L.brevis*	97.8	97.3	97.9	89.8	89.3	89.9	88.4	89.6	100												
10. *L.zymae*	98.6	98.0	97.1	90.8	90.2	91.0	89.3	89.5	98.8	100											
11. *L.acidifarinae*	98.5	98.2	97.1	90.8	90.3	91.0	89.4	89.4	98.8	99.7	100										
12. *L.spicheri*	96.3	95.8	96.8	90.5	90.5	90.9	89.3	89.1	97.3	98.0	98.1	100									
13. *L.sanfranciscensis*	90.4	89.8	90.5	87.4	87.5	87.7	86.6	87.7	89.9	90.0	89.7	89.7	100								
14. *L.buchneri*	91.3	90.7	91.8	88.5	88.0	88.5	87.0	87.4	91.7	92.2	92.3	92.5	89.8	100							
15. *L.farciminis*	88.8	88.9	88.7	86.9	86.9	86.9	85.3	87.4	88.7	88.9	88.7	88.9	86.8	87.2	100						
16. *L.paralimentarius*	90.8	91.2	91.0	88.5	88.0	88.3	86.7	88.2	90.4	90.9	90.8	90.9	87.3	87.8	98.4	100					
17. *L.alimentarius*	90.2	90.6	90.4	87.7	87.7	87.9	86.4	87.9	90.0	90.4	90.2	90.3	87.9	87.9	95.7	97.6	100				
18. *L.sakei*	90.9	91.0	91.2	89.0	89.0	89.1	88.3	88.4	90.4	90.9	90.8	90.6	87.5	89.5	89.7	90.4	90.8	100			
19. *L.curvatu*	91.4	91.5	91.6	89.6	89.5	89.6	88.8	88.8	90.7	91.3	91.3	91.1	87.2	88.7	89.4	90.9	89.9	97.9	100		
20. *L.plantarum*	90.9	91.4	91.1	87.7	87.2	87.7	86.7	87.5	90.7	90.9	90.7	90.8	87.8	88.2	89.3	90.6	90.5	90.5	90.5	100	
21. *L.delbrueckii*	86.4	86.4	86.5	86.7	87.1	86.4	84.6	85.8	86.3	86.3	86.3	86.1	83.7	84.5	84.3	84.9	84.8	85.5	85.9	84.4	100

2. Sequence identity matrix of *rpoA* gene of the strain *Lactobacillus* sp. nov. L13 compared with selected lactobacilli.

Sequence	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. **L13**	100													
2. ***L.parabrevis***	**92.0**	100												
3. ***L.hammesii***	**92.2**	**96.5**	100											
4. *L.brevis*	90.3	91.8	90.7	100										
5. *L.zymae*	87.0	86.9	86.9	86.5	100									
6. *L.spichei*	86.5	86.7	87.0	87.6	90.8	100								
7. *L.sanfranciscensis*	71.3	71.9	70.3	71.7	69.8	70.0	100							
8. *L.plantarum*	72.1	72.8	73.6	74.0	71.7	74.0	70.2	100						
9. *L.sakei*	69.4	70.5	70.7	70.2	68.8	70.0	68.3	72.8	100					
10. *L.curvatus*	69.6	70.7	71.3	70.9	68.6	70.0	69.0	74.1	96.2	100				
11. *L.panis*	69.2	70.0	70.5	71.1	68.8	69.2	70.5	67.9	64.8	66.0	100			
12. *L.reuteri*	69.4	70.9	70.9	70.9	69.0	68.5	71.7	68.6	67.3	67.7	83.8	100		
13. *L.delbruekii*	59.7	60.7	60.5	60.1	60.9	61.6	61.2	63.3	63.3	64.1	59.0	59.2	100	
14. *L.hilgardii*	73.2	72.1	71.5	72.4	70.5	71.3	73.2	73.0	70.2	70.5	73.2	72.4	59.7	100

3. Sequence identity matrix of 16S rRNA gene of *Enterococcus camilliae* sp. nov FP15-1 compared with selected enterococci.

Sequence 16S rDNA	1	2	3	4	5	6	7	8	9	10
1. **FP15_1**	100									
2. ***E.italicus***	**99.2**	100								
3. *E.sulfureus*	98.1	98.2	100							
4. *E.saccharolyticus*	98.3	98.4	97.6	100						
5. *E.faecium*	96.2	96.6	96.6	97.0	100					
6. *E.canintestini*	96.7	97.1	96.7	97.5	97.8	100				
7. *E.avium*	96.7	97.0	97.0	97.4	98.6	97.8	100			
8. *E.phoeniculicola*	95.9	96.6	96.4	96.9	97.9	97.3	97.9	100		
9. *E.gallinarum*	97.0	97.6	97.4	98.3	97.8	97.9	98.1	97.7	100	
10. *E.faecalis*	95.7	95.9	95.8	96.7	96.8	96.0	96.2	96.4	96.6	100

4. Sequence identity matrix of *rpoA* gene of *Enterococcus camilliae* sp. nov FP15-1 compared with selected enterococci

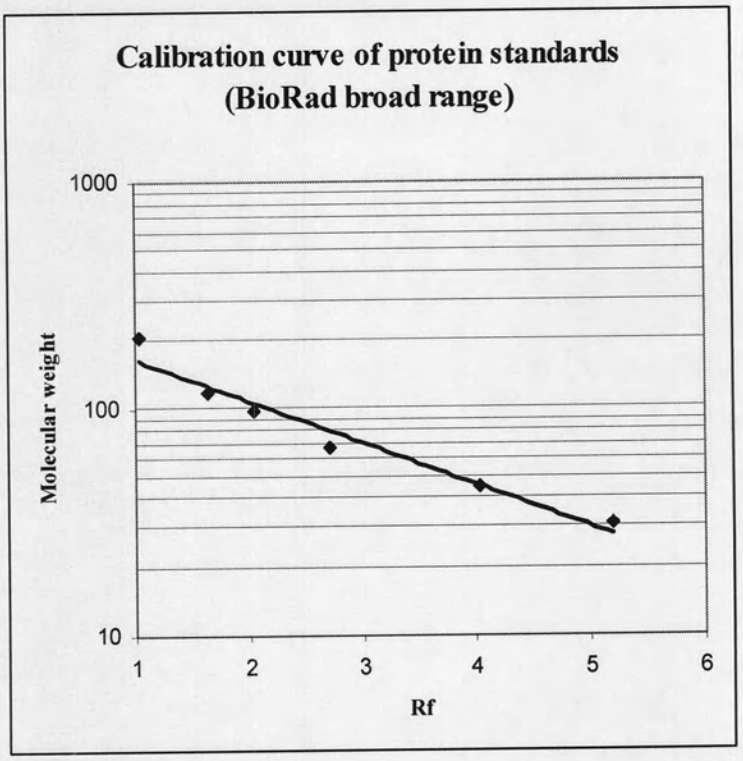
Sequence *rpoA*	1	2	3	4	5	6	7	8	9
1. **FP15_1**	100								
2. ***E.italicus***	93.8	100							
3. *E.sulfureus*	88.1	88.9	100						
4. *E.saccharolyticus*	88.2	89.4	86.0	100					
5. *E.faecium*	86.5	87.1	86.6	83.5	100				
6. *E.canintestini*	81.7	81.3	84.2	81.4	84.0	100			
7. *E.avium*	81.6	82.2	83.4	81.3	82.7	80	100		
8. *E.gallinarum*	87.8	87.8	90.2	86.1	85.5	82.9	82.1	100	
9. *E.faecalis*	84.2	84.8	85.5	83.5	83.5	80.6	86.1	84.7	100

5. Sequence identity matrix of identified genes of L13 and LSF 8-13 compared with others *gadB.*

***gadB* sequence** (1329 bp)	1	2	3	4	5	6	7	8	9	10	11
1. *E.coli gadA*	100										
2. *E.coli gadB*	98.2	100									
3. *Lc.lactis* subsp.*lactis*	51.8	52.2	100								
4. *E.faecium*	53.6	54.1	79.2	100							
5. *L.brevis* ATCC367 S49	54.0	54.1	66.5	68.1	100						
6. *L.plantarum* WCFS	54.4	54.4	65.3	68.0	72.8	100					
7. LSF8-13 *gadB*	54.4	54.4	65.3	68.0	72.8	99.7	100				
8. *L.brevis* OPK	54.0	54.1	66.6	68.1	99.8	72.9	72.9	100			
9. *L.brevis* ATCC 367 S18	49.8	49.6	60.9	59.2	57.4	58.7	58.6	57.3	100		
10. *L.brevis* IFO12005	49.8	49.5	60.8	59.5	57.4	58.8	58.6	57.2	99.4	100	
11. L13 *gadB*	49.2	49.5	57.8	58.3	56.6	57.9	57.7	56.5	72.4	**72.3**	100

6. Sequence identity (similarity) matrix of deduced amino of L13 and LSF 8-13 compared with other GADs.

Deduced amino *gadB*	1	2	3	4	5	6	7	8	9	10
1. *E.coli gadA*	100									
2. *E.coli gadB*	99.7	100								
3. *Lc.lactis* subsp.*lactis*	44.1	44.3	100							
4. *L.brevis* ATCC367 S49	43.4	43.2	70.9	100						
5. *L.plantarum* WCFS	44.3	44.5	68.6	83.3	100					
6. LSF8-13 *gadB*	44.3	44.5	68.6	83.3	99.5	100				
7. *L.brevis* OPK	43.0	43.0	70.7	98.8	82.8	82.8	100			
8. *L.brevis* ATCC 367 S18	37.8	37.8	52.9	51.8	53.3	53.1	51.8	100		
9. *L.brevis* IFO12005	37.8	37.8	53.1	52.0	53.6	53.3	52.0	99.7	100	
10. L13 *gadB*	37.1	36.9	52.0	50.6	51.5	51.3	50.4	82.2	81.9	
(%similarity)	(56.9)	(56.9)	(69.1)	(66.2)	(66.7)	(66.2)	(65.5)	(88.9)	(88.7)	100



Calibration curve of protein standards taken from the SDS-PAGE in Fig.4.28

The equation of the line is:
y = -31.5x + 159.2

Molecular weight = (slope)(mobility) + y-intercept

The mobility of purified Gad of L13 was ~3.18 cm.

Approximatly calculated MW is 59.03 kDa

VITA

Miss Sirapan Sukontasing was born on December 29, 1971 in Bangkok, Thailand. She received her Bachelor's degree of Sciences from Kasetsart University in 1992, and Master's degree of Sciences in Industrial Microbiology from Chulalongkorn University in 1996. She received the scholarship from the Royal Golden Jubilee Ph.D. Program.

Publications

Sukontasing, S., Tanasupawat, S., Moonmangmee, S., Lee, J. S., and Suzuki, K. 2007 *Enterococcus camelliae* sp. nov., isolated from fermented tea leaves in Thailand. Int. J. Syst. Evol. Microbiol. (in press)

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Sukontasing, S., Tanasupawat, S., Moonmangmee, S., and Oda, K. 2005.Protease and related products of lactic acid bacteria. TISTR seminar. 28th July 2005.