

Appendix A

Bacterial Strains Used

No	Strain	Serogroups	Source	Location	Year
1	1/08	O1	Water	Petaling Jaya	2008
2	3/08	O1	Water	Klang	2008
3	3P/08	O1	Water	Banting	2008
4	4/08	O1	Human Stool	Kota Bharu	2008
5	6/08	O1	Human Stool	Kota Kinabalu	2008
6	9/08	O1	Human Stool	Kota Kinabalu	2008
7	10/08	O1	Human Stool	Kota Kinabalu	2008
8	11/08	O1	Algae	Petaling Jaya	2008
9	21/04	O1	Human Stool	Alor Setar	2004
10	35/04	O1	Human Stool	Alor Setar	2004
11	37/04	O1	Human Stool	Kota Bharu	2004
12	38/04	O1	Human Stool	Tumpat	2004
13	40/04	O1	Human Stool	Tumpat	2004
14	52/08	O1	Human Stool	Labuan	2008
15	63/08	O1	Human Stool	Labuan	2008
16	64/08	O1	Human Stool	Kota Kinabalu	2008
17	65/08	O1	Human Stool	Kota Bharu	2008
18	66/08	O1	Human Stool	Labuan	2008
19	70/08	O1	Water	Labuan	2008
20	87/08	O1	Water	Kota Bharu	2008
21	88/08	O1	Water	Kota Kinabalu	2008
22	90/08	O1	Water	Kota Kinabalu	2008

23	123/08	O1	Human Stool	HUKM	2008
24	4370/08	O139	Water	Klang	2008
25	17/08	non-O1/non-O139	Water	Bachok	2008
26	77/08	non-O1/non-O139	Water	Klang	2008
27	81/08	non-O1/non-O139	Seafood	Selangor	2008
28	82/08	non-O1/non-O139	Seafood	Selangor	2008
29	83/08	non-O1/non-O139	Seafood	Selangor	2008
30	84/08	non-O1/non-O139	Seafood	Selangor	2008
31	85/08	non-O1/non-O139	Seafood	Selangor	2008
32	310/08	non-O1/non-O139	Seafood	Selangor	2008
33	374/08	non-O1/non-O139	Water	Morib	2008
34	933/08	non-O1/non-O139	Seafood	Selangor	2008
35	3477/08	non-O1/non-O139	Water	Klang	2008
36	4933/08	non-O1/non-O139	Water	Klang	2008
37	B2/08	non-O1/non-O139	Water	Serdang	2008
38	B4/08	non-O1/non-O139	Water	Serdang	2008
39	M1/08	non-O1/non-O139	Water	Petaling Jaya	2008
40	M2/08	non-O1/non-O139	Water	Kajang	2008
41	GB/08	non-O1/non-O139	Water	Kajang	2008
42	PSW/08	non-O1/non-O139	Water	Morib	2008
43	SW/08	non-O1/non-O139	Algae	Morib	2008

Appendix B

Media, Buffer, Solution

All media and solutions were prepared with distilled or deionised water. They were sterilized by autoclaving at 15 psi at 121°C for 20 minutes, unless otherwise stated.

B.1 Arginine Dihydrolase Test

LB broth	100 ml
L-arginine solution	1 ml
Phenol red (indicator)	

B.2 Alkaline Peptone Water (APW)

Alkaline peptone water powder	12.0 g
Distilled water up to	400 ml

B.3 Cell lysis buffer (50 mM Tris, 50 mM EDTA, pH 8.0 + 1.0% Sarcosine)

1.0M Tris, pH 8.0	25 ml
0.5M EDTA, pH 8.0	50 ml
10% Sarcosyl	50 ml
Deionised water to	500 ml

B.4 Cell suspension buffer (100 mM Tris, 100 mM EDTA, pH 8.0)

1 M Tris, pH 8.0	10 ml
0.5 M EDTA, pH 8.0	20 ml
Deionised water to	100 ml

B.5 Ethylenediaminetetraacetic acid, (0.5 M) pH 8.0

EDTA 74.44g

Deionised water to 400 ml

The pH of the solution was adjusted pH to 8.0 by adding concentrated HCl.

B.6 Luria-bertani (LB) agar

Tryptone 1.0 g

Yeast extracts 0.5 g

NaCl 0.5 g

Bacteriological agar 1.5 g

Distilled water up to 100 ml

B.7 Luria-bertani (LB) broth

Tryptone 1.0 g

Yeast extracts 0.5 g

NaCl 0.5 g

Distilled water up to 100 ml

B.8 Oxidase Test

N,N,N',N'-Tetramethyl-p-phenylenediamine-2HCl 0.10 g

Distilled water up to 10 ml

B.9 Phosphate Buffered Saline (PBS), 10 X, pH7.4

NaCl 80 g

KCl 2 g

Na₂HPO₄ 14.4 g

KH₂PO₄ 2.4 g

Deionised water to 1000 ml

The pH of the stock solution was adjusted pH to 8.3 and autoclaved. It was then diluted to 1X for routine use.

B.10 Proteinase K (10.0 mg/ml)

Proteinase K powder (Promega, Madison, USA) 100 mg

Sterile deionised water to 10 ml

B.11 Sarcosyl (10% N-Lauryl-Sarcosine)

Sodium N-lauroyl-sarcosinate Solution 10 ml

Deionised water to 100 ml

B.12 Sodium chloride (0.85%)

Sodium chloride 3.2g

Distilled water up to 400ml

B.13 String-Test

Sodium deoxycholate 0.05g

Distilled water up to 10 ml

B.14 Thiosulphate citrate bile salt (TCBS) agar

Thiosulphate citrate bile salt (TCBS) powder 8.8 g

Distilled water up to 100 ml

B.15 Triple Sugar Iron (TSI) Agar supplemented with 3% NaCl

Triple Sugar Iron powder 6.5 g

NaCl 3.0 g

Distilled water up to 100 ml

B.16 Tris, (1M) pH 8.0

Tris 48.45g

Deionised water to 400 ml

The pH of the solution was adjusted pH to 8.0 by adding concentrated HCl.

B.17 Tris-Borate EDTA Buffer (TBE), 10 X, pH8.3

Tris base 121.1 g

Othoboric Acid 61.8 g

EDTA (Ultra Pure Grade) 0.745 g

Deionised water to 1000 ml

The pH of the stock solution was adjusted pH to 8.3 and autoclaved. It was then diluted to 0.5X for routine use.

B.18 Tris-EDTA (TE) buffer (10mM Tris : 1mM EDTA, pH8.0)

1 M Tris, pH 8.0 10.0 ml

0.5 M EDTA, pH 8.0 2.0 ml

Deionised water to 1000 ml

B.19 Tryptone broth with 0% NaCl

Tryptone water powder 1.0 g

Distilled water up to 100 ml

B.20 Tryptone broth with 3% NaCl

Tryptone water powder 1.0 g

NaCl 3.0 g

Distilled water up to 100 ml

B.21 Tryptone broth with 6% NaCl

Tryptone water powder 1.0 g

NaCl 6.0 g

Distilled water up to 100 ml

B.22 Tryptone broth with 10% NaCl

Tryptone water powder 1.0 g

NaCl 10.0 g

Distilled water up to 100 ml

Appendix C

Publications

1. Cindy Shuan Ju Teh, Kek Heng Chua , Kwai Lin Thong. (2011). Genetic Variation Analysis of *Vibrio cholerae* by using Multilocus Sequencing Typing and Multi-Virulence Locus Sequencing Typing. *Infect Genet Evol.* 11: 1121-1128. (ISI cited)
2. Cindy Shuan Ju Teh, Kek Heng Chua, Kwai Lin Thong (2011). Genotypic Characterization of *Vibrio cholerae* Isolated in Malaysia Based on Multilocus Sequencing Typing (MLST) and Multi-virulence Locus Sequencing Typing (MVLST). *Int J Infect Dis.* 15 (supple1): S35. (ISI cited).
3. Cindy Shuan Ju Teh, Kek Heng Chua, Ro Osawa, Kwai Lin Thong (2011). Comparative PCR-based Fingerprinting of *Vibrio cholerae* isolated in Malaysia. *J Gen Appl Microbiol* (Accepted) (ISI cited)
4. Cindy Shuan Ju Teh, Kek Heng Chua, Kwai Lin Thong (2010). Multiple-locus variable number tandem repeat analysis of *Vibrio cholerae* in comparison with pulsed field gel electrophoresis and virulotyping. *J Biomed Biotech.* doi: 10.1155/2010/817190 (ISI cited)
5. Cindy Shuan Ju Teh, Kek Heng Chua , Kwai Lin Thong (2010). Simultaneous Differential Detection of human pathogenic and non-pathogenic *Vibrio* species using a multiplex PCR based on *gyrB* and *pntA* genes. *J Appl Microbiol.* 108: 1940-1945. (ISI cited)

6. Cindy Shuan Ju Teh, Kwai Lin Thong, Soo Tein Ngoi, Norazah Ahmad, G Balakrish Nair, T. Ramamurthy (2009). Molecular Characterization of Serogrouping and Virulence genes of Malaysian *Vibrio cholerae* Isolated from Different Sources. *J Gen Appl Microbiol.* **55**: 419-425. (ISI cited)
7. Cindy Shuan Ju Teh, Kwai Lin Thong. (2009). Virulence factors determination and molecular characterization of Malaysian *Vibrio cholerae*. S33 International Journal of Infectious Diseases, Vol 13 Supple 1, Aug 2009. ISSN 1201-9712 (ISI cited)

Appendix D

Presentations

1. Cindy Shuan Ju Teh, Kek Heng Chua, Kwai Lin Thong (2011). Genotypic Characterization of *Vibrio cholerae* Isolated in Malaysia Based on Multilocus Sequencing Typing (MLST) and Multi-virulence Locus Sequencing Typing (MVLST). 5th Ditan International Conference on Infectious Disease (DICID), Beijing, China, 13-17 July, 2011.
2. Kwai Lin Thong, Cindy Shuan Ju Teh, Kek Heng Chua. “Comparative Genomic Analysis of Malaysian *Vibrio cholerae* Based on MLVA, PFGE and Virulotyping”. Oral presentation. ISISM, Indonesia, 4-7-October, 2010.
3. Cindy Shuan Ju Teh, Kwai Lin Thong., Kek Heng Chua. “Comparative Genomic Analysis of Malaysian *Vibrio cholerae* Based on MLVA, PFGE and Virulotyping”. Oral presentation. BSGC, UM.
4. Cindy Shuan Ju Teh, Kwai Lin Thong. “Virulence Factors Determination and Molecular Characterisation of Malaysian *Vibrio cholerae*”. Oral

- Presentation. 3rd Ditan International Conference on Infectious Disease (DICID), Beijing, China, 30-1 August, 2009.
5. Cindy Shuan Ju Teh, Kwai Lin Thong. “Characterisation of Malaysian Toxigenic and Non-toxigenic *Vibrio cholerae*” 18th Malaysia Society for Molecular Biology and Biotechnology (MSMBB), Saujana Hotel, Kuala Lumpur, 18-20 August, 2009.
 6. Cindy Shuan Ju Teh, Kwai Lin Thong. “A Tetraplex-PCR Assay For Detection Of Pathogenic And Non-Pathogenic *Vibrio* spp. in Malaysia”. Poster Presentation. International Congress of Biochemistry and Molecular Biology di Shanghai, China, 2 -7 August 2009.
 7. Cindy Shuan Ju Teh, Ro Osawa, Kek Heng Chua, Kwai Lin Thong. “Comparative PCR-based Fingerprinting towards a better genomic Diversity Analysis for *Vibrio cholerae* in Malaysia”. Poster Presentation. International Congress of Malaysia Society for Microbiology. Park Royal Hotel, Penang, 1 - 4 December 2009.

Appendix E

Interlectual Property

1. A System for One Step Detection of *Vibrio* species, *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* (IP20092073).

Appendix F

Awards

1. **Gold Medal Award.** A Rapid and Easy PCR Assay for Differentiation of Human Pathogenic and Non-Pathogenic *Vibrio* Spp. Umexpo 2010. 1-3 April, UM.
2. **Gold Medal Award.** Molecular Approaches Towards the Differentiation of Biotypes, Serogroups and Virulence Genes in *Vibrio cholerae*. Umexpo 2010. 1-3 April, UM.
3. **Best of the best Award.** Development and Application of Multilocus Variable Tandem Repeat Analysis: A New Generic Technology for Subtyping and Characterisation of Bacterial Pathogens. Umexpo 2010. 1-3 April, UM.
4. **Best of Category Award.** Development and Application of Multilocus Variable Tandem Repeat Analysis: A New Generic Technology for Subtyping and Characterisation of Bacterial Pathogens. Umexpo 2010. 1-3 April, UM.
5. **Gold Medal Award.** Development and Application of Multilocus Variable Tandem Repeat Analysis: A New Generic Technology for Subtyping and Characterisation of Bacterial Pathogens. Umexpo 2010. 1-3 April, UM.
6. **Gold medal Award.** A one step PCR method for differentiation of human pathogenic and non-pathogenic *Vibrio* species. BioMalaysia 2009. 11-13 November 2009, KLCC.
7. **Silver Medal Award.** EzPlex V - A Rapid Multiplex-PCR for Simultaneous Detection of *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and other *Vibrio* spp. 20th ITEX, 17-19 May 2009, KLCC.