

CHAPTER V



RESULT

PART I : IDENTIFICATION OF *Vibrio parahaemolyticus* .

Two hundred and eleven samples were examined in this study. These 211 samples consisted of one- hundred and eighty-nine of non-clinical isolates of *Vibrio spp.*, which were randomly collected from shrimp, sediment, coastal water, and cockle samples and twenty-two strains of *Vibrio parahaemolyticus* isolated from stool samples. All isolates were tested using 23 selected biochemical methods as listed in Table 5.

Seventy isolates were identified as *Vibrio parahaemolyticus*. Of these isolates, 32 isolates showed 5 atypical biochemical characteristics. The negative results were found in 28 isolates for ornithine decarboxylation test, 6 isolates for arabinose fermentation test, 1 isolate for MR test. The positive results were found in 3 isolates for 10 % NaCl requirement test.

Table 5 : Characteristics of the 70 isolates of *Vibrio parahaemolyticus* identified.

Strain no.	Source	Oxidase*	Indole*	MR	Vp*	Citrate	Motility	TSI*	0% NaCl*	3% NaCl*	6% NaCl*	8% NaCl*	10% NaCl*	Arginine*	Ornithine*	Lysine*	Glucose*	Manitol*	Mannose	Arabinose*	Sucrose*	Lactose*	Inositol	Rhamnose
CA 1	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 5	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 6	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 10	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 11	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 12	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 13	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
CA 17	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 19	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 20	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 21	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 22	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 23	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 24	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 25	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 32	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 33	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 34	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
CA 35	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 36	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
CA 40	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
CA 41	C	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
EP 10	RP/CHN	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
EP 11	RP/CHN	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
EP 16	RP/CHN	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
EP 18	RP/CHN	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
EP 22	RP/CHN	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-

Strain no.	Source	Oxidase*	Indole*	MR	VP*	Citrate	Motility	TSI*	0% NaCl*	3% NaCl*	6% NaCl*	8% NaCl*	10% NaCl*	Arginine*	Ornithine*	Lysine*	Glucose*	Manitol*	Mannose	Arabinose*	Sucrose*	Lactose*	Inositol	Rhamnose
EP 25	RP/CHN	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
EP 26	RP/CHN	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
EP 29	RP/CHN	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
EP 35	RP/CHN	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 1	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 2	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 3	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 5	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 6	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 7	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 8	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 9	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 12	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 13	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 14	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 15	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-
SMV 16	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-
SMV 17	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 18	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-
SMV 19	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-
SMV 21	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 22	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 29	RP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 31	RP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 33	RP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 34	RP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 35	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 36	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 37	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 38	RP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-

Strain no.	Source	Oxidase*	Indole*	MR	VP*	Citrate	Motility	TSI*	0% NaCl*	3% NaCl*	6% NaCl*	8% NaCl*	10% NaCl*	Arginine*	Ornithine*	Lysine*	Glucose*	Manitol*	Mannose	Arabinose*	Sucrose*	Lactose*	Inositol	Rhamnose
SMV 39	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-
SMV 40	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-
SMV 41	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 43	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 44	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 45	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 46	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 47	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 48	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 49	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 50	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
SMV 51	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
SMV 52	SP/SRT	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-
ATCC 17802	-	+	+	+	-	-	+	K/A	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-

K/A : alkaline top and acidic bottom ; + , positive ; - , negative

C : clinical isolate ; RP : shrimp from shrimp pond ;

SP : sediment from shrimp pond ; CHN : Chonburi province ;

SRT : Suratthani province

** Test is recommended as part of the routine set for Vibrio identification*

PART II : ANTIMICROBIAL SUSCEPTIBILITY TESTING.

The results of antimicrobial susceptibility test of ciprofloxacin were summarized in Table 6. MIC distribution of *Vibrio parahaemolyticus* isolates are shown in Fig 4 - 8. For 70 *Vibrio parahaemolyticus* isolates (Fig 4.), 5 isolates have MIC \leq 0.064 $\mu\text{g/ml}$, 24 isolates have MIC = 0.128 $\mu\text{g/ml}$, 16 isolates have MIC = 0.256 $\mu\text{g/ml}$, 7 isolates have MIC = 1 $\mu\text{g/ml}$, 12 isolates have MIC = 2 $\mu\text{g/ml}$, 5 isolates have MIC = 4 $\mu\text{g/ml}$ and 1 isolates has MIC \geq 8 $\mu\text{g/ml}$. MIC distribution of 48 environmental isolates is shown in Fig. 5. Two isolates have MIC \leq 0.064 $\mu\text{g/ml}$, 5 isolates have MIC = 0.128 $\mu\text{g/ml}$, 16 isolates have MIC = 0.256 $\mu\text{g/ml}$, 7 isolates have MIC = 1 $\mu\text{g/ml}$, 12 isolates have MIC = 2 $\mu\text{g/ml}$, 5 isolates have MIC = 4 $\mu\text{g/ml}$ and 1 isolates has MIC \geq 8 $\mu\text{g/ml}$.

MIC distribution of 34 sediment isolates is demonstrated in Fig 6 comprising of 1 isolates have MIC \leq 0.064 $\mu\text{g/ml}$, 5 isolates have MIC = 0.128 $\mu\text{g/ml}$, 11 isolates have MIC = 0.256 $\mu\text{g/ml}$, 6 isolates have MIC = 1 $\mu\text{g/ml}$, 8 isolates have MIC = 2 $\mu\text{g/ml}$, 3 isolates have MIC = 4 $\mu\text{g/ml}$. For 14 shrimp isolates MIC distribution are shown in Fig 7. One isolates have MIC \leq 0.064 $\mu\text{g/ml}$, 5 isolates have MIC = 0.256 $\mu\text{g/ml}$, 1 isolates have MIC = 1 $\mu\text{g/ml}$, 4 isolates have MIC = 2 $\mu\text{g/ml}$, 2 isolates have MIC = 4 $\mu\text{g/ml}$ and 1 isolates has MIC \geq 8 $\mu\text{g/ml}$. MIC distribution of 22 clinical isolates are shown in Fig 8. Three isolates have MIC \leq 0.064 $\mu\text{g/ml}$ and 19 isolates have MIC = 0.128 $\mu\text{g/ml}$. MIC 90 and MIC 50 value are demonstrated in Table 8.

Twenty-one isolates of 70 *Vibrio parahaemolyticus* isolates were indicated to have MIC values \geq 1 $\mu\text{g/ml}$. Of these isolates, none of *Vibrio*

parahaemolyticus clinical isolates were found to have MIC values ≥ 1 $\mu\text{g/ml}$. All selected isolates were comprised of 13 sediment isolates and 8 shrimp isolates. MIC values ranged between 1-8 $\mu\text{g/ml}$. The highest MIC values, 8 $\mu\text{g/ml}$, was isolated from shrimp in Chonburi province.



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Table 6 : MIC of 70 *Vibrio parahaemolyticus* isolates.

Strain no.	Source	MIC ($\mu\text{g/ml}$)	Strain no.	Source	MIC ($\mu\text{g/ml}$)	Strain no.	Source	MIC ($\mu\text{g/ml}$)
CA 1	C	0.064	EP 16	RP/CHN	2	SMV 21	SP/SRT	0.128
CA 5	C	0.064	EP 18	RP/CHN	2	SMV 22	SP/SRT	0.256
CA 6	C	0.128	EP 22	RP/CHN	1	SMV 29	RP/SRT	0.256
CA 10	C	0.128	EP 25	RP/CHN	8	SMV 31	RP/SRT	0.256
CA 11	C	0.128	EP 26	RP/CHN	4	SMV 33	RP/SRT	0.256
CA 12	C	0.128	EP 29	RP/CHN	4	SMV 34	RP/SRT	0.256
CA 13	C	0.128	EP 35	RP/CHN	0.064	SMV 35	SP/SRT	0.256
CA 17	C	0.128	SMV 1	SP/SRT	2	SMV 36	SP/SRT	1
CA 19	C	0.128	SMV 2	SP/SRT	2	SMV 37	SP/SRT	4
CA 20	C	0.128	SMV 3	SP/SRT	0.064	SMV 38	RP/SRT	0.256
CA 21	C	0.128	SMV 5	SP/SRT	0.128	SMV 39	SP/SRT	0.256
CA 22	C	0.128	SMV 6	SP/SRT	0.128	SMV 40	SP/SRT	0.256
CA 23	C	0.128	SMV 7	SP/SRT	0.128	SMV 41	SP/SRT	0.256
CA 24	C	0.128	SMV 8	SP/SRT	0.256	SMV 43	SP/SRT	1
CA 25	C	0.128	SMV 9	SP/SRT	0.256	SMV 44	SP/SRT	2
CA 32	C	0.128	SMV 12	SP/SRT	1	SMV 45	SP/SRT	3
CA 33	C	0.064	SMV 13	SP/SRT	1	SMV 46	SP/SRT	2
CA 34	C	0.128	SMV 14	SP/SRT	0.128	SMV 47	SP/SRT	0.256
CA 35	C	0.128	SMV 15	SP/SRT	1	SMV 48	SP/SRT	1
CA 36	C	0.128	SMV 16	SP/SRT	0.256	SMV 49	SP/SRT	2
CA 40	C	0.128	SMV 17	SP/SRT	0.256	SMV 50	SP/SRT	4
CA 41	C	0.128	SMV 18	SP/SRT	0.256	SMV 51	SP/SRT	2
EP 10	RP/CHN	2	SMV 19	SP/SRT	2	SMV 52	SP/SRT	2
EP 11	RP/CHN	2						

C : clinical isolate ; *RP* : shrimp from shrimp pond ;

SP : sediment from shrimp pond ; *CHN* : Chonburi province ;

SRT : Suratthani province

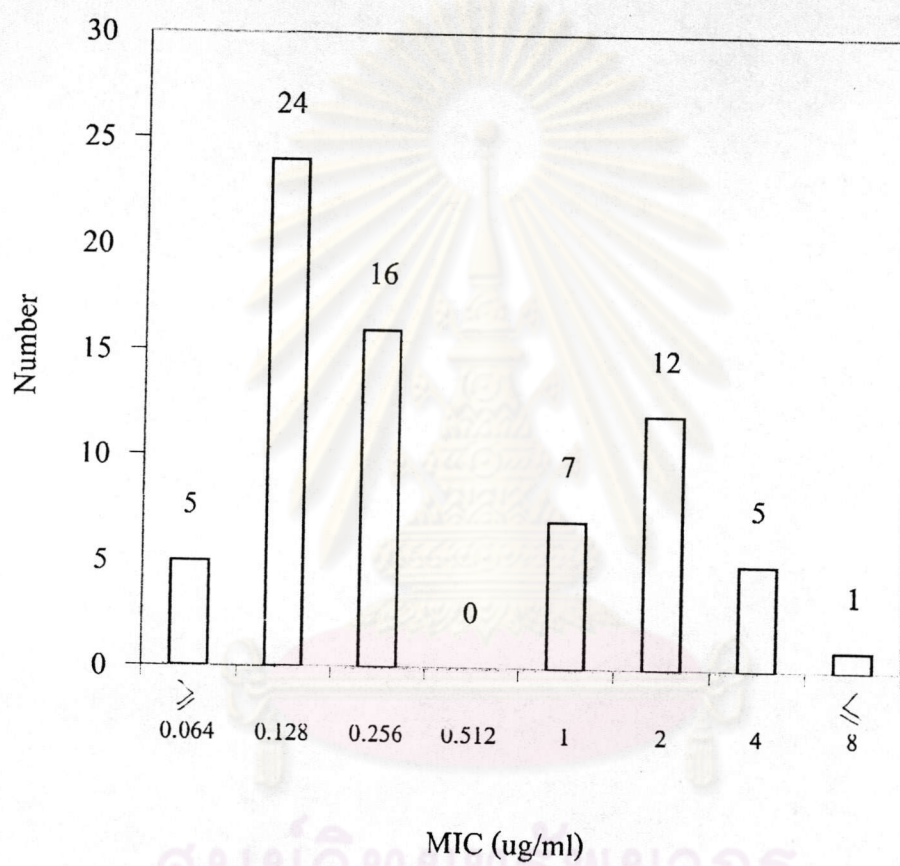


Fig. 4 MIC distribution of 70 *Vibrio parahaemolyticus* isolates

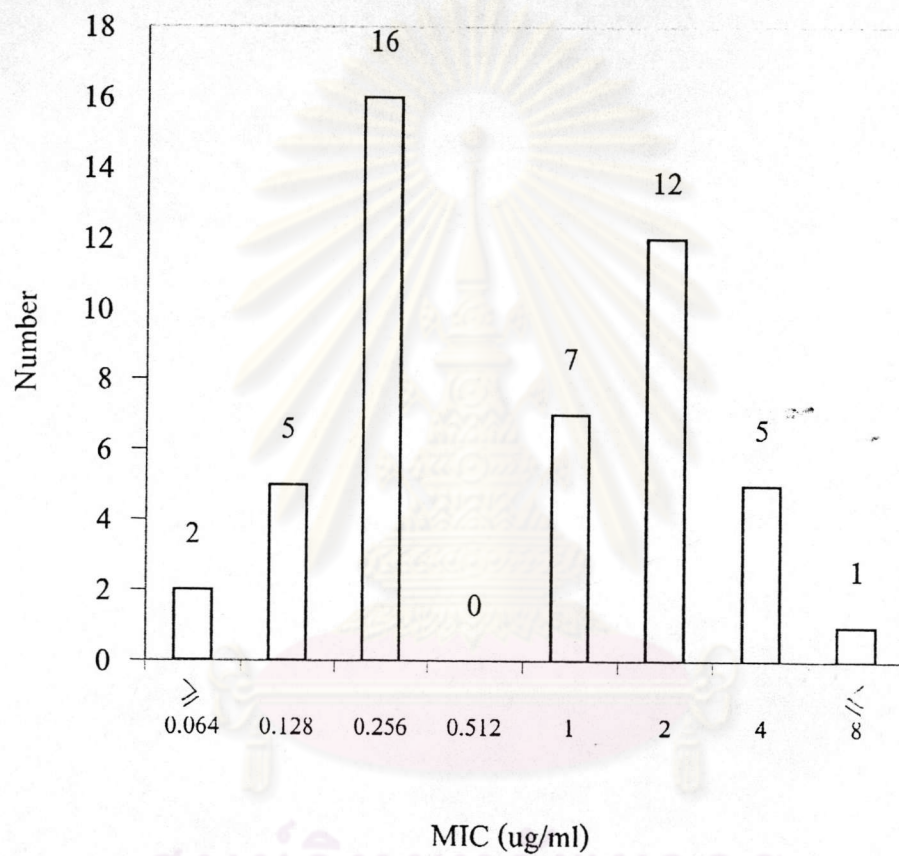


Fig. 5 MIC distribution of 48 environmental isolates

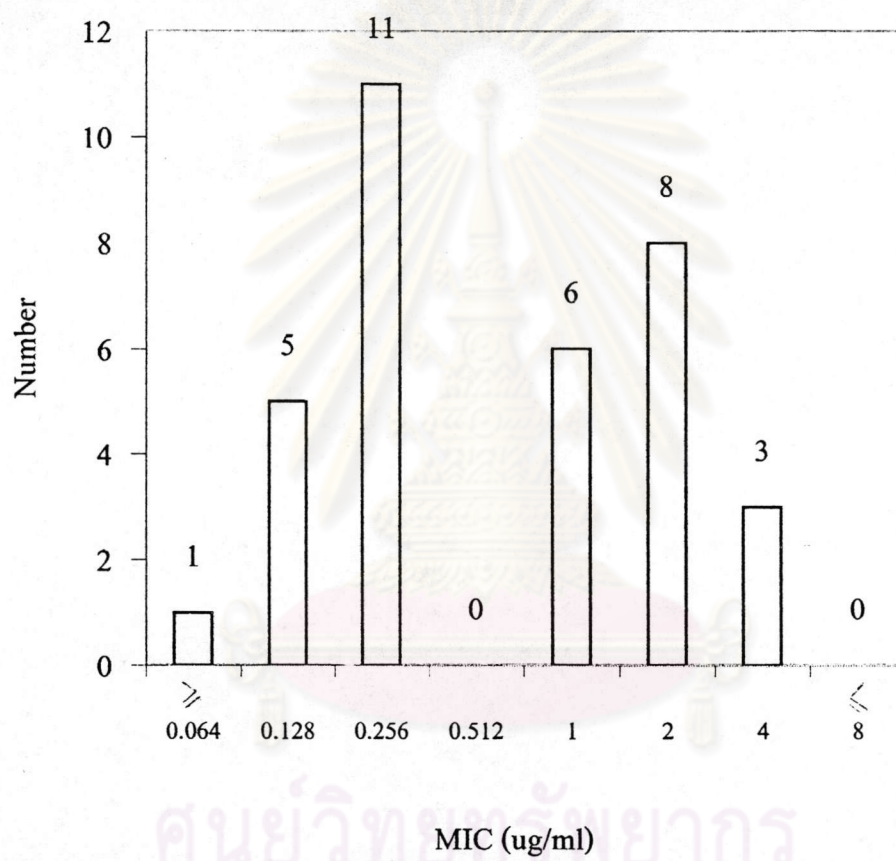


Fig. 6 MIC distribution of 34 sediment isolates

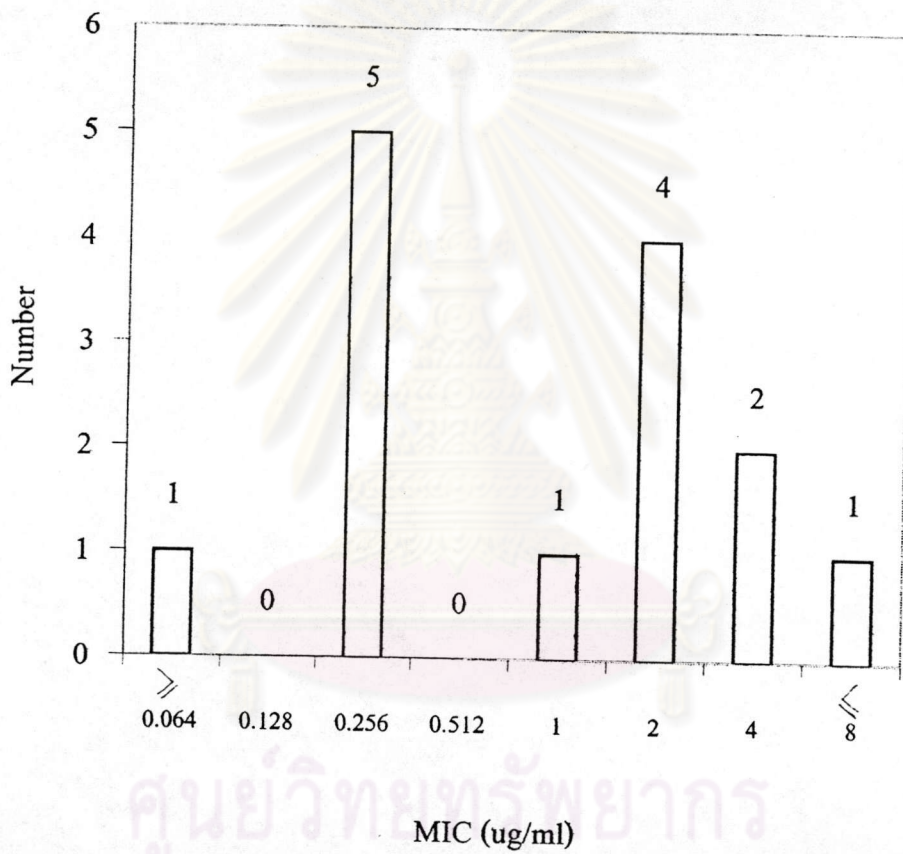


Fig. 7 MIC distribution of 14 shrimp isolates

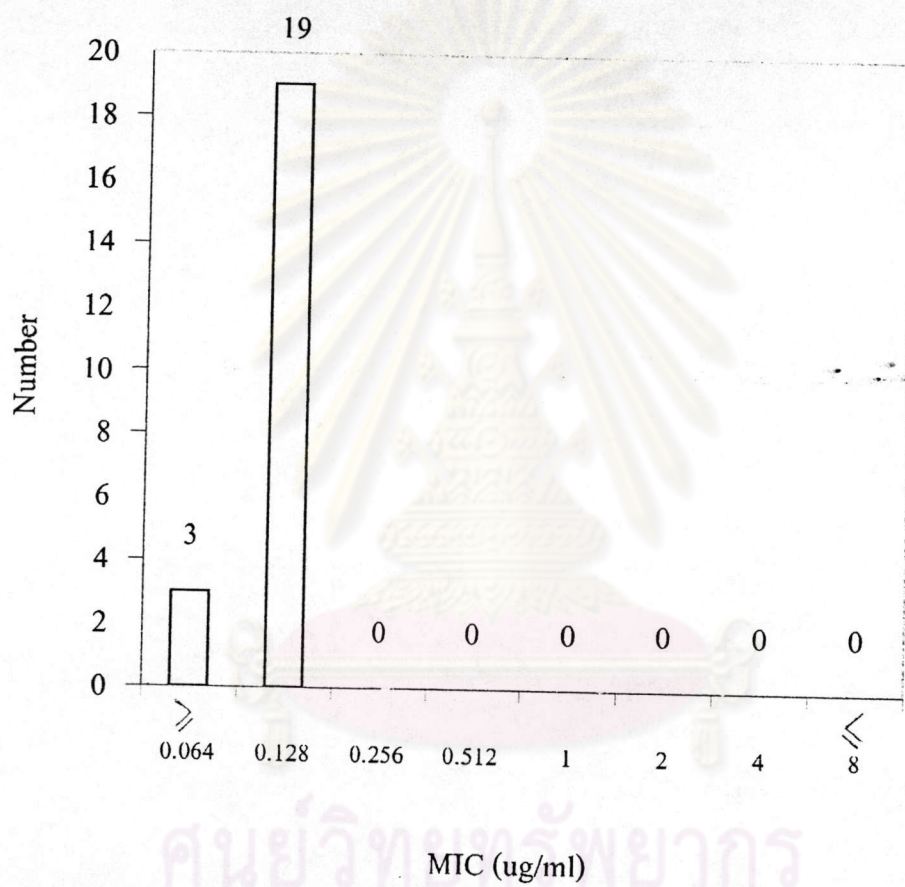


Fig. 8 MIC distribution of 22 clinical isolates

Table 7 : MIC₅₀ and MIC₉₀ of *Vibrio parahaemolyticus* isolates from different sources

Source of <i>V. parahaemolyticus</i> Isolates (n)	MIC range (µg/ml)	MIC ₉₀ (µg/ml)	MIC ₅₀ (µg/ml)
Environmental isolates (48)	0.064-8	3.3	1
: sediment isolates (34)	0.064-4	2	0.5
: shrimp isolates (14)	0.064-8	4	1.5
Clinical isolates (22)	0.064-0.128	0.128	0.128
All isolates (70)	0.064-8	2	0.256

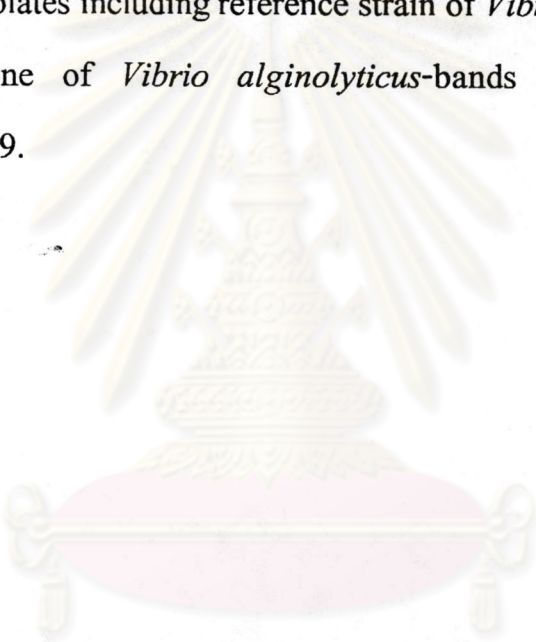
(n) : number of testing isolates

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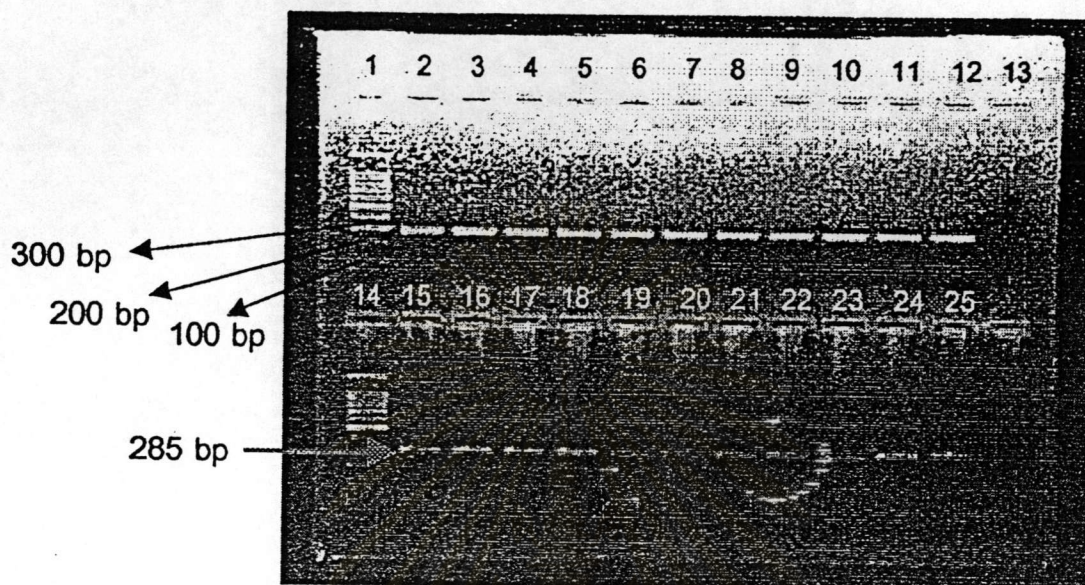
**PART III : CONFIRMATION OF *Vibrio parahaemolyticus*
BY PCR.**

PCR assays using *gyrB* primer described by Venkateswaran et al., 1997 were performed to support biochemical identifications of 21 *Vibrio parahaemolyticus* isolates which have MIC values ≥ 1 $\mu\text{g/ml}$.

Specific 285-bp amplicon for *Vibrio parahaemolyticus* were amplified in all twenty-one isolates including reference strain of *Vibrio parahemolyticus* ATCC 17802. None of *Vibrio alginolyticus*-bands were detected as demonstrated in Fig 9.



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**Fig 9. Agarose gel electrophoresis of specific
285-bp amplicon.**

Lane 1 & 14 : 100bp DNA ladder

Lane 2-11 : EP 10, 11, 16, 18, 22, 25, 26, 29,
SMV 1, SMV 2

Lane 12 : *Vibrio parahaemolyticus* ATCC 17802

Lane 13 : *Vibrio alginolyticus* ATCC 17749

Lane 15-25 : SMV 19, 37, 43, 44, 45, 46, 48, 49,
50, 51, 52

PART IV : DNA SEQUENCING.

The QRDRs of the *gyrA* and *parC* of ciprofloxacin resistant mutants were amplified by single step PCR with specific primers. PCR was expected to yield amplicons of 200 and 214 bp for *gyrA* QRDR and the *parC* QRDR, respectively (Fig 10). The nucleotide sequences were determined by automate sequencing method. Mutations detected within the amplified regions of the mutant strains are summarized in Table 9.

The mutations in *gyrA* were found in 19 isolates at codon 83 resulting in amino acid changes from Ser (AGT) to Lle (ATT), however, 200 bp amplicon of *gyrA* QRDR cannot be amplified in two isolates, SMV37 and SMV43. For *parC* gene, the point mutations were found at codon 85 in all isolates resulting in amino acid changes from Ser (TCT) to Phe (TTT), excepted SMV48 which has mutation only in *gyrA*. In additional, silent mutations were found at codon 79 in *gyrA* QRDR of SMV 50 and SMV 51 with no amino acid changes but base changes from CCA to CCG.

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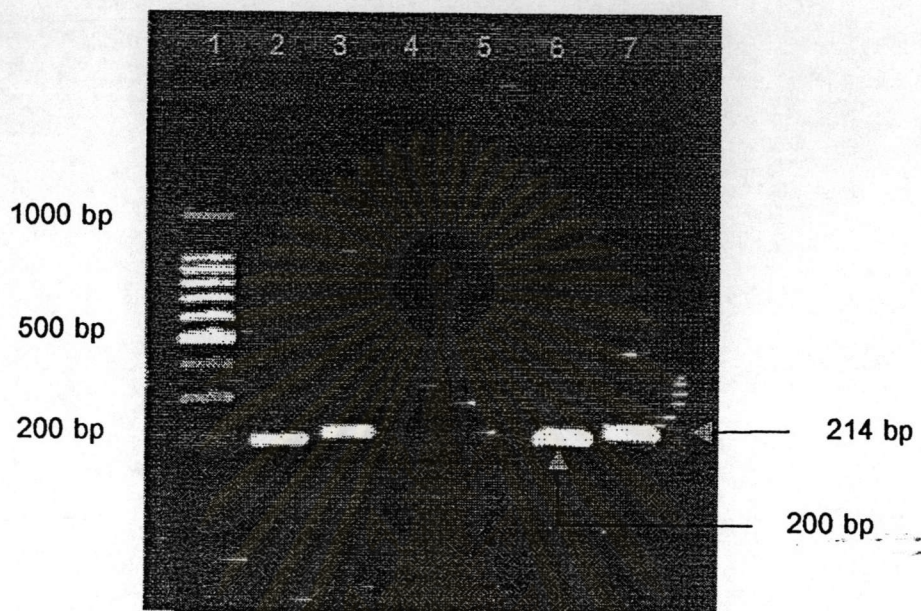


Fig. 10 Agarose electrophoresis showed amplicons of 200 and 214 bp for *gyrA* QRDR and the *parC* QRDR, respectively.

Lane 1 : 100bp DNA ladder.

Lane 2 : *gyrA* QRDR of *Vibrio parahaemolyticus* ATCC 17802.

Lane 3 : *parC* QRDR of *Vibrio parahaemolyticus* ATCC 17802.

Lane 6 : *gyrA* QRDR of PCR product of EP10.

Lane 7 : *parC* QRDR of PCR product of EP10.

**Table 8 : Mutations detected in *gyr A* and *par C* sequences of
MIC ≥ 1 $\mu\text{g/ml}$ of 21 *Vibrio parahaemolyticus* isolates.**

Strain No.	Source ^a	MIC (ug/ml)	Mutation in <i>gyrA</i>			Mutation in <i>par C</i>			Silent mutation		
			Position	Base Change	Aa change	Position	Base change	Aa change	Position	Base change	Aa change
EP 25	RP/CHB	8	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 37	SP/SRT	4	ND	ND	ND	85	TCT→TTT	Ser→Phe	-	-	-
EP26	RP/CHB	4	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
EP29	SP/SRT	4	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 45	SP/SRT	4	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 50	SP/SRT	4	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	79	CCA	CCG
EP 16	RP/CHB	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 1	SP/SRT	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 2	SP/SRT	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 44	SP/SRT	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 46	SP/SRT	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 49	SP/SRT	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 52	SP/SRT	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
EP 10	RP/CHB	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
EP 11	RP/CHB	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
EP 18	SP/SRT	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 19	SP/SRT	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 51	SP/SRT	2	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	79	CCA	CCG
EP 22	RP/CHB	1	83	AGT→ATT	Ser→Ile	85	TCT→TTT	Ser→Phe	-	-	-
SMV 43	SP/SRT	1	ND	ND	ND	85	TCT→TTT	Ser→Phe	-	-	-
SMV 48	SP/SRT	1	83	AGT→ATT	Ser→Ile	-	-	-	-	-	-

^a RP, shrimp ; SP, sediment ;

CHB, Chonburi province ; SRT, Suratthani province.

Position : Residue number counted from the N-terminal residue.

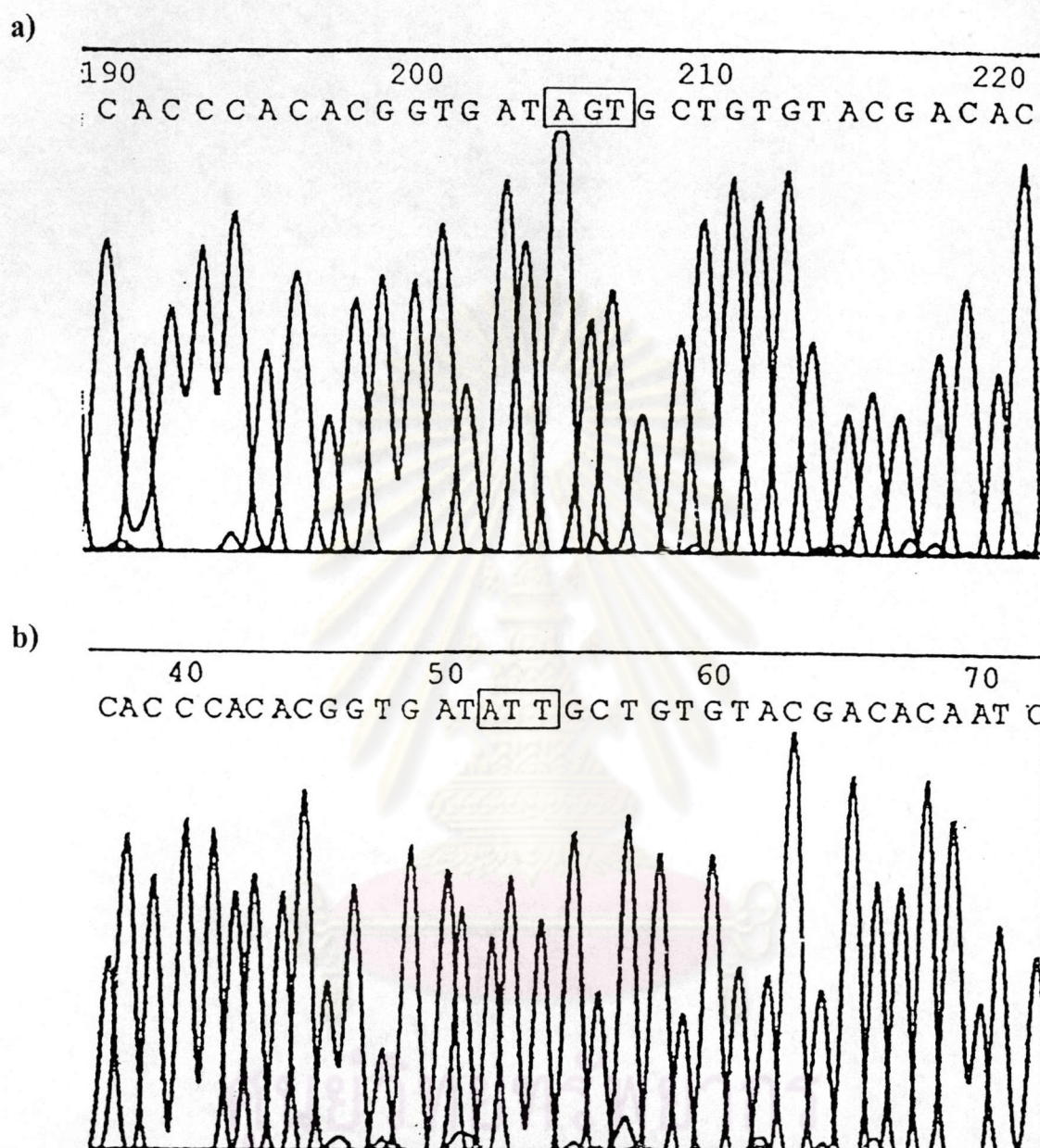


Fig 11. The chromatogram obtained from automate sequencing showed differentiation between nucleotide sequences within *gyrA* QRDR of *Vibrio parahaemolyticus* ATCC 17802 (a) and a ciprofloxacin resistant mutant (b) at the codon 83 (AGT→ATT).

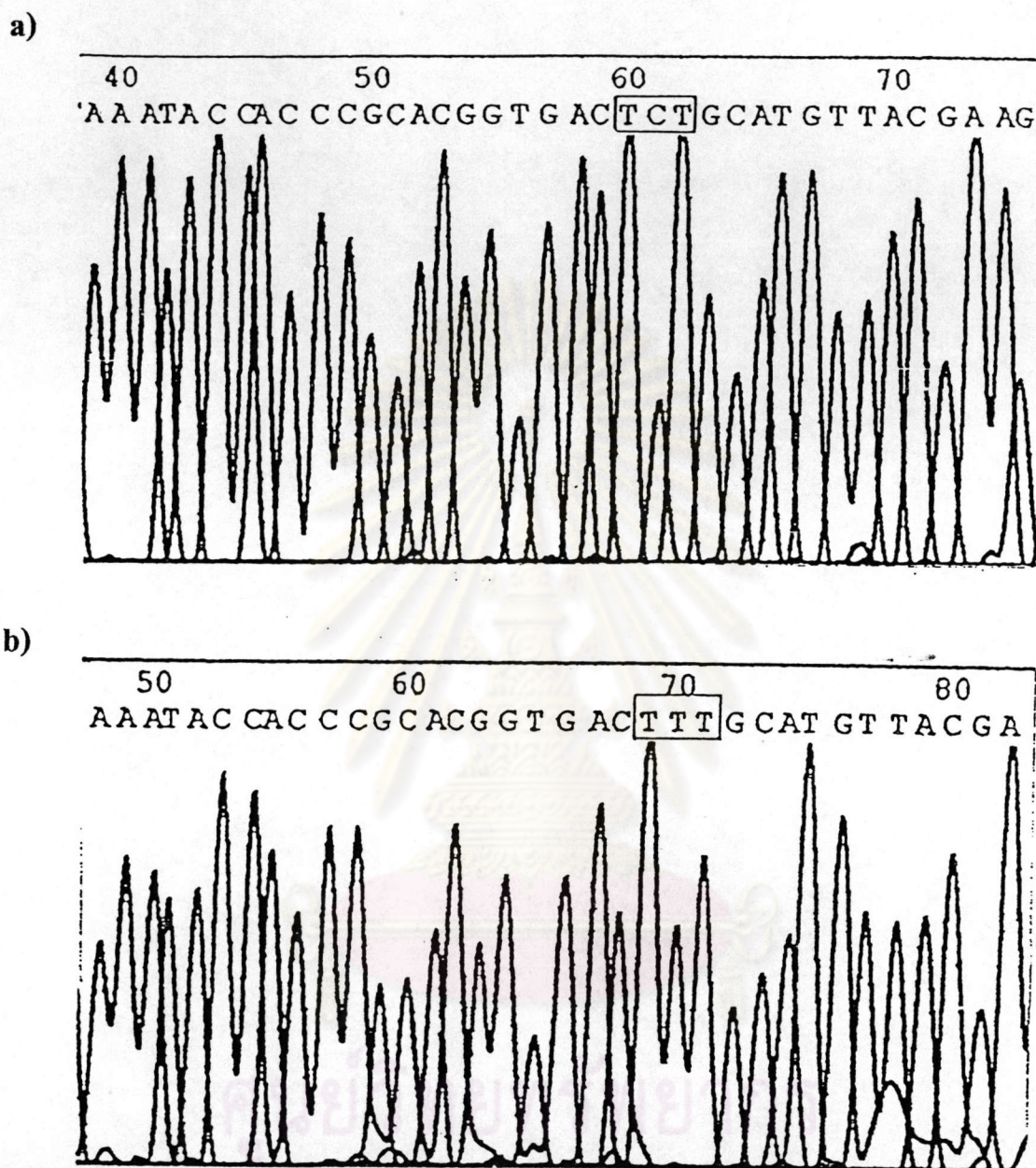


Fig 12. The chromatogram obtained from automate sequencing showed differentiation between nucleotide sequences within *parC* QRDR of *Vibrio parahaemolyticus* ATCC 17802 (a) and a ciprofloxacin resistant mutant (b) at the codon 85 (TCT→TTT).

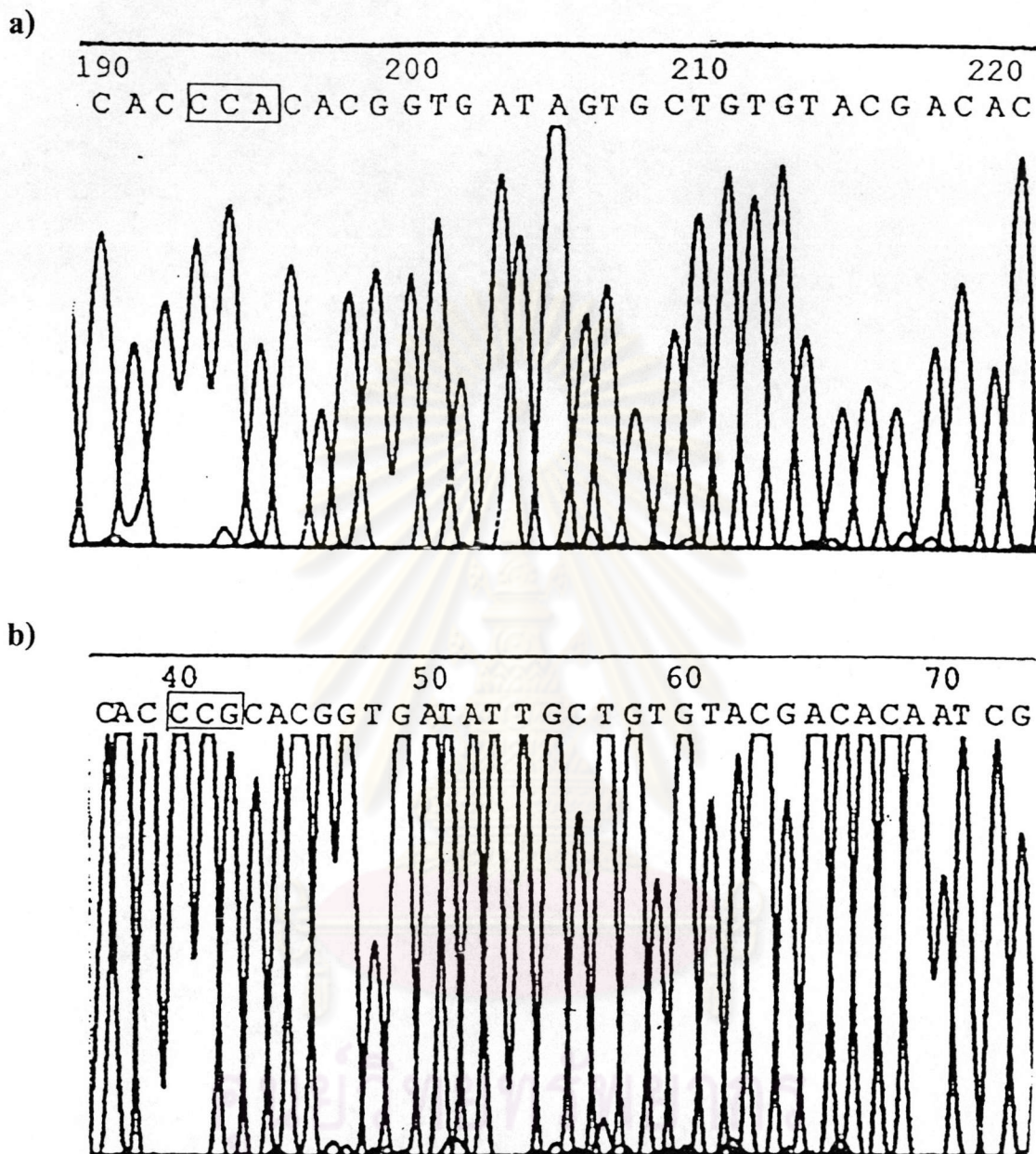


Fig 13. The chromatogram obtained from automate sequencing showed silent mutation within *gyrA* QRDR of a ciprofloxacin resistant mutant - SMV51 (b) at the codon 79 (CCG) comparing with *Vibrio parahaemolyticus* ATCC 17802 (CCA) (a).