CHAPTER 4

RESULTS

4.1 ISOLATION, SCREENING AND SELECTED OF MERCURY-RESISTANT BACTERIAL ISOLATES

From natural source mercury-resistant bacteria 272 strains were isolated from 61 samples collected from different sites. In all cases, the total number of mercury-resistant bacteria were mostly found in sediment samples from industry area than municipal waste and not found mercury-resistant bacteria from natural samples. Mercury resistance of all strains isolated were determined by an agar dilution method with a multiple inoculator system, the results were shown in **Table 4.1.** Sixty percent of bacterial isolates resisted mercury concentration 50 μg/ml, while only 1 percent of bacterial strains resisted mercury concentration 250 μg/ml And other metal resistant were also tested. The result was shown in **Table 4.2.** A total of 272 bacterial strains isolate were screened for mercury chloride-volatilizing ability by using the X-ray film method. The mercury-volatilizing bacterium, E. coli KP245 (pRR130), which support from Dr. Nakamura, K. was used as controls of the method. Of those strains, 259 strains were found to volatilize mercury chloride. Some result was shown in **Figure 4.13**. Two of them which were resistant to mercury in high level (250 μg/ml) and volatilized mercury chloride containing in the medium were chosen to be a selected bacterial isolated strains. Identification of selected bacterial isolated were perform by growing on selective media and by some biochemical test (**Table 4.3** and **4.4, Figure 4.1-4.6**) and named, HgR-11 and HgR-14 for the futher study, they were identified as ***Acinetobacter*** sp..

4.2 RESISTANCE TO OTHER METALS BY THE SELECTED BACTERIAL STRAINS

The selected strains, HgR-11 and HgR-14 were found to be sensitive (less than 200μg/ml) to a number of other heavy metals, i.e., Cd, Cr, Cu, Ni and Ag, but resistant to Zn and Mn, Detailed result is summarized in **Table 4.2.**

4.3 EFFECT OF SOME ENVIRONMENTAL FACTORS ON GROWTH OF THE SELECTED BACTERIAL STRAINS

The optimum pH of those selected bacterial isolates were found to be 8 and optimum temperature for both strains were shown to be 35^0C, see **Table 4.6 Figure 4.8** and **4.9**. The effect of mercury concentration were present in **Table 4.7** and **4.8**

4.4 EFFECT OF SOME FACTORS ON VOLATILIZATION CAPACITY OF THE SELECTED BACTERIAL STRAINS

The percentage of mercury loss from the medium containing 50 μg/ml were reported as efficiency of mercury volatilization by the selected bacterial isolates. The highest efficiency were found at pH 7-9 and temperature at 25-40^0C in both selected strains. The result were shown in **Table 4.9**, **Figure 4.10** and **4.11.**

4.5 REDUCTION OF MERCURY AT DIFFERENT TIME

The reduction of mercury was determined by analyzed remaining mercury at different time. It was found that mercury concentration in the medium were reduced quite rapidly in the first 2 hours. The result were presented in **Table 4.10** and **Figure 4.12**

4.6 RECOVERY OF METALS

The loss of mercury from the medium as a vapor by selected bacterial strains were confirmed by trapped in acid potassium permanganate solution. The result indicated that in first trap solution can be recover mercury by converted them into soluble form at efficiency 98%.The result was presented in **Table 4.11.**

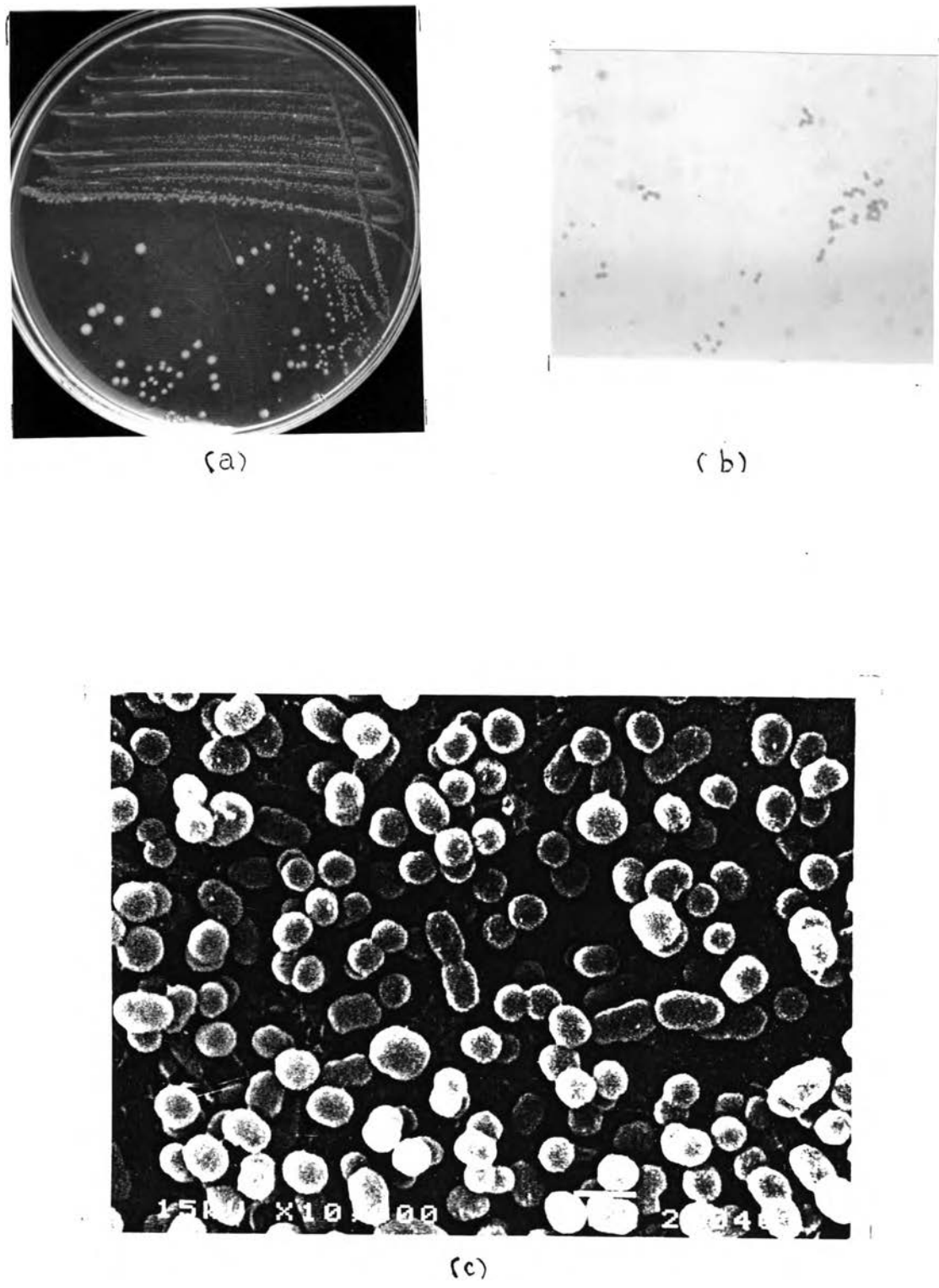
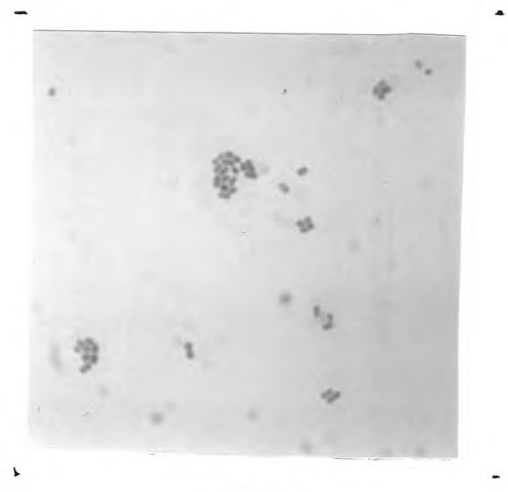
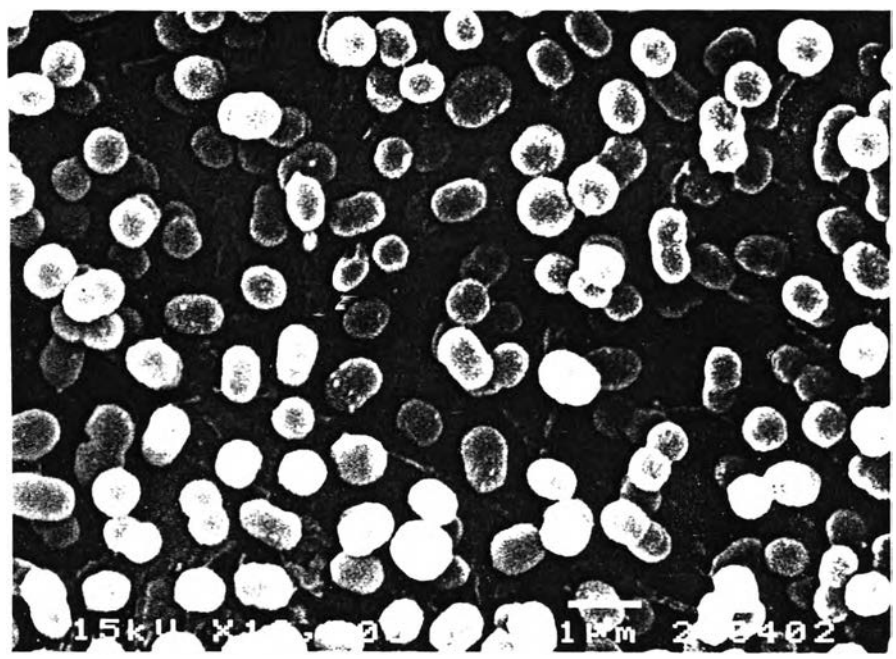


Figure 4.1 Colonial characteristic on 1/10 TSA (a) gram-staining (b) and cells morphology (x10,000) of HgR-11 strain (c).

(a)



(b)



(c)

Figure 4.2 Colonial characteristic on 1/10 TSA (a) gram-staining (b) and cells morphology (x10,000) of HgR-14 strain (c).

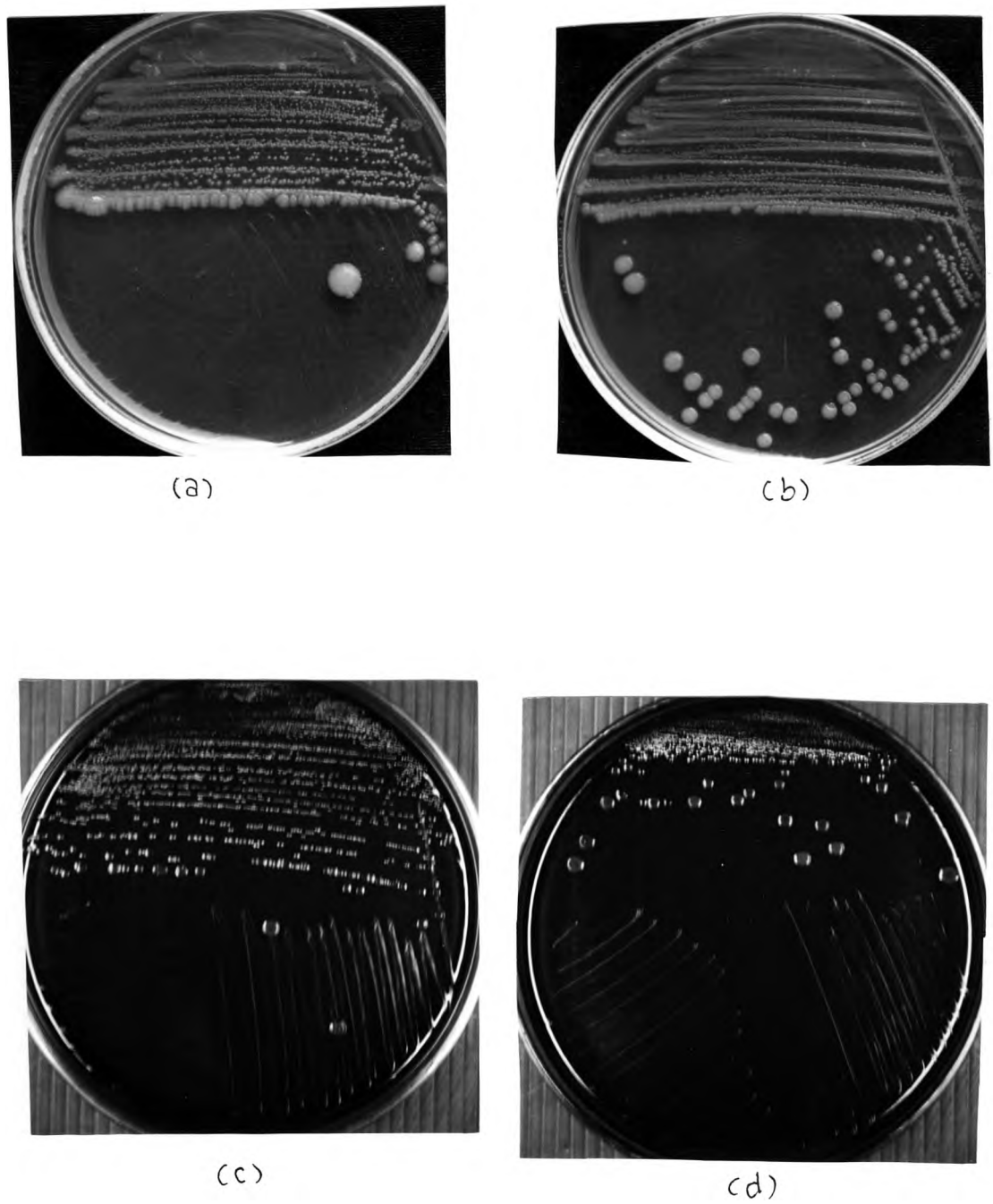
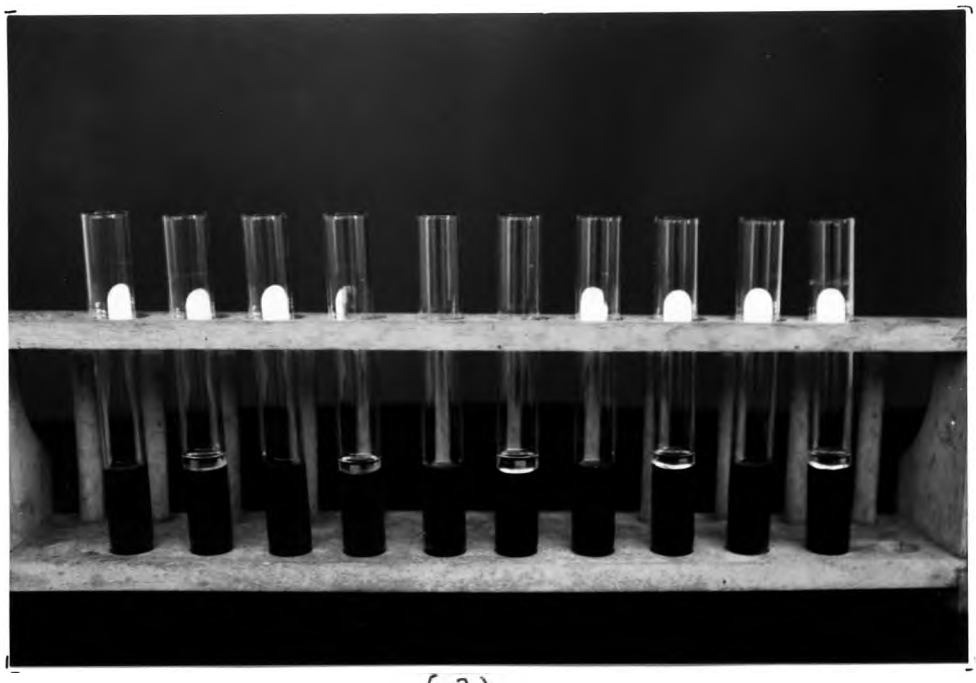
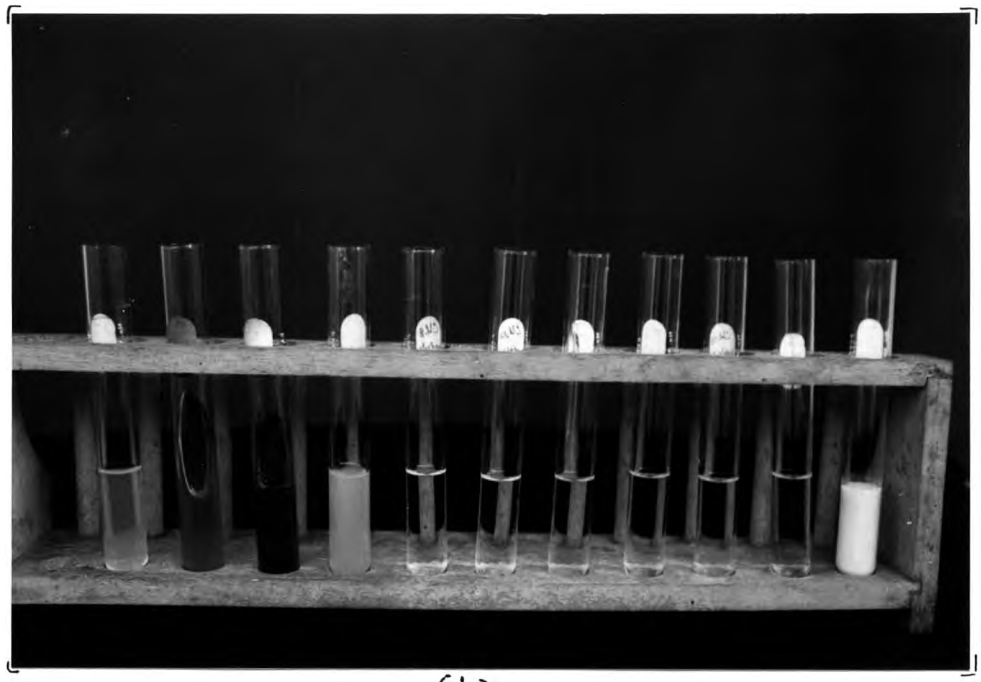


Figure 4.3 Colonial characteristic on McConkey agar of HgR-11 strain (a) HgR-14 (b) and on E.M.B.agar of HgR-11 strain (c) HgR-14 strain (d)



(a)



(b)

Figure 4.4 Control of O-F test (a) from the left is glucose, dextose, maltose and sucrose, respectively and control of biochemical test (b) from the left is Motility, TSI, Citrate, Urease, KCN, Nitrate, Indole, MR-VP and litmus milk test, respectively.

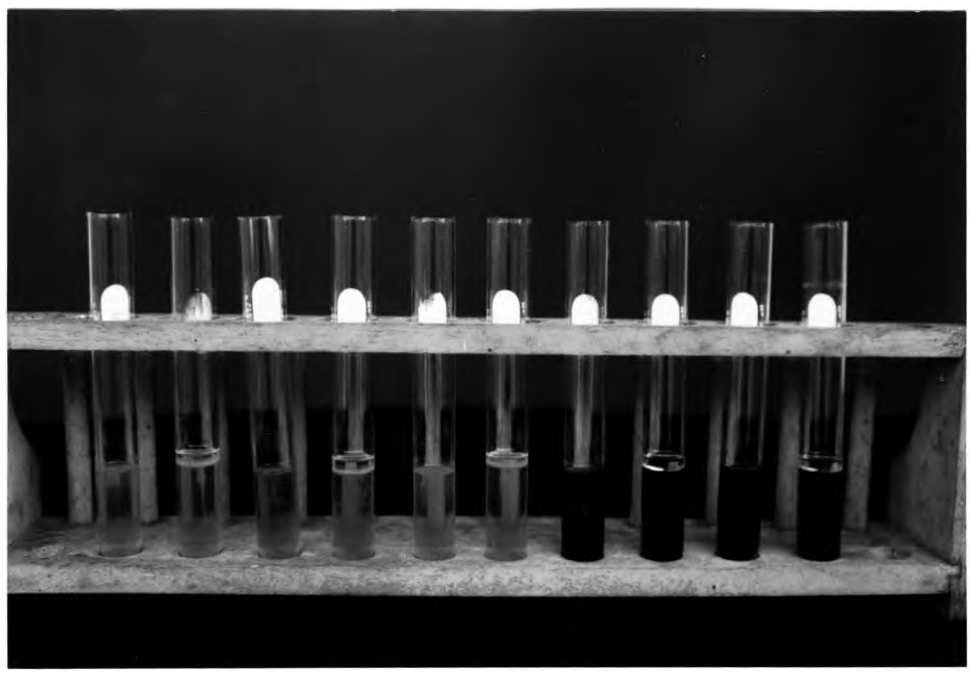
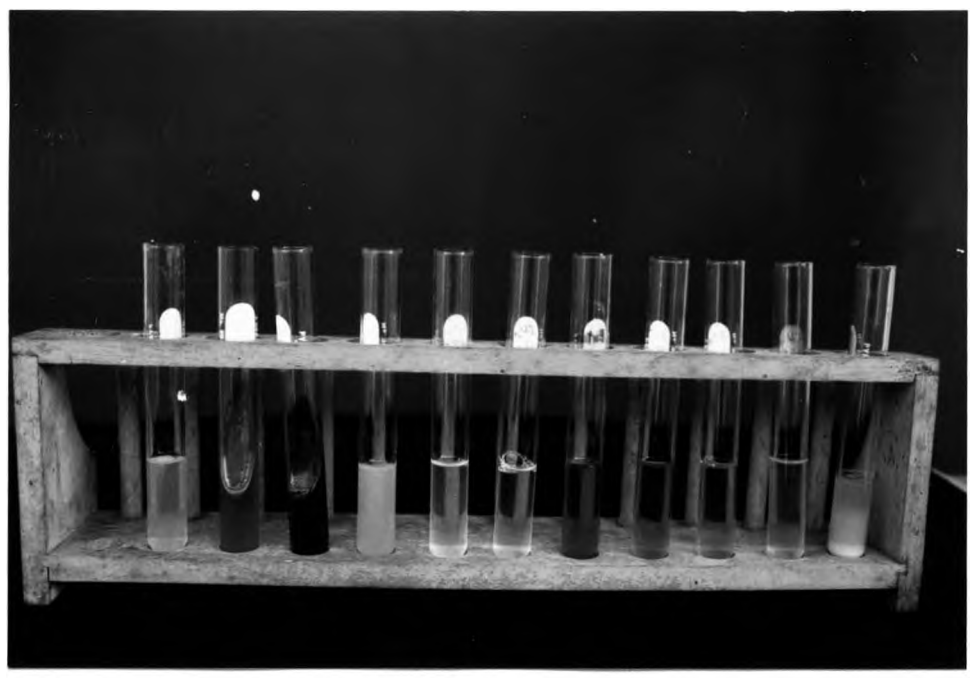


Figure 4.5 The result of OF test by HgR-11 strain and HgR-14 strain

(a)



(b)

Figure 4.6 The result of biochemical test by HgR-11 strain (a) and HgR-14 strain (b).

Table 4.1 Mercury resistance of 272 mercury-resistant bacteria isolates from different samples.

Hg Concentration (μg/ml)	Number of strains	%
50	165	60.7
100	88	32.4
150	11	4.0
200	5	1.8
250	3	1.1
Total	272	100

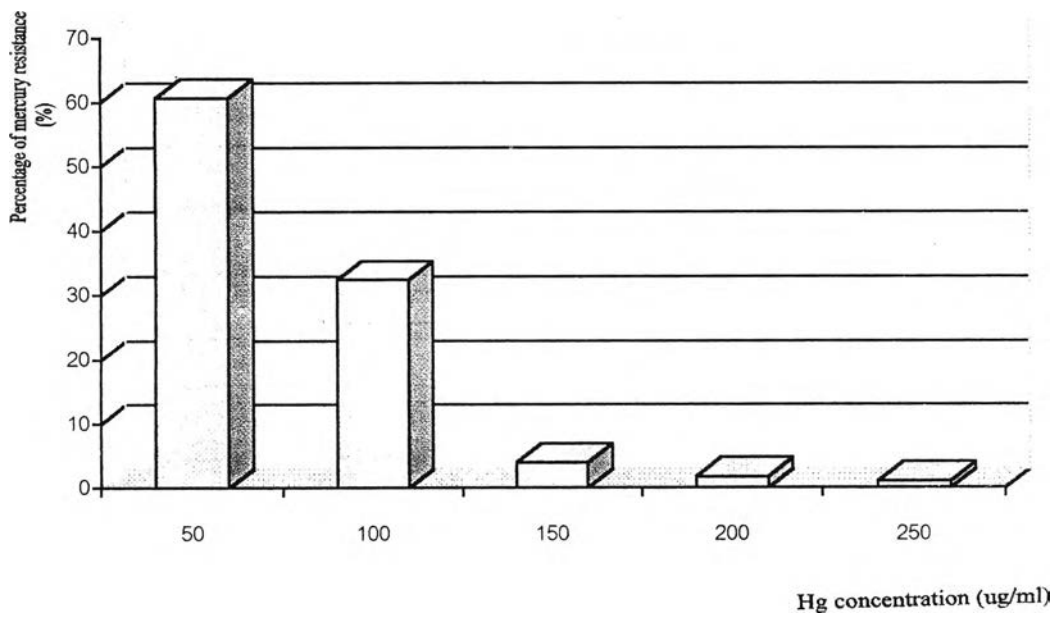


Figure 4.7 Percentage of mercury resistance of 272 mercury- resistant Bacterial isolates from diferanct samples.

Table 4.2 Other heavy metal resistance of bacterial isolates and reference Bacteria.

Strains	Concentration (μg/ml) of solutions of metal compounds							
	Hg	Cd	Cr	Cu	Zn	Ni	Mn	Ag
Hg-R11	250	<100	200	200	400	100	800	<100
Hg-R14	250	<100	100	200	200	100	800	<100
E. coli	<100	<100	200	200	100	<100	800	<100
S. macescens	<100	<100	100	<100	100	<100	800	<100

Table 4.3 Some characteristics and identification of the mercury-resistant bacterial isolates.

Bacterial Isolates	Sources (Sampling site)	Character of bacterial isolates		Identify as
		Colony	Morphology	
HgR-11	S42	~2-3 mm, circular raised, intrie	Coccobacilli	***Acinetobacter*** sp.
HgR-14	S44	~2-3 mm, circular raided, entire	Coccobacilli	***Acinetobacter*** sp.

Table 4.4 Microbial characteristics of the isolate

Characteristic	Results	
	Hg-R11	Hg-R14
Cell morphology	Coccobacilli	Coccobacilli
Gram-stain	-	-
Motility	-	-
Catalase	-	+
Oxidase	-	-
OF-glucose	F	F
-dextose	F	F
-lactose	F	F
-maltose	-	-
-sucrose	-	-
KCN	+	+
Urease	-	-
Citrate	+	+
Nitrate reduction	-	-
Indole	-	-
MR	-	-
VP	-	-
Litmus milk	Peptonization	Peptonization
Gelatinase	+	+
TSI	K/N, H_2S-	K/N, H_2S-

-: negative; +: positive; F: Fermentation; K: alkaline (media chance to redish) ; N: changeless color of media; H_2S: hydrogen sulfide.

Table 4.5 Stability of mercury resistance after 20 times of repeated subculturing in two strains of mercury-resistant bacterial isolates.

Bacterial Isolates	Resistance to mercury (μg/ml)	Stability of resistance
Hg-R11	250	
-induced with 5 μg/ml of Hg		> 20
-without induced		< 17
Hg-R14	250	
-induced with 5 μg/ml of Hg		> 20
-without induced		< 14

Table 4.6 Effects of pH and temperature on growth of the bacterial Isolates

Bacterial isolates	Initial no. Of organisms (cells x 10^6/ml)	Number of organisms (cells x 10^8/ml)									
		pH						Temperature (°C)			
		5	6	7	8	9	10	25	30	35	40
Hg-R11	0.70	-	0.01	3.70	71.00	29.90	0.19	15.40	15.90	71.60	5.50
Hg-R14	1.75	-	0.05	13.40	44.00	11.80	0.84	26.10	35.30	39.00	10.80

-: not found at dilution 10^{-1}

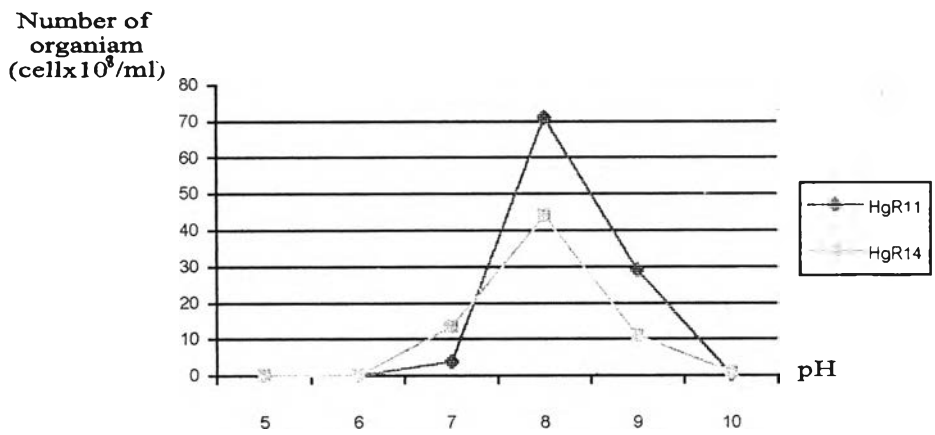


Figure 4.8 Effect of pH on growth of the bacterial isolates

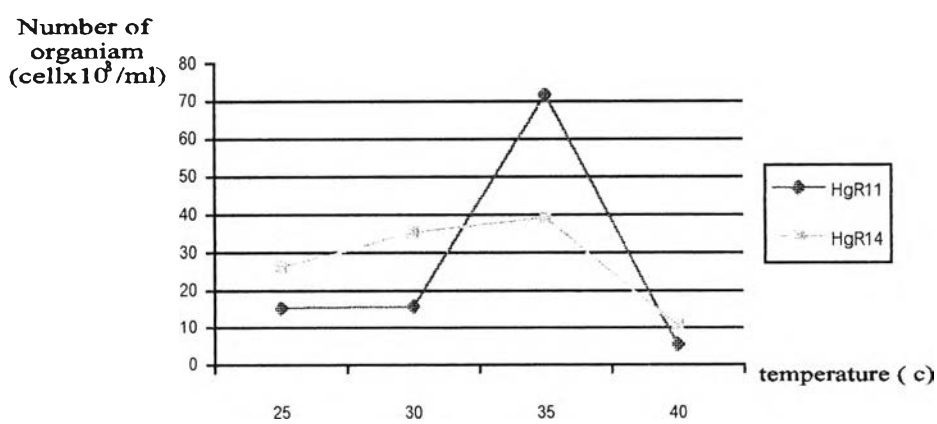


Figure 4.9 Effect of temperature on growth of the bacterial isolates

Table 4.7 Effect of mercury concentration on growth of the bacterial isolates

Time (hour)	Number of organisms (cells x 10^7/ml)							
	Hg-R11				Hg-R14			
	0	4	8	50	0	4	8	50*
	0.09	0.02	0.01	0.01	0.05	0.04	0.01	0.01
2	0.35	0.02	0.03	0.03	0.45	0.05	0.05	0.04
4	5.33	0.57	0.61	0.17	4.27	0.71	0.70	0.15
6	9.04	4.31	3.03	0.24	8.93	3.22	2.17	0.29
8	11.10	7.01	7.11	0.66	9.31	8.81	5.47	0.62
12	74.40	76.30	62.20	8.80	24.30	22.90	12.10	7.30
24	146.00	106.00	139.00	55.0	69.70	78.30	78.70	35.30

*: concentration of mercury (μg/ml)

Table 4.8 Effect of high concentration of mercury on growth of the bacterial isolates.

Time (hour)	Number of organisms (cells/ml)					
	Hg-R11			Hg-R14		
	25	100	150	50	100	150*
0	$3.62x10^6$	$<10^3$	$<10^2$	$2.04x10^6$	$1.45x10^4$	$<10^2$
1	$6.05x10^6$	$1.50x10^3$	$<10^2$	$7.00x10^6$	$1.55x10^4$	$<10^2$
2	$3.30x10^7$	$6.50x10^3$	$<10^2$	$8.30x10^6$	$2.15x10^4$	$<10^2$
3	$1.41x10^7$	$5.90x10^4$	$<10^2$	$1.37x10^7$	$1.69x10^5$	$<10^2$
4	$1.43x10^7$	$3.09x10^6$	$<10^2$	$1.43x10^7$	$7.86x10^6$	$<10^2$
6	$4.79x10^7$	$1.12x10^7$	$4.40x10^3$	$2.16x10^7$	$1.37x10^7$	$7.11x10^3$
24	$8.6x10^8$	$2.65x10^8$	$9.97x10^7$	$6.07x10^8$	$7.10x10^8$	$1.15x10^8$
48	$6.77x10^8$	$3.67x10^8$	$7.50x10^7$	$7.63x10^8$	$5.47x10^8$	$1.56x10^8$

*: concentration of mercury (μg/ml)

Table 4.9 Effect of pH and temperature on volatilization of bacterial isolates

Selected strain	Initial Conc. of Hg (µg/ml)	Concentration of mercury (µg/ml)							
		pH				Temperature (°C)			
		6	7	8	9	25	30	35	40
HgR11	50	12± 0.18[a] (76.00)[b]	0.44± 0.06 (99.10)	0.70± 0.02 (98.58)	0.28± 0.02 (99.44)	0.73± 0.04 (98.53)	0.63± 0.02 (98.72)	0.58± 0.03 (98.82	2.38± 0.05 (95.24)
HgR14	50	13.213 ± 0.16 (73.57)	0.57± 0.03 (98.86)	0.17± 0.02 (99.66)	0.53± 0.07 (98.94)	0.33± 0.02 (99.33)	0.33± 0.04 (99.32)	0.17± 0.02 (99.64	0.31± 0.03 (99.37)

a: Remaining of mercury concentration ± SD. in 3 replicates after 24 hr incubate.
b: Percent of mercury loss from the medium.

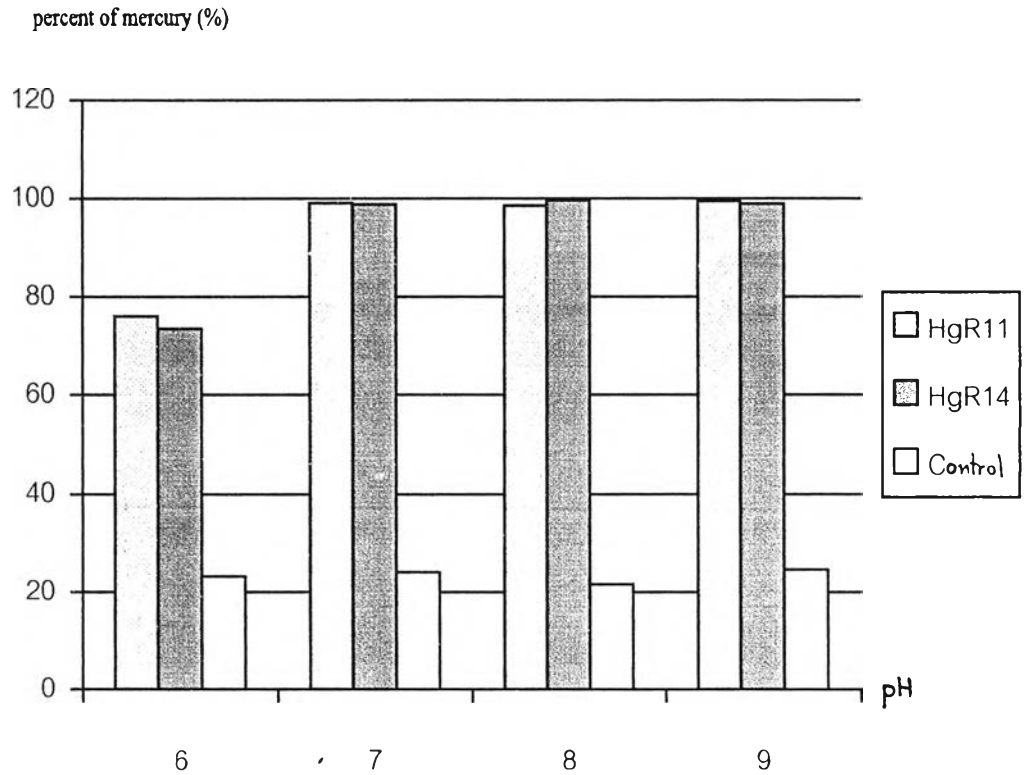


Figure 4.10 Percentage of mercury removal at difference pH

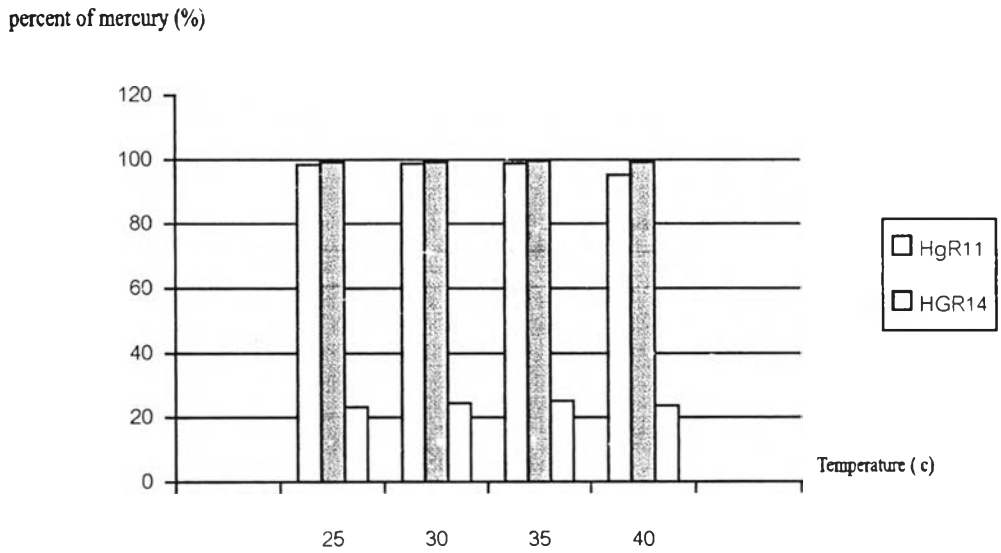


Figure 4.11 percentage of mercury removal at different temperature

Table 4.10 Remaining concentration of mercury at different time

Time (hour)		0	1	2	3	4	6	8	10	24
Conc Hg (µg/ ml)	HgR-11	50	10.4	8.2	6.3	4.1	4.6	4.4	3.7	1.4
	HgR-14	50	15.9	9.3	9.5	6.1	5.7	4.3	2.8	0.7
	C[b]	50	46.8	47.2	45.1	43.9	42.7	41.8	40.1	38.2

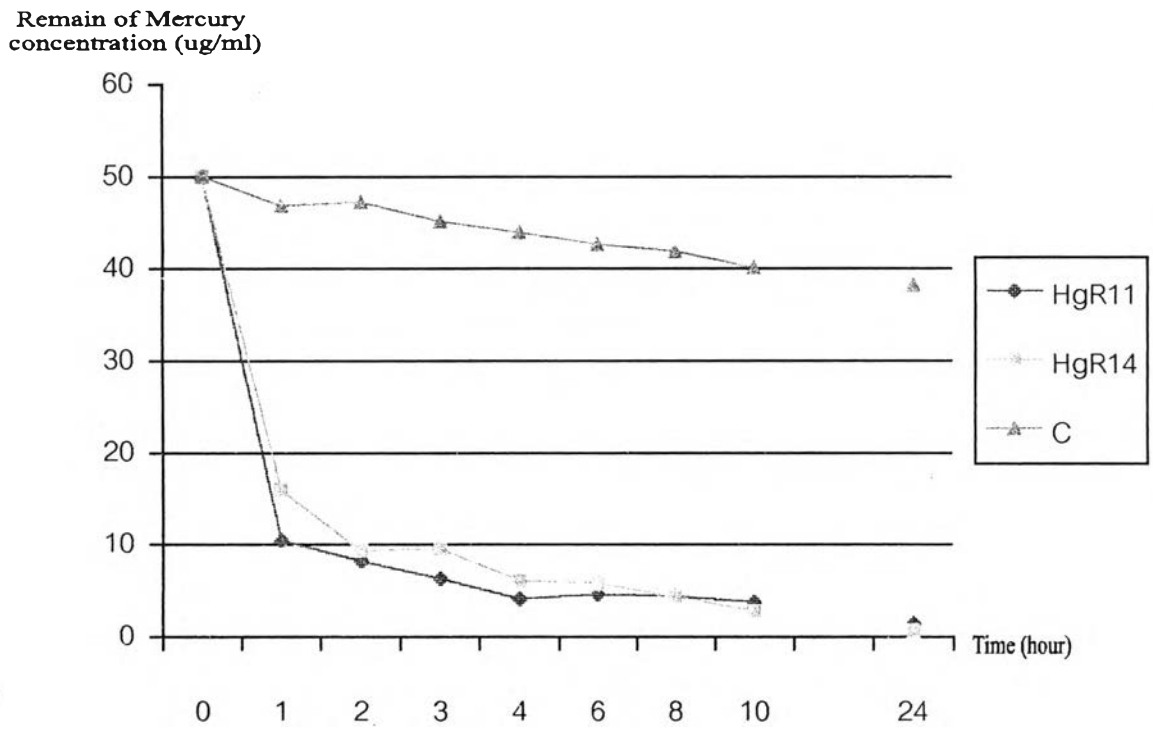
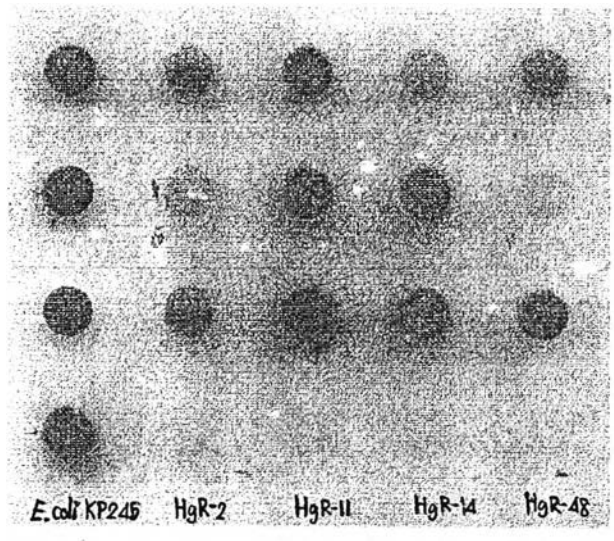


Figure 4.12 The reduction of mercury at difference time



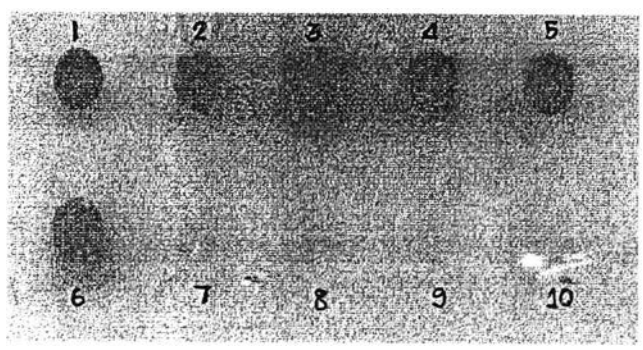


Figure 4.13 Black spot of mercury vapor detect by X-ray film method ***E.coli*** KP245 pRR130 (1and 6) HgR-2 (2) HgR-11 (3) HgR-14 (4) HgR-48 (5) buffer solution (7and 10) ***E.coli*** sensitive strain (8) and ***S. marcescens*** sensitive strain (9).

Table 4.11 Mercury recovery efficiency of successive potassium permanganate in sulfuric acid in 5 days batch culture

Selected Strain	Initial mercury concentration (mg/L)	Concentration of Hg in trapped solution (μg/ml)		Total Hg^o recovery efficiency (%)
		First trap column	Second trap column	
HgR-11	150	143.07	0.24	95.38
HgR-14	150	147	0.09	98.00