CHAPTER V

RESULTS

1. Determination of the clarithromycin susceptibility of MAC tested by NCCLS recomended method, broth microdilution

Of the 100 MAC isolates, 97 isolates (97%) were susceptible to clarithromycin (MIC, 0.5 – 8 μg/ml), and the remaining 3 isolates (3%) were resistance to clarithromycin. All resistant MAC isolates had a MIC > 256 μg/ml (Table 4).

Table 4. Number of clarithromycin susceptible and resistance isolates tested by broth microdilution

No. of MAC isolates	No. of MAC susceptible to clarithromycin (%)	No. of MAC resistance to clarithromycin (%)
100	97 (97%)	3 (3%)

2. Comparison of BACTEC MGIT 960 and E test with NCCLS recommended method, broth microdilution, for detection of clarithromycin resistance in MAC isolates

The qualitative susceptibility results from three susceptibility tests (i.e., resistance and susceptibility) based on the broth microdilution interpretive breakpoint suggested by NCCLS are given in Table 5. Excellent agreement was demonstrated for all method (100%).

For MICs determined by E test and broth microdilution method, 7 isolates had MICs of 12-16 μg/ml and 8 μg/ml by E test and broth microdilution method, respectively (Table 6). According to Lebrun et al. (27), MIC ≤8 μg/ml was considered as the breakpoint for determining susceptibility of MAC to clarithromycin and ≥64 μg/ml for determining resistance. If E-test-determined MIC was evaluated according to Lebrun et al. (27), 7 isolates would be classified as intermediate to clarithromycin by the E test whereas the broth microdilution indicated them to be susceptible (Table 7).

Table 5. Comparison of three susceptibility testing method for detection of clarithromycin resistance in 100 MAC isolates according to the broth microdilution interpretive breakpoint suggested by NCCLS

Category (microgram/milliliter)	No. of isolates tested by:			%Agreement
	Broth microdilution	BACTEC MGIT 960	E test	
Susceptible (≤16)	97	97	97	100
Intermediate (32)	0	0	0	100
Resistance (≥64)	3	3	3	100

Table 6. MIC determined by broth microdilution and E test of 7 MAC isolates

Strain	MIC (microgram/milliliter)	
	Broth microdilution	E test
MAC016	8	16
MAC023	8	16
MAC024	8	12
MAC026	8	12
MAC032	8	16
MAC044	8	16
MAC052	8	12

Table 7. Comparison of the broth microdilution and E test for detection of clarithromycin resistance in 100 MAC isolates according in each interpretive breakpoints methods

Category	No. of isolates tested by:		%Agreement
	Broth microdilution [a]	E test [b]	
Susceptible	97	90	93
Intermediate	0	7	0
Resistance	3	3	100

[a] According to the broth microdilution interpretive breakpoint suggested by NCCLS.

[b] According to the interpretive breakpoints used by Lebrun et al.

3. Comparison of broth microdilution-determined and E-test-determined MICs.

The agreement between MICs determined by the E test and the broth microdilution, within ±1 $\log_2$ dilution was 95% (Table 8). Within ±2 $\log_2$ dilution, agreement with the broth microdilution-determined MICs increased to 100%. The MICs obtained by both methods are shown in Table 9. The data are reported as MIC ranges and MICs required to inhibit 50% and 90% of the isolates (MIC_{50} and MIC_{90} respectively). An example of clarithromycin MIC determined by the E test technique for a clarithromycin susceptible MAC isolate is given in the Figure 9.

Table 8. Agreement between E test and broth microdilution MICs for clarithromycin tested against 100 MAC isolates

No. of Strain	No. of E-test MICs that are same as or different from those of Broth microdilution MICs for the following dilution: (%agreement)					%Agreement (within +/- 1 log2 dilution)
	-2	-1	same	1	2	
100	0	9 (9)	23 (23)	63 (63)	5 (5)	95%

Table 9. MICs of clarithromycin for 100 MAC isolates by the broth microdilution method and E test

MIC (microgram/milliliter)	No. of isolates tested by:	
	Broth microdilution	E test
<0.25	0	0
0.25	ND	0
0.5	3	2
1	7	4
1.5	ND	1
2	21	7
3	ND	4
4	48	24
6	ND	14
8	18	34
12	ND	3
16	0	4
32	0	0
64	0	0
128	0	0
256	0	0
>256	3	3
MIC ranges (μg/ml)	0.5 - >256 μg/ml	0.5 - >256 μg/ml
MIC_{50}	4 μg/ml	6 μg/ml
MIC_{90}	8 μg/ml	8 μg/ml

ND, not determined.

Figure 9. Clarithromycin MIC determination of clarithromycin susceptible MAC isolate with the E test method.

4. PCR amplification of 23S rRNA gene

Primers 23SFII and 23SRI, designed to amplify domain V of 23S rRNA gene fragment from MAC. The 813-bp DNA fragment was clearly visualized on an agarose gel. As shown in Figure 10.

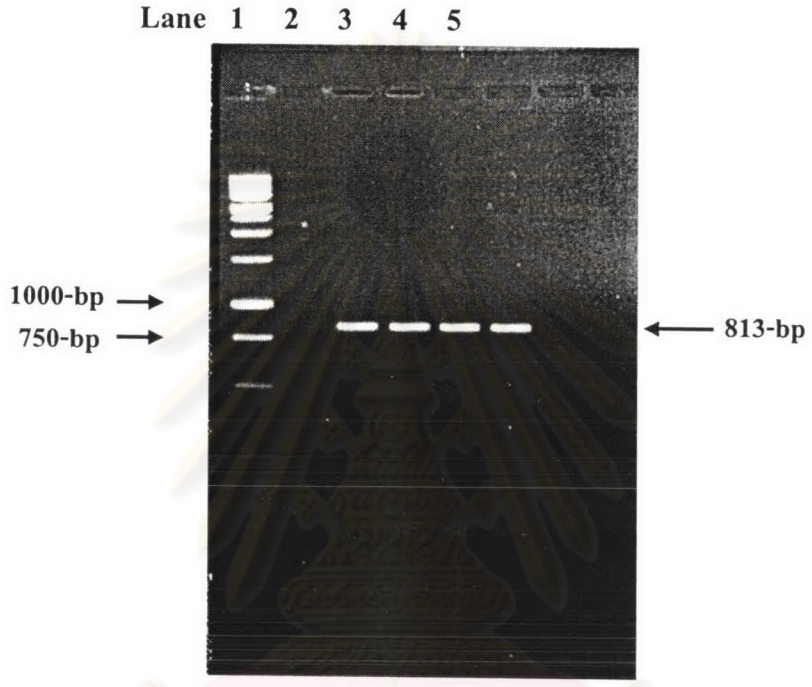

Figure 10. Amplification of MAC DNA. Lanes : 1, 3000-bp DNA ladder; 2, negative control; 3, MAC DNA-positive control; 4-6, MAC DNA from clinical isolates.

5. Domain V region of 23S rRNA sequencing result

In sequencing analysis, reference strain *M. avium* ATCC 25291 and 3 susceptible clinical isolates had no mutation in the domain V region of the 23S rRNA gene (Table 10). Single point mutation in the domain V of the 23S rRNA gene was observed in 3 resistance clinical isolates with high MIC value (>256 μg/ml). Two of them showed the transition of A to G (A→G) and 1 showed the transversion of A to C (A→C) at position 2058. In figure 11 the wild type strain, figure 12 was indicated by an arrow the position of A2058G mutation, figure 13 was indicated by an arrow the position of A2058C mutation. Moreover, no mutation in the domain V region of the 23S rRNA gene were observed in 7 clinical isolates that had E-test MIC 12-16 μg/ml (Table 10).

Table 10. Results of sequencing the domain V of 23S rRNA gene, and phenotypic determination

Strain	MIC (microgram/milliliter)			Category	23S rRNA gene mutation
	Broth microdilution	BACTEC MGIT 960	E-test		
M. avium ATCC 25291	1-2	2	1-2	susceptible	wilde type (A2058,2059)
MAC042	1	≤16	2	susceptible	wilde type (A2058,2059)
MAC076	2	≤16	4	susceptible	wilde type (A2058,2059)
MAC014	4	≤16	6	susceptible	wilde type (A2058,2059)
MAC003	>256	>64	>256	resistance	A2058G
MAC029	>256	>64	>256	resistance	A2058G
MAC047	>256	>64	>256	resistance	A2058C
MAC016	8	≤16	16	susceptible	wilde type (A2058,2059)
MAC023	8	≤16	16	susceptible	wilde type (A2058,2059)
MAC024	8	≤16	12	susceptible	wilde type (A2058,2059)
MAC026	8	≤16	12	susceptible	wilde type (A2058,2059)
MAC032	8	≤16	16	susceptible	wilde type (A2058,2059)
MAC044	8	≤16	16	susceptible	wilde type (A2058,2059)
MAC052	8	≤16	12	susceptible	wilde type (A2058,2059)

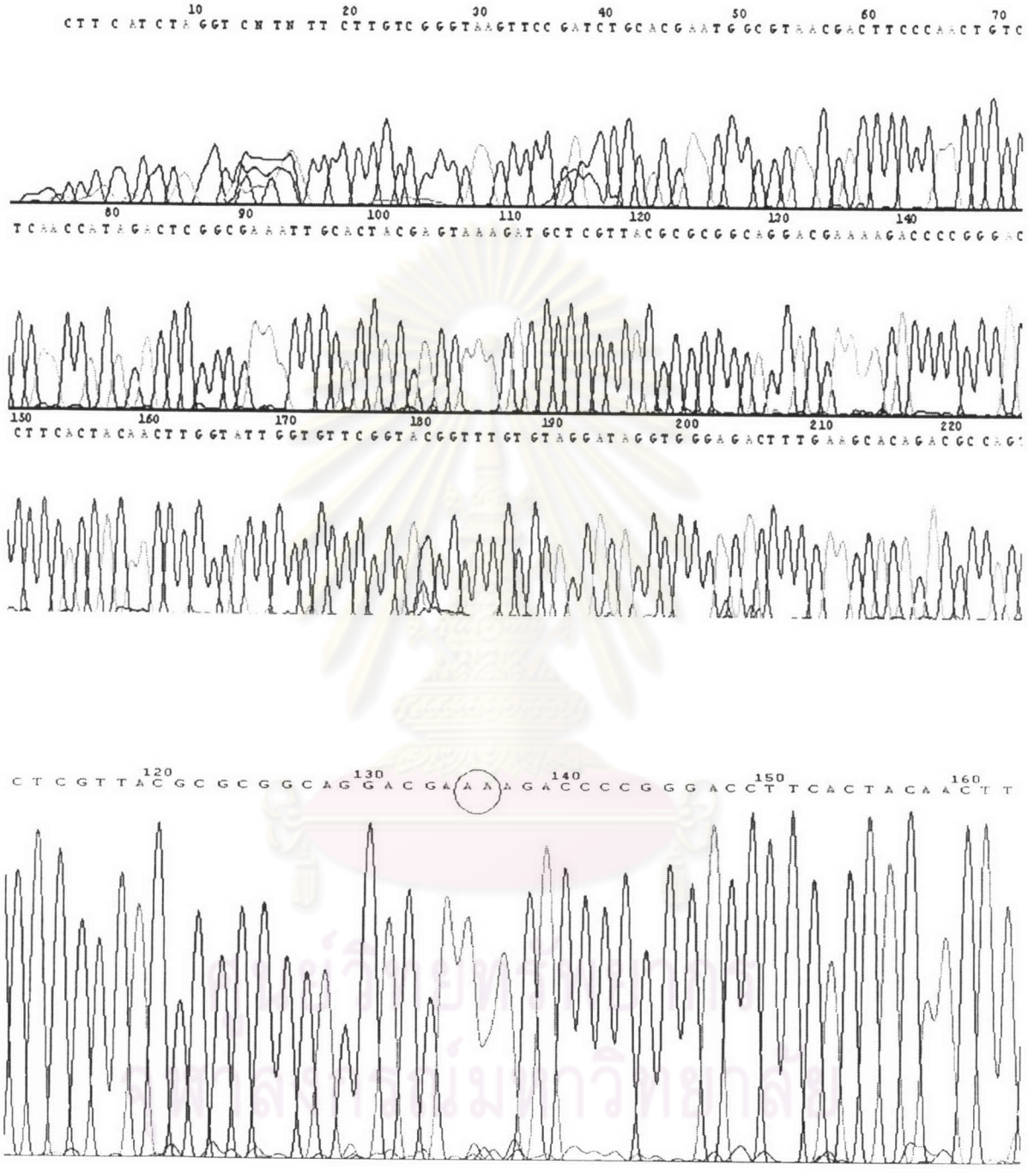

Figure 11. Nucleotide sequence of the 420 bp amplicon from 23S rRNA gene from MAC-susceptible isolate.

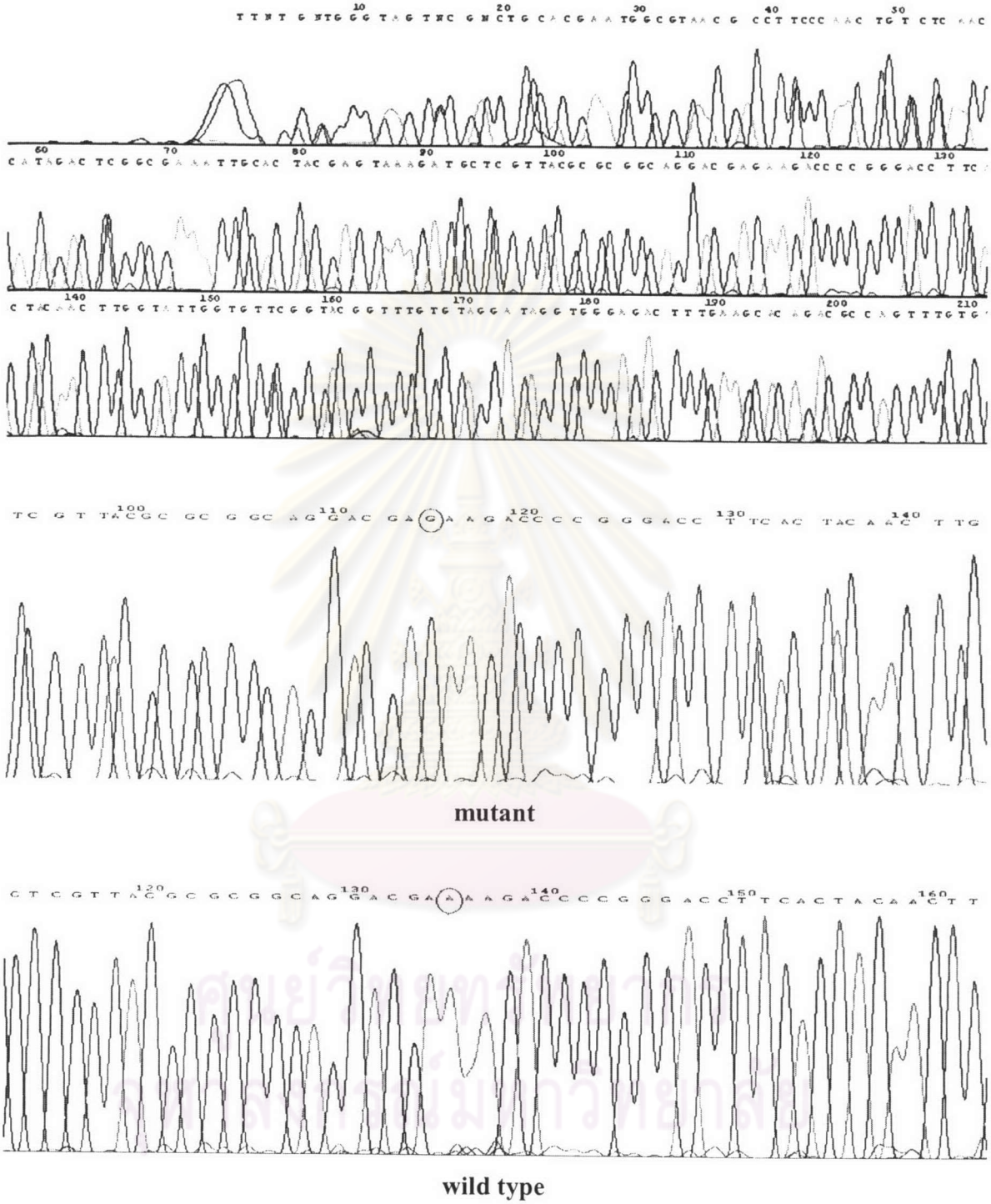

Figure 12. Nucleotide sequence of the 420 bp amplicon from 23S rRNA gene from MAC-resistance isolate. The circle indicate a single point mutation, that is a A2058G transition.

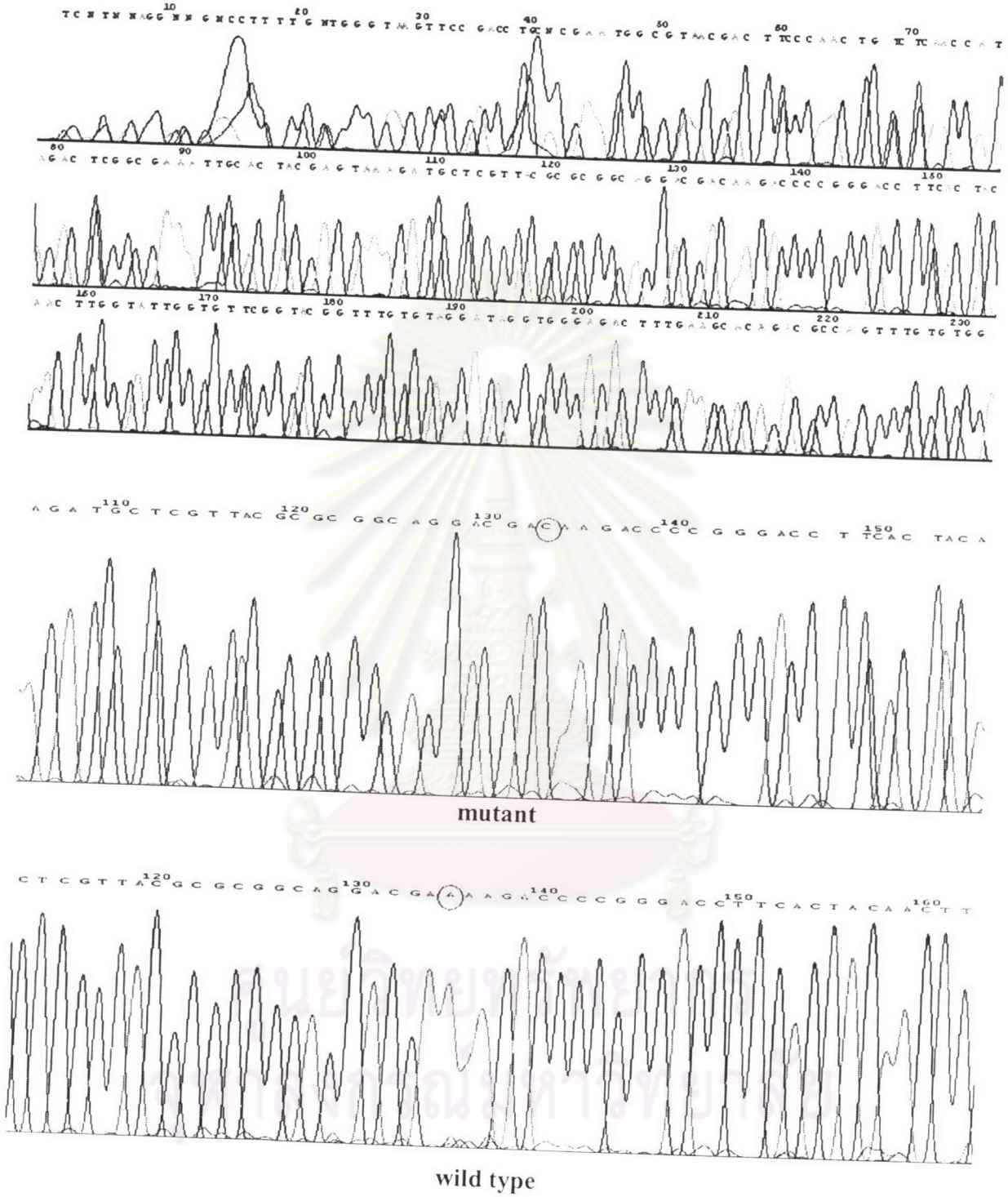

Figure 13. Nucleotide sequence of the 420 bp amplicon from 23S rRNA gene from MAC-resistance isolate. The circle indicate a single point mutation, that is a A2058C transition.