

CHAPTER V

RESULT

MIC screening for study strain *C. albicans*

C. albicans 6 clinical isolates from oral cavities of HIV-infected patients were selected for screening the MIC value by E-test. Using Etest to determine MIC of fluconazole, the result show in the table 5. One with susceptible strain (K44.1, 8 µg/ml), one is susceptible dose dependent, SDD (K49.1, 48µg/ml), four of resist strain K39.1, K54.1 and K78.1 (>256 µg/ml), K51.1 (96µg/ml). The susceptible with the least MIC, K44.1 was selected for this study.

Table 5. Minimal inhibition Concentration (MIC) against isolates of *C. albicans* clinical isolated from oral lesion of 6 HIV-infected patients in this reserch.

Strains of <i>C. albicans</i>	Drug and Dose of treatment	MIC (mg/ml)	Interpretation
K 39.1	Diflucan* 400 mg/day	>256	R
K44.1	Diflucan* 50 mg/day	8	S
K49.1	Nizoral**	48	SDD
K 51.1	Diflucan* 400 mg/day	96	R
K 54.1	Sporal*** 200 mg/day	>256	R
K 78.1	Diflucan*	>256	R

MIC determination during the interval time of culture.

Five groups of the cultured yeast in RPMI-1640 medium plus fluconazole at different concentrations, 8, 16, 24 and 32 $\mu\text{g/ml}$, of original MIC values were performed as described in chapter IV: material and methods. MIC values of each group in each interval time were measured by microdilution test, the gold standard test follows the NCCLS M27-P protocol.

The results showed that on day 0, all cultures have a MIC value of 2 $\mu\text{g/ml}$, the same level as *C. albicans* K44.1 original strain. These results confirm that between the single cell isolation method the MIC of study strain *C. albicans* has not changed.

At day 14 of culture, the MIC level of the cultured group in drug-free media did not change, 2 $\mu\text{g/ml}$, the same level as day 0. While all remaining strains that were cultured in fluconazole plus medium, the MIC level increased to 32 $\mu\text{g/ml}$, a susceptible dose-dependent level (Figure 10).

The MIC of *C. albicans* strains 11 and 18 reached 64 $\mu\text{g/ml}$ on day 29, whereas the same MIC level was shown in all remaining cultured strains (Figure 11).

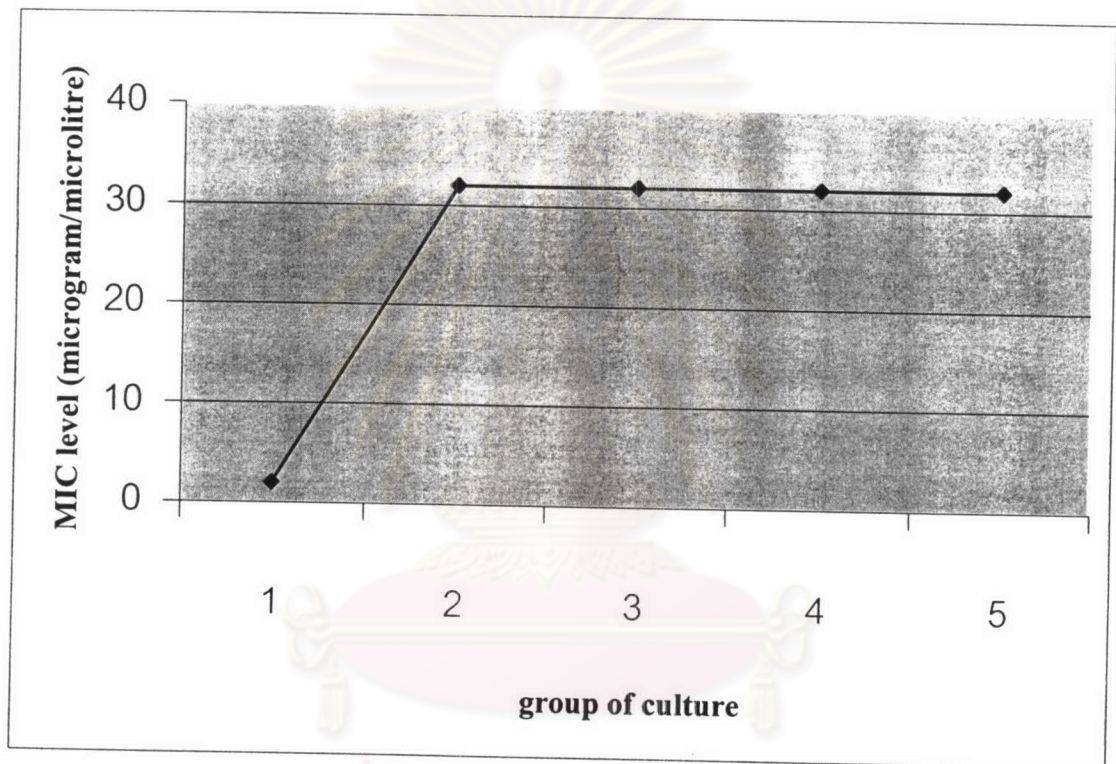
At day 50, the MIC level of strains 11 and 18 dropped to 32 $\mu\text{g/ml}$, while strains 22 and 23, which were cultured in drug concentrations of 32 $\mu\text{g/ml}$, increased to 64 $\mu\text{g/ml}$ and remained stable until the end of the culture study. The MIC levels of other strains did not change (Figure 12). The MIC results of all strains from day 0 until day 60 are shown in table 6.

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Table 6. The fluconazole MIC level ($\mu\text{g/ml}$) of experimental *C. albicans* detected by microdilution test

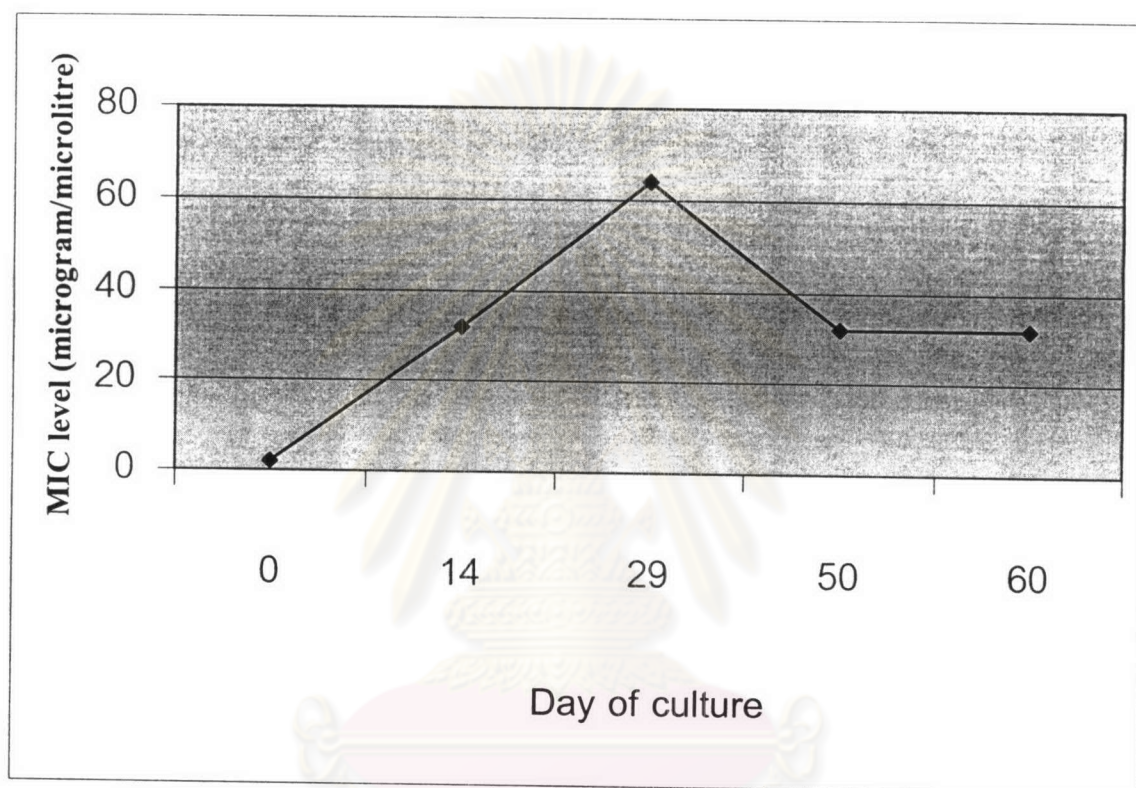
Group	Strain	Culture day				
		0	14	29	50	60
1	1	2	2	2	2	2
	2	2	2	2	2	2
	3	2	2	2	2	2
	4	2	2	2	2	2
	5	2	2	2	2	2
2	6	2	32	32	32	32
	7	2	32	32	32	32
	8	2	32	32	32	32
	9	2	32	32	32	32
	10	2	32	32	32	32
3	11	2	32	64	32	32
	12	2	32	32	32	32
	13	2	32	32	32	32
	14	2	32	32	32	32
	15	2	32	32	32	32
4	16	2	32	32	32	32
	17	2	32	32	32	32
	18	2	32	64	32	32
	19	2	32	32	32	32
	20	2	32	32	32	32
5	21	2	32	32	32	32
	22	2	32	32	64	64
	23	2	32	32	64	64
	24	2	32	32	32	32
	25	2	32	32	32	32

Figure 10. The pattern of MIC level of experimental *C. albicans* at day14. (Group 1) was cultured in drug free medium, group 2-5 were cultured in drug presence media at concentration 8, 16, 24 and 32 $\mu\text{g/ml}$, respectively).



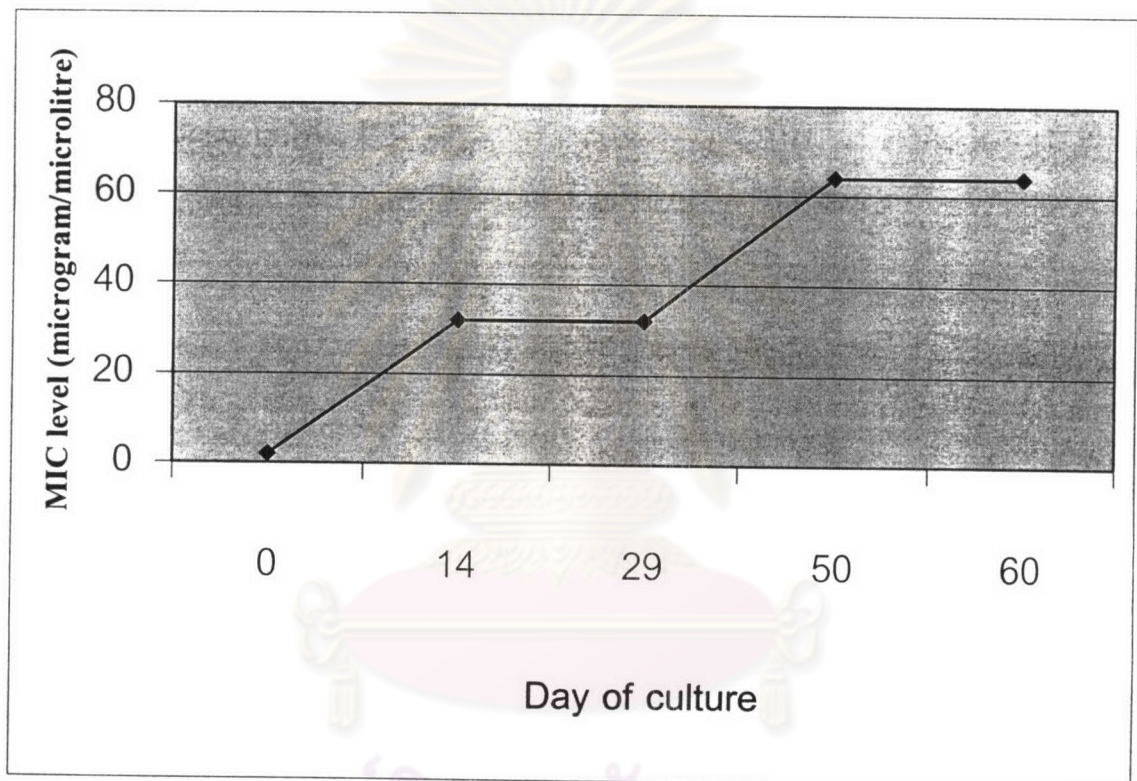
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Figure 11. The pattern of MIC level of experimental *C. albicans* strain 11 and 18.



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Figure 12. The pattern of MIC level of experimental *C. albicans* strain 22 and 23. The MIC was measured at each interval time of culture.



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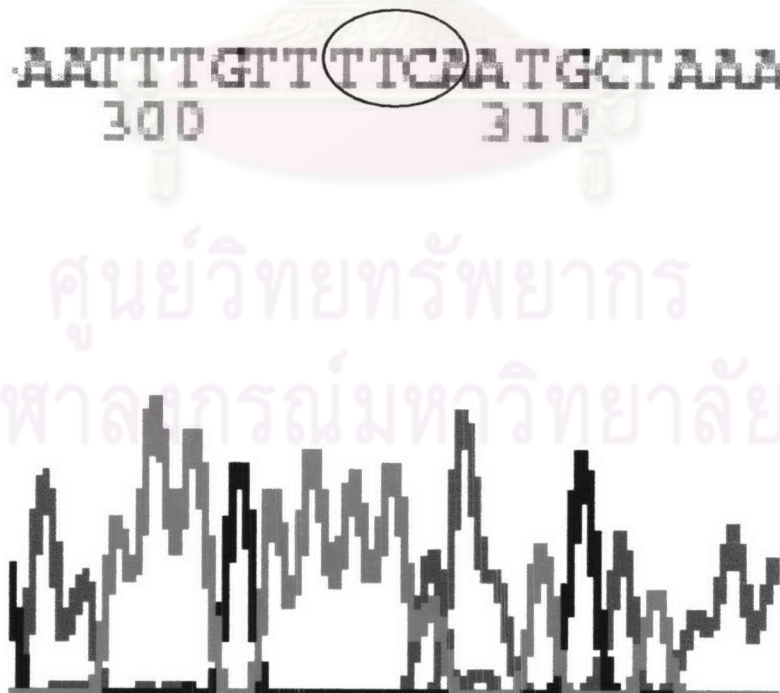
ERG11 sequence analysis

We selected 15 strain of culture for study the sequence of *ERG11* gene. Including *C. albicans* K44.1 original strain, strain 1 and 11 from day14, strain 1,11, 18 And 21 from day 29 and strain 1, 11, 22 and 23 from day 50 and day 60. All sequences were compared to the Lai and Kirch sequence, the *ERG16* gene of *C. albicans* from GeneBank X13296, this sequence recommended by Marichal and co-workers.

The sequence of *C. albicans* K44.1, the original strain, show the point mutation at base position 315 of reference sequence, base T change to C, but it is a silence mutation because there is no change in amino acid sequence, phenylalanine. And the mutation at this position was found in all strain from day 0 through day 60.

For other sequences show, The point mutation that found in these series was silence mutation because the sequences have not show the point mutation that can cause the change of amino acid level.

Figure 13. The point mutation at position 315 of *C. albicans* K 44.1.



There are no point substitution mutation that correlated with fluconazole drug resistant in this isolates when the sequence were compared by CastalX program.

mRNA expression analysis by Northern blot.

The expression of four resistant related genes, *CDR1*, *CDR2*, *MDR1*, and *ERG11*, was analysed by detected the presence of mRNA level. To prove this expression, the Northern dot blot was performed. The amount of 10 µg RNA were blotted on Nitrocellulose membrane and then probed with 5,000 pg of each specific as described in materials and methods. A culture of each induction group from each time point (day 14, 29, 50, 60) was randomly selected and also the original strain, K44.1 was used in this study. The result showed that *the original strain* strain demonstrated lower signal of *CDR1*, *MDR1*, and *ERG11* 1 except *CDR2* gene.

Figure 14. The expression of mRNA of original *C. albicans* K44.1.



The study of mRNA in the induction group

The presence of mRNA of resistant related gene, *CRD1*, *CDR2*, *MDR1* and *ERG11* were detected by northern dot blot. The result show in table 7

Table 7 . The expression of *CRD1*, *CDR2*, *MDR1* and *ERG11* mRNA for each group at each time point.

A. *CDR1* mRNA

Group	<i>CDR1</i> mRNA			
	Day 14	Day29	Day50	Day60
Group 1	+	+	+	+
Group 2	+	+	+	+
Group 3	+	+	+	+
Group 4	+	+	+	+
Group 5	+	+	+	+

B. *CDR2* mRNA

Group	<i>CDR 2</i> mRNA			
	Day 14	Day29	Day50	Day60
Group 1	-	-	-	-
Group 2	+	+	+	+
Group 3	+	+	+	+
Group 4	+	+	+	+
Group 5	+	+	+	+

Table 7 (continue) . The expression of *CRD1*, *CDR2*, *MDR1* and *ERG11* mRNA for each culture groups at each time point.

C. *MDR1* mRNA

Group	<i>MDR1</i> mRNA			
	Day 14	Day29	Day50	Day60
Group 1	+	+	+	+
Group 2	+	+	+	+
Group 3	+	+	+	+
Group 4	+	+	-	+
Group 5	+	+	+	+

D. *ERG11* mRNA

Group	<i>ERG11</i> mRNA			
	Day 14	Day29	Day50	Day60
Group 1	+	+	+	+
Group 2	+	+	+	+
Group 3	+	+	+	+
Group 4	+	+	+	+
Group 5	+	+	+	+

+ express

- not express

From table , the result show that all culture group from all time point were presence of the *CRD1*, *CDR2* , *MDR1* and *ERG11* mRNA. *CDR2* gene that not express in group 1 from all time point whereas the other groups, group 2-5 show the expression of *CDR2*

Table 8 summarize the expression of resistant relate gene from all groups of culture.

Table 8. Summarize the expression of *CRD1*, *CDR2*, *MDR1* and *ERG11* mRNA for each cultures group.

Group	Expression of mRNA			
	<i>CDR1</i>	<i>CDR2</i>	<i>MDR1</i>	<i>ERG11</i>
Group 1	+	-	+	+
Group 2	+	+	+	+
Group 3	+	+	+	+
Group 4	+	+	+	+
Group 5	+	+	+	+

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