CHAPTER VI

DISCUSSION

The MIC result of 6 *C. albicans* from oral cavities HIV infected pateints that show the strain have been treate with antifungal drug show MIC value higher than strain that never treat before enroll to this study. *C. albicans* K39.1, K54.1 and K78.1 have MIC level over than 256 μg/ml while K51.1 and K49.1 show MIC is 96 μg/ml. and 48 μg/ml, respectively. Although *C. albicans* K44.1 and K40.1 have been treat with diflucan (fluconazole) show the MIC value 8 μg/ml, a susceptible level. And K52.1, the strain that never treat with any drug before, it show the susceptible level , 0.125 μg/ml. This result were supported by other investigators, the risk factor that the previous exposure to oral azole for develop of resistant.

To avoid the genetic diversity regardly the resistant gene, single cell from the same strain was performed and the first generation (day 0) of each cell was reconfirmed its MIC by E test. In the same level, was revealed 8 μg/ml by E test and 2 μg/ml by microdilution broth in all 25 cells.

The fluconazole treated cells were monitored to observe their drug induction ability at four times interval. The yeasts were grown in the same drug concentration from the first day till day 60. After that from day14 until day 60, all populations exposed to fluconazole adapted to the the presence of drug, showing increase MIC level up to 32 and some strain up to 64 μg/ml. While the culture in the absence of drug, MIC level not change until day60. These result were supported by Cowen and his colleages' report, no change in MIC for any populations grown without drug while the population grown with fluconazole show increasing MIC values and the pattern of MIC were different among the isolate (62). While in our study the pattern of MIC value is increasing in the same way of all populations

The experiment evolution approch offered no outside genotype entered these populations, and there was no genetic change between individual yeast cells within these populations, mutation was only possible source of genetic variability. Under these condition, mutation that confer an adaptive adventage in the presence of the drug have the

opportunity to increase in frequency response to natural selection during the course of the experiment and the environmental condition were controlled so the other factors from the environmental was not interfere the culture. The innoculum size was controlled to minimize the genetic drift (62).

Although, the point mutation of *ERG11* gene, the gene encoded the target enzyme of fluconazole and other azole drug, have been report that involve to contribute drug resistant. In our study, the result of *ERG11* gene sequencing shows that the mechanisms of resistant in this series were not cause by mutation in this gene. So the other mechanisms may cause to resistant to fluconazole in this populations.

The mRNA level analysis show that the gene that have been report that involve in the drug resistant including *CDR1, CDR2, MDR1* and *ERG11* may involve in resistant mechanisms in these population. In our study the expression of these mRNA were detected the function of genes. The susceptible strain *C. albicans* K44.1 show three gene are express, *CDR1, MDR* and *ERG11* while *CDR2* was not express. For group1 culture, absence of drug, the expression of these gene same as original strain. Group 2 show in this group the function all of gene were detected and it begin to detect the function of *CDR2*. The remaining group were found the expression of these resistant gene. This experiment revealed only the expression ability of these genes not the level of the expression. Thus, the relation among these genes needed to be performing furthermore.