

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

A Search For Contingency Genes
In *Candida albicans*

A thesis presented in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
in the Institute of Fundamental Sciences
Massey University, Palmerston North
New Zealand

Synodalia Chrisma Wattimena

2014

Abstract

Many microbial pathogens have been known to use repeats in their cell wall proteins to generate diversity, and this has been found to contribute to their virulence. In bacteria, these genes are called contingency genes, and function to facilitate adaptation of bacteria to the host environments as they invade different host parts and to evade the host's constantly evolving immune system. In the diploid *Candida albicans*, few genes have been classified as contingency genes due to the variation in the length of their repeat regions in different clinical isolates. This study attempts to answer a question of whether *YWP1*, *HWP1*, and *EAP1* of *C. albicans* are contingency genes. These three genes encode cell wall proteins and contain repeats. For this purposes, allelic distributions of the genes in the general purpose genotype (GPG) and non-GPG strains (two groups with different genetic backgrounds), in commensal and infection strains, and in strains isolated from different sites of the humans body were examined. Based on the allelic distributions of the genes in GPG and non-GPG strains, it can be inferred that *YWP1* and *HWP1* can be categorized as contingency genes, while *EAP1* cannot be categorized as a contingency gene. The allelic distributions of the genes in commensal and infection strains indicate that *YWP1*, *HWP1*, and *EAP1* do not act as contingency genes when *C. albicans* state changes from commensal to pathogenic. Although the allelic distributions of the genes cannot distinguish commensal from infection strains, the non-random association between alleles of *YWP1*, *HWP1*, and *EAP1* does distinguish these two groups, i.e. the *YWP1*-*HWP1*-*EAP1* association is stronger in commensal strains that it is in infection strains. Based on the allelic distribution of the genes in strains isolated from different sites of the human body, it can be inferred that *YWP1* and *EAP1* do not act as contingency genes, but *HWP1* may act as a contingency gene, when *C. albicans* moves to particular sites of the human body.

Acknowledgments

I would like to thank Dr. Jan Schmid and Dr. Mark Patchett as my supervisors, who introduce me to *Candida albicans* field. I found this field interesting, and am ready to continue this study in years to come. I am thankful for Dr. Mark Patchett for his helpful comments in shaping my thesis. I am grateful for the encouragement I received from Dr. Kathryn Stowell as IMBS graduate advisor in many occasions. I really appreciate the helps I received from Richard Fong, Lorraine Berry, and Trish McLenachan from the Allan Wilson Centre Genome Service at Massey University for helpful discussions on the sample preparations. I would also like to thank the members of Dr. Schmid's Lab; special thanks for Zhuo Zhou for her collaboration work with me in preparing some samples, in particular the 300 generation sample, Pany Vijayan, Dennys and Yeying Wang for introducing me to many new stuffs in the beginning of my program, and Shuguang Zhang for many helpful discussions. I am really grateful for the generous funding from NZAID (the Ministry of Foreign Affairs & Trade) for my PhD program, and for Sylvia Hooker, Sue Flynn, Olive Pimental, and Jamie Hooper from Massey International Office for their supports. My special thanks go to my family in Ambon, Indonesia for their prayers and supports. Finally, I would like to give my very special Thanks to my daughter Wilda, and my husband Philip, for their supports, loves, understandings, helps, and prayers.

Contents

Abstract	i
Acknowledgments	ii
Contents	iii
List of Tables	ix
List of Figures	xii
1 Introduction	1
1.1 Literature Review	1
1.1.1 <i>Candida albicans</i> and Humans Host	1
1.1.2 Cell Wall of <i>C. albicans</i>	2
1.1.3 Repeat Sequences	5
1.1.4 Contingency Genes	6
1.2 Research Background	6
1.3 Research Questions	10
2 Materials and Methods	12
2.1 Fungal and Bacterial Strains	12
2.1.1 Fungal Strains	12
2.1.2 Bacterial Strains	18
2.2 Media and Solutions	18
2.2.1 Media	18
2.2.2 Solutions	20
2.3 Culture Conditions	21
2.3.1 Short-term Cultures	21

2.3.2	Long-term Cultures	21
2.4	<i>E. coli</i> Competent Cell Preparation	22
2.5	Primers	22
2.6	PCR	23
2.6.1	PCR (Gel Electrophoresis)	23
2.6.2	Gel Electrophoresis	23
2.6.3	PCR (Genotyping)	24
2.6.4	Genotyping	25
2.7	DNA Sequencing	25
2.7.1	PCR	25
2.7.2	Ligation	26
2.7.3	Transformation	26
2.7.4	Selection of Transformed Cells	26
2.7.5	Sample Preparation for Sequencing	27
2.7.6	Sequencing Data Analysis	27
2.8	Preparations and Analysis of Isolates Derived from Repeated Sub- Culture (300 Generations) of A single Parental Strains of <i>C. Albicans</i>	27
2.9	Statistical Analysis	28
3	Alleles of the YWP1 Gene	29
3.1	Identification of the Repeat Units in the <i>YWP1</i> Gene	30
3.2	Results of Allelic Characterization of the <i>YWP1</i> Gene For Repeat Re- gion 1	32
3.2.1	Allelic Characterization of the <i>YWP1</i> Gene For Repeat Re- gion 1 in GPG and non-GPG strains (Infection Strains)	32
3.2.2	Allelic Characterization of the <i>YWP1</i> Gene For Repeat Re- gion 1 in Commensal Strains	32
3.2.3	No Variability in Alleles of the <i>YWP1</i> Gene For Repeat Re- gion 1	34
3.3	Results of Allelic Characterization of the <i>YWP1</i> Gene	37
3.3.1	Allelic Characterization of the <i>YWP1</i> Gene in GPG and non- GPG Strains (Infection Strains)	37
3.3.2	Alleles of the <i>YWP1</i> Gene in Commensal Strains	42
3.3.3	Diversity of Alleles of the <i>YWP1</i> Gene	48

3.3.4	Analysis of the Combination of the Two Alleles of <i>YWP1</i> in an Individual Strain	50
3.3.5	Allelic Distribution of <i>YWP1</i> in Strains Isolated From Different Sites of the Humans Body	56
3.4	Discussion	58
3.4.1	Variability in the number of <i>YWP1</i> repeats	58
3.4.2	GPG and non-GPG strains have distinct predominant allele combinations, but share the same predominant alleles of <i>YWP1</i>	62
3.4.3	The number of <i>YWP1</i> repeats does not alter when <i>C. albicans</i> state changes from commensal to pathogenic	66
3.4.4	The number of <i>YWP1</i> repeats does not alter when <i>C. albicans</i> moves to particular sites of the humans body	67
3.4.5	The homozygous and heterozygous alleles of <i>YWP1</i>	68
4	Alleles of the <i>HWP1</i> Gene	70
4.1	Identification of the Repeat Units in the <i>HWP1</i> Gene	71
4.2	Allelic Characterization of the <i>HWP1</i> Gene in GPG and Non-GPG Strains (Infection Strains)	71
4.3	Allelic Characterization of the the <i>HWP1</i> Gene in Commensal Strains	79
4.4	Diversity of Alleles of the <i>HWP1</i> Gene	83
4.5	Analysis of the Combination of the Two Alleles of the <i>HWP1</i> Gene in An Individual Strain	83
4.6	Allelic Distribution of the <i>HWP1</i> Gene in Strains Isolated From Different Sites of the Humans Body	91
4.7	Discussion	93
4.7.1	Variability in the number of <i>HWP1</i> repeats	93
4.7.2	GPG and non-GPG strains have the same predominant alleles and allele combinations of <i>HWP1</i>	95
4.7.3	The number of <i>HWP1</i> repeats does not alter when <i>C. albicans</i> state changes from commensal to pathogenic	97
4.7.4	The number of <i>HWP1</i> repeats may require to alter when <i>C. albicans</i> moves to particular sites of the humans body	98
4.7.5	The homozygous and heterozygous alleles of <i>HWP1</i>	99

5	Alleles of the <i>EAP1</i> Gene	100
5.1	Identification of the Repeat Units in the <i>EAP1</i> Gene	101
5.2	Results of Allelic Characterization of the <i>EAP1</i> Gene For Repeat Region 1	101
5.2.1	Allelic Characterization of the <i>EAP1</i> Gene For Repeat Region 1 in GPG and Non-GPG Strains (Infection Strains)	103
5.2.2	Allelic Characterization of the <i>EAP1</i> Gene For Repeat Region 1 in Commensal Strains	107
5.2.3	Diversity of Alleles of the <i>EAP1</i> Gene For Repeat Region 1	114
5.2.4	Analysis of the Combination of the Two Alleles of the <i>EAP1</i> Gene For Repeat Region 1 in An Individual Strain	118
5.2.5	Allelic Distribution of the <i>EAP1</i> Gene For Repeat Region 1 in Strains Isolated From Different Sites of the Humans Body	123
5.3	Results of Allelic Characterization of the <i>EAP1</i> Gene For Repeat Region 2	123
5.3.1	Allelic Characterization of the <i>EAP1</i> Gene For Repeat Region 2 in GPG and non-GPG Strains (Infection Strains)	126
5.3.2	Allelic Characterization of the <i>EAP1</i> Gene For Repeat Region 2 in Commensal Strains	135
5.3.3	Diversity of Alleles of the <i>EAP1</i> Gene For Repeat Region 2	140
5.3.4	Analysis of the Combination of the Two Alleles of the <i>EAP1</i> Gene of For Repeat Region 2 in An Individual Strain	140
5.3.5	Allelic Distribution of the <i>EAP1</i> Gene For Repeat Region 2 in Strains Isolated From Different Sites of the Humans Body	146
5.4	Allele Combinations of the <i>EAP1</i> Gene For Pairs of Repeat Regions 1 and 2	149
5.4.1	Genotypes of the <i>EAP1</i> Gene in GPG and Non-GPG Strains	149
5.4.2	Genotypes of the <i>EAP1</i> Gene in Commensal Strains	152
5.5	Discussion	152
5.5.1	Variability in the number of <i>EAP1</i> repeats	152
5.5.2	GPG and non-GPG strains have distinct predominant alleles and allele combinations of <i>EAP1</i>	154
5.5.3	The number of <i>EAP1</i> repeats does not alter when <i>C. albicans</i> state changes from commensal to pathogenic	157

5.5.4	The number of <i>EAP1</i> repeats does not alter when <i>C. albicans</i> moves to particular sites of the humans body	158
5.5.5	The homozygous and heterozygous alleles of <i>EAP1</i>	159
6	Interconnectedness Between Alleles	161
6.1	Genotypes of Pairs of Genes	162
6.1.1	<i>YWP1-HWP1</i> Genotypes	162
6.1.2	<i>YWP1-EAP1</i> Genotypes	163
6.1.3	<i>HWP1-EAP1</i> Genotypes	172
6.2	<i>YWP1-HWP1-EAP1</i> Genotypes	185
6.3	Diversity of Genotypes of Pairs of Genes	192
6.4	Non-Random Association Between Alleles of Different Genes	192
6.4.1	Linkage Disequilibrium : A Measure of Non-Random Association Between Alleles of Different Genes	194
6.4.2	Linkage Disequilibrium Between Alleles of Different Genes	196
6.4.3	Summary of Linkage Disequilibrium Between Alleles of Different of Genes	199
6.5	Discussion	202
6.5.1	Distribution of genotypes of the pairs of genes	202
6.5.2	Genetic background influences the gene interconnectedness	204
6.5.3	The gene interconnectedness distinguishes commensal strains from infection strains	207
7	Detection of New Alleles	210
7.1	Alleles of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> in Strain RIHO30 from Serial Transfer	210
7.2	Alleles of the <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> Genes in Strains from an AIDS Patient with Recurrent Candidiasis	211
7.3	Discussion	212
7.3.1	Prediction of mutation rate of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i>	212
7.3.2	A contingency gene has a low mutation rate	216
7.3.3	No change in <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> alleles occurred during recurrent candidiasis over a four-year period	219
8	Summary and Conclusion	221
8.1	Summary	221

8.1.1	Variability in the number repeats in <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i>	222
8.1.2	Allelic distributions of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> in GPG and non-GPG strains	223
8.1.3	Allelic distributions of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> in commensal and infection strains	225
8.1.4	Allelic distributions of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> in strains isolated from different sites of the humans body	226
8.1.5	Are <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> contingency genes ?	226
8.1.6	The gene interconnectedness distinguishes commensal from infection strains	227
8.1.7	Are <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> hypermutable genes ?	227
8.2	Conclusions and Future Directions	228
8.2.1	Conclusions	228
8.2.2	Future Directions	229

Bibliography	233
---------------------	------------

List of Tables

2.1	List of GPG infection strains used in this study	13
2.2	List of Non-GPG infection strains used in this study	15
2.3	List of GPG commensal strains used in this study	17
2.4	Primers for sequencing of the <i>YWP1</i> gene	23
2.5	Primers for PCR amplification and the sequencing of repeat regions of the <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> genes	24
3.1	Frequency of alleles of <i>YWP1</i> in GPG and non-GPG strains	38
3.2	Frequency of allele combinations of <i>YWP1</i> in GPG and non-GPG strains	42
3.3	Frequency of alleles of <i>YWP1</i> in commensal strains	44
3.4	Frequency of allele combinations of <i>YWP1</i> in commensal strains . . .	48
3.5	Index of diversity λ of the alleles and the allele combinations of <i>YWP1</i> for GPG, non-GPG, and commensal strains	50
3.6	The number of homozygous alleles in GPG, non-GPG, and commensal strains compared to the expected number by chance	55
3.7	The mean (\pm standard deviation) of the differences in the number of repeats between the two alleles of the <i>YWP1</i> gene in an individual strain from observed data, data expected from random alleles, and the significance of the difference between the two means	56
3.8	Number of different alleles detected in some <i>C.albicans</i> genes	61
4.1	Frequency of alleles of the <i>HWP1</i> gene in GPG and non-GPG strains	73
4.2	Frequency of allele combinations of <i>HWP1</i> in GPG and non-GPG strains	77
4.3	Frequency of alleles of <i>HWP1</i> in commensal strains	79
4.4	Frequency of allele combinations of <i>HWP1</i> in commensal strains . . .	82
4.5	Index of diversity λ of the alleles and the allele combinations of <i>HWP1</i> for GPG, non-GPG, and commensal strains	83

4.6	The number of homozygous alleles in GPG, non-GPG, and commensal strains compared to the expected number by chance	89
4.7	The mean (\pm standard deviation) of the differences in the number of repeat units between the two alleles of <i>HWP1</i> in an individual strain among strains of interest from observed data, data expected from random alleles, and the significance of the difference between the two means	91
5.1	Frequency of alleles of <i>EAP1</i> for repeat region 1 in GPG and non-GPG strains	104
5.2	Frequency of allele combinations of <i>EAP1</i> for repeat region 1 for GPG and non-GPG strains	108
5.3	Frequency of alleles of <i>EAP1</i> for repeat region 1 in commensal strains	111
5.4	Frequency of allele combinations of <i>EAP1</i> for repeat region 1 in commensal strains	114
5.5	Index of diversity of alleles and allele combinations of <i>EAP1</i> for repeat region 1 for GPG, non-GPG, and commensal strains	118
5.6	The number of homozygous strains in GPG, non-GPG, and commensal strains compared to the expected number by chance	121
5.7	The mean (\pm standard deviation) of the differences in the number of repeat units between the two alleles of the <i>EAP1</i> gene for repeat region 1 in an individual strain from observed data, data expected from random alleles, and the significance of the difference between the two means for infection and commensal strains	122
5.8	Frequency of alleles of <i>EAP1</i> for repeat region 2 in GPG and non-GPG strains	128
5.9	Frequency of allele combinations of <i>EAP1</i> for repeat region 2 in GPG and non-GPG strains	131
5.10	Frequency of alleles of <i>EAP1</i> for repeat region 2 in commensal strains	134
5.11	Frequency of allele combinations of <i>EAP1</i> for repeat region 2 in commensal strains	134
5.12	Index of diversity of the alleles and the allele combinations of <i>EAP1</i> for repeat region 2 in GPG, non-GPG, and commensal strains	140
5.13	The number of homozygous strains in GPG, non-GPG, and commensal strains compared to the expected number by chance	143

5.14	The mean (\pm standard deviation) of the differences in the number of repeat units between the two alleles of <i>EAP1</i> for repeat region 2 in an individual strain from observed data, data expected from random alleles, and the significance of the difference between the two means for GPG, non-GPG, and commensal strains	146
6.1	The index of diversity of genotypes of pairs of genes in GPG, non-GPG, and commensal strains	194
6.2	The index of association between alleles of different genes for GPG, non-GPG, and commensal strains	199
6.3	The index of association between alleles of different genes for GPG, non-GPG, and commensal strains	199
7.1	Results of the allele characterization of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> (1) in strain RIHO30 at zero and 300 generations	211
8.1	Summary of the number of different alleles and allele combinations of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i>	223
8.2	The predominant alleles and allele combinations of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> in GPG and non-GPG strains	224
8.3	The predominant alleles and allele combinations of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> in GPG and non-GPG strains	225
8.4	The summary of whether there is any indication that <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> are involved in adaptation, thus contingency genes	226

List of Figures

1.1	A neighbour-joining tree of 266 <i>C. albicans</i> strains	8
3.1	Identification of the repeat units in <i>YWP1</i> using MacVector software	31
3.2	The gel electrophoresis fragment separation of repeat region 1 of <i>YWP1</i> for some GPG and non-GPG strains	33
3.3	The gel electrophoresis fragment separation of repeat region 1 of <i>YWP1</i> for some commensal strains	35
3.4	Results of the sequencing of the <i>YWP1</i> gene in the area containing repeat region 2 and the associated amino acid sequences	36
3.5	Distribution of alleles of <i>YWP1</i> for GPG and non-GPG strains . . .	39
3.6	Distribution of alleles of <i>YWP1</i> for GPG and non-GPG strains isolated from oral sites	40
3.7	Distribution of allele combinations of <i>YWP1</i> for GPG and non-GPG strains	41
3.8	Distribution of allele combinations of the <i>YWP1</i> gene in GPG and non-GPG strains isolated from oral sites	43
3.9	Distribution of alleles of <i>YWP1</i> for commensal strains	45
3.10	Distribution of alleles of <i>YWP1</i> for commensal strains, where all strains were isolated from oral sites	46
3.11	Distribution of allele combinations of <i>YWP1</i> for commensal strains .	47
3.12	Distribution of allele combinations of <i>YWP1</i> for commensal, where all strains were isolated from oral sites	49
3.13	Index of diversity λ of the alleles and the allele combinations of <i>YWP1</i> for GPG, non-GPG, and commensal strains	51
3.14	Distribution of differences in the number of repeats between the two alleles of <i>YWP1</i> in an individual strain for infection and commensal strains	53

3.15	Frequency of homozygotes and heterozygotes of <i>YWP1</i> for GPG, non-GPG, and commensal strains	54
3.16	Distribution of alleles <i>YWP1</i> in strains isolated from different sites of the humans body	57
3.17	Distribution of allele combinations of <i>YWP1</i> in strains isolated from different sites of the humans body	59
3.18	Distribution of allele combinations of <i>YWP1</i> of non-GPG strains of branches B and C	64
4.1	Identification of the repeat unit in the <i>HWP1</i> gene using Macvector software	72
4.2	Distribution of alleles <i>HWP1</i> for GPG and non-GPG strains	74
4.3	Distribution of alleles of <i>HWP1</i> for GPG and non-GPG strains isolated from oral sites	75
4.4	Distribution of allele combinations of <i>HWP1</i> for GPG and non-GPG strains	76
4.5	Distribution of allele combinations of <i>HWP1</i> for GPG and non-GPG strains isolated from oral sites	78
4.6	Distribution of alleles of <i>HWP1</i> for commensal strains	80
4.7	Distribution of alleles of <i>HWP1</i> for commensal strains, where all strains were isolated from oral sites	81
4.8	Distribution of allele combinations of <i>HWP1</i> for commensal strains	84
4.9	Distribution of allele combinations of <i>HWP1</i> for commensal and infection strains, where all strains were isolated from oral sites	85
4.10	Index of diversity λ of the alleles and the allele combinations of <i>HWP1</i> for GPG, non-GPG , and commensal strains	86
4.11	Distribution of differences in the number of repeat units between the two alleles of <i>HWP1</i> in an individual strain for GPG, non-GPG, and commensal strains	87
4.12	Frequency of homozygotes and heterozygotes of <i>HWP1</i> for GPG, non-GPG, and commensal strains	88
4.13	Distribution of alleles of <i>HWP1</i> in strains isolated from different sites of the humans body	92
4.14	Distribution of allele combinations of <i>HWP1</i> in strains isolated from different sites of the humans body	94

5.1	Identification of the repeat unit in <i>EAP1</i> using MacVector software .	102
5.2	Distribution of alleles of <i>EAP1</i> for repeat region 1 in GPG and non-GPG strains	105
5.3	Distribution of alleles of <i>EAP1</i> for repeat region 1 for GPG and non-GPG strains isolated from oral sites	106
5.4	Distribution of allele combinations of <i>EAP1</i> for repeat region 1 for GPG and non-GPG strains	109
5.5	Distribution of allele combinations of <i>EAP1</i> for repeat region 1 for GPG and non-GPG strains isolated from oral sites	110
5.6	Distribution of alleles of <i>EAP1</i> for repeat region 1 for commensal strains	112
5.7	Distribution of alleles of <i>EAP1</i> for repeat region 1 for commensal strains isolated from oral sites	113
5.8	Distribution of allele combinations of <i>EAP1</i> for repeat region 1 for commensal strains	115
5.9	Distribution of allele combinations of <i>EAP1</i> for repeat region 1 for commensal strains isolated from oral sites	116
5.10	Index of diversity of the alleles and the allele combinations of <i>EAP1</i> for repeat region 1 for GPG, non-GPG, and commensal strains	117
5.11	Distribution of the differences in the number of repeat units between the two alleles of <i>EAP1</i> for repeat region 1 in an individual strain for GPG and non-GPG strains of infection strains and (GPG) commensal strains	119
5.12	Frequency of homozygotes and heterozygotes of <i>EAP1</i> for repeat region 1 for GPG, non-GPG, and commensal strains	120
5.13	Distribution of alleles of <i>EAP1</i> for repeat region 1 in strains isolated from different sites of the humans body	124
5.14	Distribution of allele combinations of <i>EAP1</i> for repeat region 1 in strains isolated from different sites of the humans body	125
5.15	Distribution of alleles of <i>EAP1</i> for repeat region 2 for GPG and non-GPG strains	129
5.16	Distribution of alleles of <i>EAP1</i> for repeat region 2 for GPG and non-GPG strains isolated from oral sites	130
5.17	Distribution of allele combinations of <i>EAP1</i> for repeat region 2 for GPG and non-GPG strains	132

5.18	Distribution of allele combinations of <i>EAP1</i> for repeat region 2 for GPG and non-GPG strains isolated from oral sites	133
5.19	Distribution of alleles of <i>EAP1</i> for repeat region 2 for commensal strains	136
5.20	Distribution of alleles of <i>EAP1</i> for repeat region 2 by the number of repeat units for commensal strains isolated from oral sites	137
5.21	Distribution of allele combinations of <i>EAP1</i> for repeat region 2 for commensal strains	138
5.22	Distribution of allele combinations of <i>EAP1</i> for repeat region 2 for commensal strains isolated from oral sites	139
5.23	Index of diversity of the alleles and the allele combinations of <i>EAP1</i> for repeat region 2 in GPG, non-GPG, and commensal strains	141
5.24	Distribution of the differences in the number of repeat units between the two alleles of <i>EAP1</i> for repeat region 2 in an individual strain for GPG, non-GPG, and commensal strains	142
5.25	Frequency of homozygotes and heterozygotes of <i>EAP1</i> for repeat region 2 for GPG, non-GPG, and commensal strains	144
5.26	Distribution of alleles of <i>EAP1</i> for repeat region 2 in strains isolated from different sites of the humans body	147
5.27	Distribution of allele combinations of <i>EAP1</i> for repeat region 2 in strains isolated from different sites of the humans body	148
5.28	Distribution of genotypes of <i>EAP1</i> for GPG strains	150
5.29	Distribution of genotypes of <i>EAP1</i> for non-GPG strains	151
5.30	Distribution of genotypes of <i>EAP1</i> for commensal strains	153
6.1	Distribution of <i>YWP1-HWP1</i> genotypes for GPG strains	164
6.2	Distribution of <i>YWP1-HWP1</i> genotypes for non-GPG strains	165
6.3	Distribution of <i>YWP1-HWP1</i> genotypes for commensal strains	166
6.4	Distribution of <i>YWP1-EAP1</i> (1) genotypes for GPG strains	168
6.5	Distribution of <i>YWP1-EAP1</i> (1) genotypes for non-GPG strains	169
6.6	Distribution of <i>YWP1-EAP1</i> (1) genotypes for commensal strains	170
6.7	Distribution of <i>YWP1-EAP1</i> (2) genotypes for GPG strains	173
6.8	Distribution of <i>YWP1-EAP1</i> (2) genotypes for non-GPG strains	174
6.9	Distribution of <i>YWP1-EAP1</i> (2) genotypes for commensal strains	175
6.10	Distribution of <i>YWP1-EAP1</i> genotypes for GPG, non-GPG, and commensal strains	176

6.11	Distribution of <i>HWP1-EAP1</i> (1) genotypes for GPG strains	177
6.12	Distribution of <i>HWP1-EAP1</i> (1) genotypes for non-GPG strains . . .	178
6.13	Distribution of <i>HWP1-EAP1</i> (1) genotypes for commensal strains . .	179
6.14	Distribution of <i>HWP1-EAP1</i> (2) for GPG strains	181
6.15	Distribution of <i>HWP1-EAP1</i> (2) genotypes for non-GPG strains . . .	182
6.16	Distribution of <i>HWP1-EAP1</i> (2) genotypes for commensal strains . .	183
6.17	Distribution of <i>HWP1-EAP1</i> genotypes for GPG, non-GPG, and com- mensal strains	184
6.18	Distribution of <i>YWP1-HWP1-EAP1</i> (1) genotypes for GPG and non- GPG strains	187
6.19	Distribution of <i>YWP1-HWP1-EAP1</i> (1) genotypes for commensal strains	188
6.20	Distribution of <i>YWP1-HWP1-EAP1</i> (2) genotypes for GPG strains .	190
6.21	Distribution of <i>YWP1-HWP1-EAP1</i> (2) genotypes for commensal strains	191
6.22	The index of diversity of genotypes of pairs of genes in GPG, non-GPG, and commensal strains	193
6.23	Index of association between alleles of different genes for GPG and non-GPG strains	200
6.24	Index of association between alleles of different genes for commensal strains	201
6.25	The percentages of the predominant genotypes of pairs of genes and the predominant allele combinations of individual genes which form the genotypes for infection and commensal strains	204
6.26	The percentages of the predominant genotypes of three genes and the predominant allele combinations of individual genes which form the genotypes for infection and commensal strains	205
7.1	Results of the allele characterization of <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> for isolates from an AIDS patient with recurrent candidiasis	213
7.2	Prediction of the mutation rate for <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> in strain RIHO30 based on the VARScore calculation	215
7.3	The range of the VARScore predicted mutation rates for <i>YWP1</i> , <i>HWP1</i> , and <i>EAP1</i> in strain RIHO30 based on the range of the number of re- peats observed	218

Chapter 1

Introduction

1.1 Literature Review

1.1.1 *Candida albicans* and Humans Host

Candida albicans is a normal member of the microflora on the mucosal surfaces of most healthy persons. It is uniquely associated with humans or animals, and rarely found in other environments such as soil. Although it is not a dangerous organism and most of the time is not able to infect healthy people, overgrowth of this fungus can cause superficial as well as life-threatening systemic infections in immunosuppressed patients due to cancer treatments or diseases such as AIDS [1]. For these patients, candidiasis is frequently caused by a resident strain and is triggered by changes in the host immune system [2, 3]. Therefore, *C. albicans* is well adapted to humans environments. However, there is evidence that *C.albicans* strains can be transmitted between persons and then replace existing commensal strains in immunosuppressed patients [4].

In order to colonize and infect humans, *C. albicans* cell has to come into contact with the humans body. There are several known ways for *C. albicans* to enter the humans body. For examples, contact with carriers [5] and transfer via contaminated food [6]. After it is in contact with the humans body, the cells must adhere on host surfaces in order to replicate and then colonize the body. Without this adherence,