EPITOPES OF COMMENSAL FUNGI CROSS-REACTING WITH HUMAN ANTIGENS ARE POTENTIAL MEDIATORS OF AUTO-IMMUNITY

A PROJECT REPORT

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE

OF

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by

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CANDIDATE'S DECLARATION

I, Gunjan Sachdeva, Roll No. 2K19/MSCBIO/08 student of M.Sc Biotechnology, hereby certify that the work which is presented in the major project entitled "Epitopes of commensal fungi cross-reacting with human antigens are potential mediators of auto-immunity" in partial fulfilment of the requirement for the award of the degree of Masters of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during the period of 7 Jan 2021 to 28 May 2021 under the supervision of Dr. Asmita Das. The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other university.

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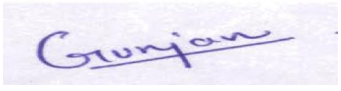

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CERTIFICATE

This is to certify that the M.Sc Dissertation entitled "**Epitopes of commensal fungi cross-reacting with human antigens are potential mediators of auto-immunity**" in partial fulfilment of the requirement for the award of the degree of Masters of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during the period of 7 Jan 2021 to 28 May 2021. The information and data enclosed in this dissertation is original and has not been submitted elsewhere for honouring of any other degree.

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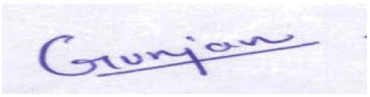

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ABSTRACT

The human body is the reservoir of both commensal as well as pathogenic fungi, that play an essential role in regulation of homeostasis and balance the susceptible conditions towards various diseases. The immune system had evolved to keep a check on these fungi and prevent them from causing life-threatening disorders. When the balance that is formed between the fungi and the immune system disrupts, it leads to dysbiosis due to which the fungal infection flares up. It can lead to various autoimmune reactions, which are caused due to cross-reactivity of epitopes of fungi with the auto-antigens of humans, as in the case, where the generation of antibodies against Aspergillus Noc2 peptide can cross-react with human IFN-γ due to the sequence homology between the two.

This study aims to explore different fungi that are present as commensals in humans. Proteins of these fungi are to be studied and the potential epitopes present in these fungi are to be screened for cross reactivity with host epitopes. Their sequence homology would be searched with auto-antigens of humans, in order to look for the cross-reactive antigens which can serve as biomarkers for certain autoimmune diseases in the future.

KEYWORDS: Commensal fungi, Autoimmune reactions, Cross-reactive, Epitopes, Aspergillus Noc2 peptide, IFN-γ, Auto-antigens of humans

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LIST OF SYMBOLS, ABBREVIATIONS

ABBREVIATIONS	NAME
MHC	Major Histocompatibility Complex
IBS	Irritable bowel syndrome
IBD	Inflammatory bowel diseases
CD	Crohn's disease
PAMPs	Pathogen associated molecular patterns
PRRs	Pathogen recognition receptors
TLRs	Toll-like receptors
ROS	Reactive-oxygen species
Treg cells	Regulatory T cells
TCR	T-Cell Receptor
APC	Antigen presenting Cell
IFN-γ	Interferon γ
NSAIDs	Nonsteroidal anti-inflammatory drugs

CHAPTER 1

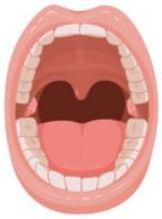
1. INTRODUCTION

The microbiome is made up of a large number of microorganisms that comprise not only microbes but also their gene products, enzymes, and several other components. Bacteria are dominant microbes in microbiome, but apart from it, fungus, virus, parasites and protozoans also play substantial role in numerous activities. Interestingly, fungal species cover up only 0.1% of total microbiome composition, but they maintain their balance with other microbes through their size and help in adjusting health and disease conditions [1] [2].

Fungi are ubiquitous organisms in environment from ancient times and they pave their way in human body either by inhalation or though ingestion along with food. Numerous studies have supported that fungal microbes hold the ability to colonize human body through specific interactions which can establish them either as commensals or pathogens. Commensal fungi bring about various essential functions such as maintenance of immune system through homeostasis, interaction with bacteriome, mediation of disease susceptibilities and much more [3] [4]. Moreover, Researchers are still diving to understand the importance of mycobiome. Figure 1 emphasizes the presence of commensal fungi across numerous body-sites in humans.

Communication between commensal fungi and immune system shows that both stems of immune system keep a check on the number of fungi, induce immune tolerance, and prevent disease susceptibility. However, disruption of balance between host and commensal can promote the development of diseases like Alzheimer, Parkinson, autoimmune disease and other pathological conditions [4] [5].

Commensal fungi antigens are continuously sampled by immune system and presented to adaptive immune system that can mount local and systemic response. Local and systemic responses are often generated via adaptive immune system when the antigens from commensal fungi are presented through immune system [6]. Host-commensal fungi immune interactions can provoke the chances of autoimmunity through various mechanisms such as cross-reactive T and B-cells, epitope spreading and through by-stander activation. In autoimmune response T and B cell cross-reactivity becomes a major concern**.** This study marks the examination of sequences from commensal fungi in stimulation of T-cells and B-cells showing cross-reactivity with human antigens**.** [7].

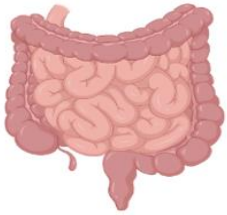


ORAL

Candida spp
Cladosporium spp.
Aspergillus spp.
Saccharomyces spp
Fusarium spp.
Aureobasidium spp
Penicillium spp
Pichia spp

Aspergillus spp.
Candida spp.
Clavispora spp.
Cladosporium spp.
Pneumocystis spp.
Wallemia spp

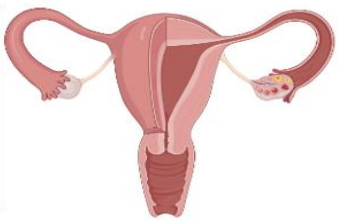
LUNGS



INTESTINE

Candida spp.
Saccharomyces spp.
Fusarium spp.
Penicillium spp.
Pichia spp.
Cladosporium spp.
Malassezia spp.
Cyberlindnera jadinii
Aspergillus

Candida spp.
Cladosporium spp.
Pichia spp.
Aspergillus spp.
Rhodotorula spp



VAGINAL

SKIN

Malassezia spp.
Aspergillus spp.
Candida spp.

Fig 1.1 | Commensal fungi across various regions of the body

1.1 Literature Review

1.1.1 Autoimmunity

The body generally distinguishes between one's own auto-antigens and exogenous non-self-antigens and therefore does not initiate an immunologic attack against itself. Autoimmune reactions represent a situation in which immunologically competent host cells or antibodies attack on auto-antigens, causing functional or structural damage [8]. Autoimmune disorders are caused by autoantibodies in case of Hashimoto's thyroiditis or due to the presence of self-reactive T-cells that primarily destroy self-proteins as in case of rheumatoid arthritis. Self-reactive B and T lymphocytes, cause destruction when stimulated by environmental or genetic factors. The development of autoimmune disease is influenced by three elements such as hormonal, viral, and genetic. All these elements can influence gene expression, which can either directly or indirectly interact with essential immunoregulatory functions. Autoimmune disorders are frequently classified as systemic or organ-specific, based on whether they impact a particular organ or numerous systems in the host [9]. Another form of classification incorporates the immunological component that is responsible for the majority of the damage such as T cells and antibodies. The majority of autoimmune disorders are polygenic, which means that afflicted people inherit numerous genetic variants that contribute to disease. Autoimmunity develops as a result of a mix of genetic and environmental factors. MHC genes makes the biggest contribution; additional genes are thought to impact the recruitment of auto-reactive B and T-cells. Infections caused by fungi can influence the development as well as worsening of autoimmunity. Furthermore, can increase the possibility of autoimmunity through a range of processes, that includes increased expression of co-stimulators in organs and cross-reactivity between auto-antigens and microbial antigens [10].

1.1.2 Fungi as Mycoflora

As fungus make up such a small percentage of the overall commensal organisms in humans, they have received far less attention. The majority of these fungi are also unculturable. As a result, fungal mycoflora studies are critical for describing these fungi and paving the way for the study of the "good fungi." In addition, several fungal pathogens are “pathobionts,” which are harmless in normal mode but have virulence factors. Fungi, aren't all bad. Some are beneficial to the body's complex workings, while others are beneficial in small amounts but harmful if they become too numerous or abundant [11]. They only make up 0.1 percent of

host microbial species, however they make up for it by being bigger. Irritable bowel syndrome (IBS), as well as inflammatory bowel diseases (IBDs) including Crohn's disease (CD) and ulcerative colitis, have all been linked to fungi. *Saccharomyces* fungus in the gut are advantageous to host health in a variety of ways—healthy persons have enough of these fungus, and those with severe gut-related health disorders like Crohn's disease possess few. *Candida albicans* has been linked to a variety of serious health problems, including irritable bowel syndrome, weight reduction resistance, and systemic infections. They cause no damage and might be beneficial when their populations are kept low. Mycobiome research is presently concentrated on the fungus found in healthy and diseased conditions, and much more work is needed to understand how the mycobiome interact with the host [12] [13].

1.1.3 Commensal fungi and Immunity

Fungus Pathogen associated molecular patterns (PAMPS) are responsible to elicit the immune reaction by interacting with host Pathogen recognition receptors PRRs. Various receptors like dectin-1, dectin-2, toll-like receptors (TLRs) regulate the cross-talk essential for maintaining immune homeostasis. Expression of these receptors occur at distal sites where the interaction with fungi is maximum and includes, gastro-intestinal tract, mucosal cells in respiratory tract, skin etc [13],[14]. Dectin-1, expressed on subsets of myeloid originated cells, specifically recognize fungal β-glucans and trigger the release of cytokines and reactive-oxygen species (ROS) against fungi. However, dectin-1 in genetically prone mice causes autoimmune arthritis due to production of T-lymphocytes [15]. Dectin-2, binds with mannans present in cell wall of fungi and stimulate pro-inflammatory cytokine response that can clear pathogenic fungi [16]. Apart from these, TLRs also hold a significant role, they not only recognize cell wall components of fungi but also their genetic material and recruit several mechanisms to mount an immune response against them [17].

Along with commensal fungi adaptive immune system also co-evolves in order to generate either a protective or non-protective immunity against fungi. Nevertheless, it has been reported that T-cell responses can be altered by fungi in order to induce fungal persistence. Different subsets of $CD4^+$ T cells get activated after encountering fungi such as TH1 cells which release cytokines to promote phagocytosis, antibodies production against fungal microbes, while TH2 subset responses prove to be deleterious as they inhibit the TH1 responses and promote the persistence of fungi. TH17 subsets gets activated in response to fungal infection through

MYD88 and SYK-CARD9 signalling pathways. Release of IL-17A from TH17 cells accelerate the mobility of neutrophils and defensins in response to fungal infection. Additionally, it has been proved that fungal TH17 cells can cross-react with C. albicans due to a phenomenon known as epitope mimicry [18], [19].

Immune tolerance to Commensal fungi can also be achieved through the regulatory T (Treg) cells, which promote immunosuppression by decreasing protective immune response and promoting fungal persistence. Thus, Balance between inflammation and tolerance induced by both innate and adaptive immunity towards fungi is regulated by Treg cells [20], [21].

1.1.4 Commensal fungi and Molecular mimicry

Many mechanisms are highlighted in numerous researches in which microbes like bacteria, viruses, fungus can induce autoimmunity in host. First, bystander activation, in which T cells get activated against numerous self-antigens due to increased circumstances of dispensation and presentation that further induces epitope spreading**.** Second, superantigens in which microbial proteins can bind to MHC class II α chain as well as with T-Cell Receptor (TCR) variable domain. Superantigens, regardless of their antigenic specificity, can activate a huge number of T-cells due to their unique binding capabilities. Superantigens can be either exogenous or endogenous. Third, Epitope mimicry employed by numerous microbes (commensals/pathogens) to evade the immune response or be tolerized by the host [22]. In epitope mimicry commensals show resemblance immunologically with host sequences and because of underlying insignificant differences in antigens between two of them, the immune response can be induced by commensal epitope against host antigens [23]. Apart from this, several other factors also govern induction and auto-immune response followed by disruption of tolerance. Probability of binding of a specific peptide to MHC after processing is unknown. T cells and B-cells exist against self-antigens. Further, B-cells can promote pathogenesis of autoimmune reactions as they can act as Antigen presenting Cell (APC) that can present the epitope of antigen (mimicry antigen) to T-cells and are also able to disrupt the balance of self-tolerance [24], [25].

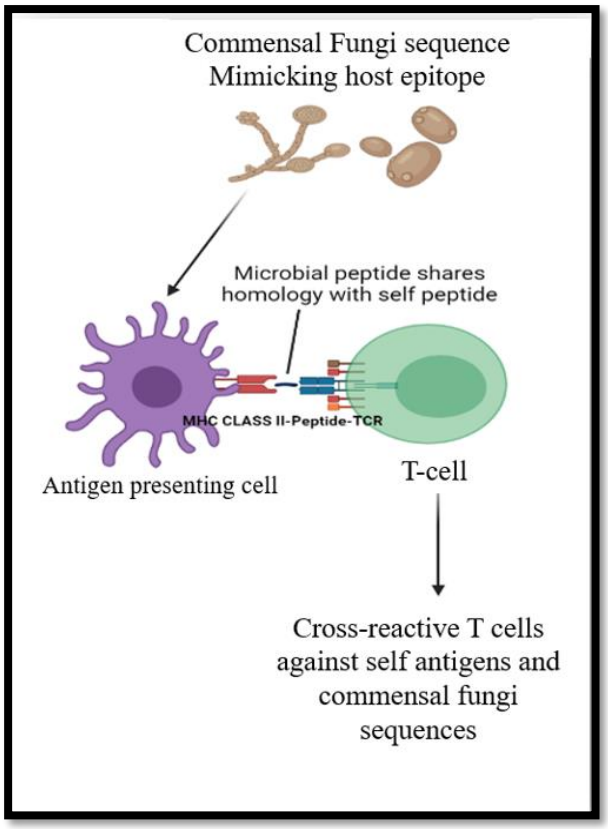


Figure 1.2 | Cross-reactivity of T-cells with commensal fungi and self-antigens as probable cause of auto-immune disease

During homeostasis mycobiota associated antigens are continuously recognized by the adaptive immune system**.** This process generates a pool of T-cells and B-cells that can cross-react with antigens of self-due to similarity between host and commensal fungi sequences. In case of aspergillus infection, it was found that antibodies against Aspergillus Noc2 peptide can cross-react with IFN-γ due to common epitope sequence [26]. Epitope mimicry becomes the root cause of major autoimmune disorders such as systemic lupus erythematosus, diabetes mellitus 1, rheumatoid arthritis, multiple sclerosis [27].

Huge number of antigens are presented by commensal fungi, it shapes antigen specific immune response after interacting with considerable range of human T-cells. Generally, a reasonable antigen-specific modulation of immune response is provided by cross-reactivity of mycobiota [28]. Therefore, settling protection of immune system in humans and pathology regarding specificity imparted by antigens is extremely important and association of prospective cross-reactivity with operational activity and specificity that is pathogenicity versus protection.

1.1.5 Treatment of Autoimmunity

Treatments cannot heal autoimmune disorders, but they could moderate the hyperactive immune reaction and lessen or eliminate inflammation and pain. Among the medications used to treat autoimmune disorders can be: usage of immunosuppressive and nonsteroidal anti-inflammatory drugs (NSAIDs). Another approach is blockage of co-stimulation of T cells so that they won't become activated due to absence of a costimulatory signal, resulting in anergy. Expanding the number of regulatory T cells is another key strategy which can induce immunosuppression. According to research, constant exposure to auto-antigens can boost the number of antigen-specific regulatory T cells, that could then suppress continuous autoimmune reactions Such self-antigens could be altered to efficaciously induce self-tolerance [9] [10].

CHAPTER 2

2. MATERIAL AND METHOD

2.1 Material

Databases used:

1. Immune Epitope and Database and Analysis Resource (IEDB)
2. Universal Protein Resource (UniProt)
3. Basic Local Alignment Search Tool (BLAST)
4. Protein Data Bank (PDB)

Software's used

1. Peptide structure prediction server (PEP-FOLD 3): To analyse Peptide structures
2. HawkDock: a server that employs structural modelling, using computational docking, predict and evaluate the protein-protein complex
3. MM/GBSA (molecular mechanics/ Generalized Born surface area): that can determine the absolute binding configuration of a protein-protein complex by predicting free binding energies.

Workflow

Selected specific epitopes of commensal fungi using

Immune Epitope and Database and Analysis Resource (IEDB)

↓

Protein sequence retrieval From Uniprot

↓

Prediction of B-cell epitopes through IEDB

↓

Prediction of MHC CLASS II T-Cell epitope through IEDB

↓

Basic Local Alignment Search Tool (BLAST)

To check similarity between Commensal fungi sequences and human auto-antigens

↓

Peptide Fold Prediction Server (Pep- fold 3)-RPBS

↓

Structure of MHC class II and TCR Retrieved from

Protein Data Bank

↓

Molecular Docking

2.2 Method:

Finding potential antigenic epitopes of commensal fungi: These epitopes can be associated with autoimmune diseases in humans. Antigenic epitopes of commensal fungi are searched in Immune Epitope and Database and Analysis Resource **(**IEDB) database (http://www.iedb.org/)

Protein sequence retrieval: Commensal fungi (*C. albicans, C. tropicalis* and *S. cerevisiae*) antigenic epitopes protein sequences was retrieved from Universal Protein Resource (Uniprot) (https://www.uniprot.org).

B-cell epitope prediction: IEDB (http://www.iedb.org/) predicts the likelihood of certain protein regions of commensal fungi binding to the B cell receptor.

T-Cell epitopes (Class II MHC): The IEDB MHC II prediction tool (http://tools.iedb.org/mhcii/result/) was used to analyze commensal fungi peptides binding to MHC class II molecules.

BLAST Search: blastp was performed in between Commensal fungi T-cell epitope sequences and human protein sequences to look for similarity in between the two (https://blast.ncbi.nlm.nih.gov)

Peptide structure prediction server (PEP-FOLD 3): Pep-fold 3 tool used to analyse peptide structures of T-cell epitopes of commensal fungi and are converted to PDB format

Protein Data Bank (PDB): Structure of MHC class II and T-cell receptor was retrieved from PDB (https://www.rcsb.org)

Molecular docking: For docking. the T-cell epitopes peptides in PDB format and the structure of class II MHC was used. HawkDock tool (http://cadd.zju.edu.cn/hawkdock/) has been employed for docking purpose. The output files were analysed to find the peptides with high binding affinity energy. Same procedure of molecular docking was performed with T-cell epitopes peptides and T-Cell Receptor.

Receptor -protein structure visualization: The peptide-receptor complex is visualized in HawkDock tool using 3djmol.s (http://cadd.zju.edu.cn/hawkdock/)

CHAPTER 3

3. RESULTS AND DISCUSSION

3.1 Potential antigenic epitopes of commensal fungi

Commensal fungi such as *Candida albicans*, *Candida tropicalis* and *Saccharomyces cerevisiae*. were reviewed from literature. These fungi were studied since they were found commonly as commensals in the normal human mycobiota.

Table 3.1 | Antigenic epitopes of commensal fungi

a. Candida albicans

Details	Epitope	Antigen	Organism
1068833	MIVENVPLL	Trafficking protein particle complex subunit	Candida albicans

b. Candida tropicalis

Details	Epitope	Antigen	Organism
1068773	LILENYMFA	PH domain-containing protein	Candida tropicalis

c. Saccharomyces cerevisiae

Details	Epitope	Antigen	Organism
1068767	LIIENAPLI	Ribosome quality control complex subunit 2	Saccharomyces cerevisiae (baker's yeast)

Above mentioned epitopes of commensal fungi (*Candida albicans*, *Candida tropicalis*, and *Saccharomyces cerevisiae*) were found to be associated with auto-immune diseases as obtained from Immune Epitope and Database and Analysis Resource (IEDB).

3.2 Antigenic Epitopes Protein Sequence retrieval

Protein sequences of antigenic epitopes of commensal fungi (*Candida albicans, Candida tropicalis,* and *Saccharomyces cerevisiae*) were searched in the UniProt database for further prediction of probable B and T cell epitopes.

a. *Candida albicans:* Trafficking protein particle complex subunit

MSNDDIILPSVSSLSKLTINDVSKSGFGYNPSIGPISNTITLESSSVLLNKRTISLTPTS
SDSIYDRNIITKKPHEINLSSLSFLFCEIISWAHSNSKGIQDLENRLNGLGYQIGQRYLE
LCKIREGFKNSKREIRLLEMLQFIHGPFWKLIFGKTANELEKSQDLPNEYMIVENVPLLN
RFISIPKEYGDLNCSAFVAGIIEGALDNSGFNADVTAHTVATDANPLRTVFLIKFDDSVL
IRESLRFG

b. *Candida tropicalis*: PH-domain containing protein

MATSPTSFHFEKQTILPSSDPKSPFFCNLPPYDTKPIDRLVEFFKYWKYFIKAILYYFKE
IVLVKELEANLNYQLISAVQFPGFKDLPPKILQDISINNGTNSPKASTPTNELKKTLSNS
SVSTVGTTSSDKRPGLFKQKSNGSNTSFLKAANPLHKRNVSLNSLRQVTTAVGAVAAPPT
PPQPPVPTNTLPPIPKLEPTSDVRIPETYFPDDSLYTNFPSMLLSSHQSAFNNSYKLSKE
LNTKLIPRLEMLLKQLSHKIKEIKTSLKNESFANDDLLKDISKTGQVLSAYMEAVELYSK
DIPVTKKCLSDGEEIGVLDDPLLVKLRVDYRLKNQLILENYMFASYINLQNISRDLFTYV
LKELTWVVDKFGKLNFNSEYYQFLKSKVSASSTQDWKYYISHNSCFVNTYESTPENPKRE
NRSVKSIVLPYTNSIHSKCIRFGILYKKSKLMKSYTRHYYVLSCNYLHEFRFDEDVNVAS
KKSKDKIGGFVGHDDEPLKSYNLNEYSISCKDSDGFKFVLTKNNNKSSKKTFKCATETDF
NNWFADLSDLLKFGNNHYERYSFVQKKVHLKKSYTLPEKRGGFKLELDNLSTPALTGMFT
PKIQTPKDSPTEENPFEGMLSDLKVHTASGTTPTETPSKMTPEGSSANLALDAQHRDYLK
LQQAFMKQQQEILDLKTKEAQTMELIQKKLENIQEQQSPYLGPARNSSDSLSSFVMPQQT
VHAAHQVISNHLQQHSDLPVNFDFGETDGNKTDQSVPTLLVSQDH

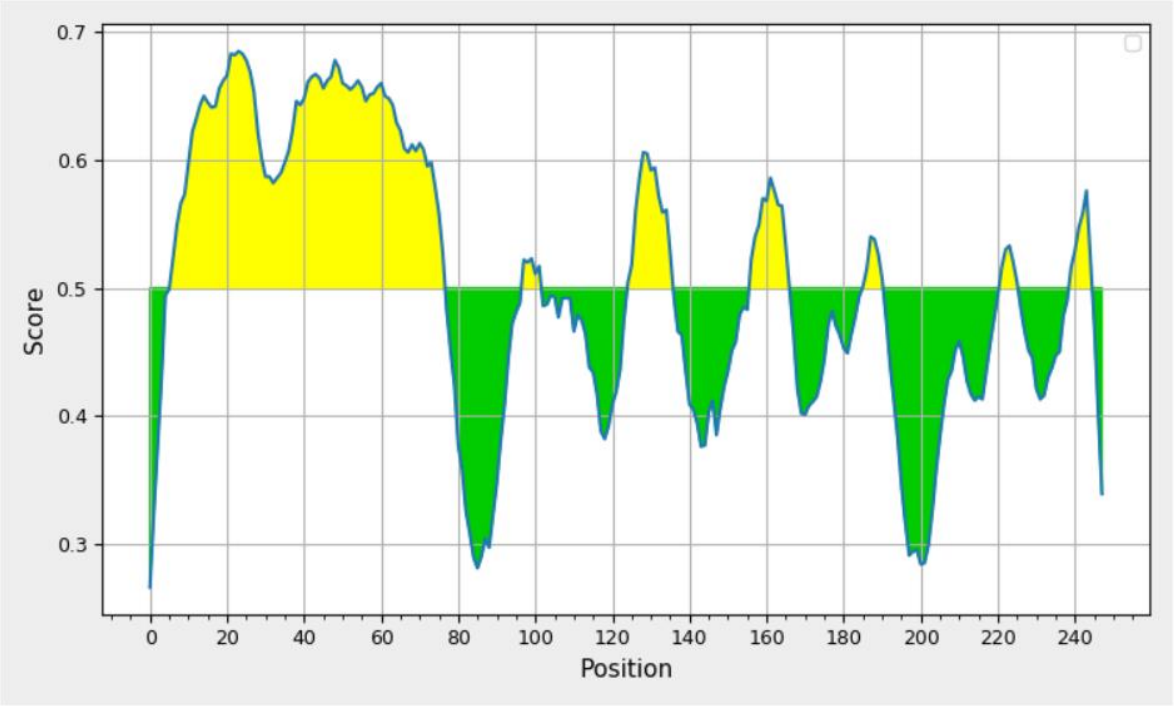
c. *Saccharomyces cerevisiae*: Ribosome quality control complex subunit 2

MKQRISALDLLLLARELKQDLEGYRLSNIYNIADSSKQFLLKFNKPDSKLNVVVDCGLRI
YLTEFSRPIPPTPSGFVVKLRKHLKAKRLTALKQVDQDRILVLQFADGHFYLVLEFFSAG
NVILLDENRRIMALQRVVLEHENKVGQIYEMFDESLFTTNNESADESIEKNRKAEYTSEL
VNEWIKAVQAKYESDITVIKQLNIQGKEGAKKKKVKVPSIHKLLLSKVPHLSSDLLSKNL
KVFNIDPSESCLNLLEETDSLAELLNSTQLEYNQLLTTTDRKGYILAKRNENYISEKDTA
DLEFIYDTFHPFKPYINGGDTDSSCIIEVEGPYNRTLDKFFSTIESSKYALRIQNQESQA
QKKIDDARAENDRKIQALLDVQELNERKGHLIIENAPLIEEVKLAVQGLIDQQMDWNTIE
KLIKSEQKKGNRIAQLLNLPLNLKQNKISVKLDLSSKELNTSSDEDNESEGNTTDSSSDS
DSEDMESSKERSTKSMKRKSNEKINVTIDLGLSAYANATEYFNIKKTSAQKQKKVEKNVG
KAMKNIEVKIDQQLKKKLKDSHSVLKKIRTPYFFEKYSWFISSEGFLVMMGKSPAETDQI
YSKYIEDDDIYMSNSFNSHVWIKNPEKTEVPPNTLMQAGILCMSSSEAWSKKISSSPWWC
FAKNVSKFDGSDNSILPEGAFRLKNENDQNHLPPAQLVMGFGFLWKVKTSGNEDNGDDDE
EEEEEEEEEEEEEEEEEEEEEEKEEEEKEEEQQQDEDDSNEVNGLEKGGDSNDSTKNNS
FEHDNLEKDIEKHCTISSDTDSDSGNAKAKNDNSSTQRILDEPGVPISLIENINSNVRGK
RGKLKKIQKKYADQDETERLLRLEALGTLKGIEKQQQRKKEEIMKREVREDRKNKREKQR
RLQALKFTKKEKARVNYDKHKSELKPSLDKGDVVDDIIPVFAPWPALLKYKYKVKIQPGS
AKKTKTLTEILHYFKSRPLDGSSTDNEMDWPQEHEMIKGLKEQDLVLLLCVDKLKVTIAG
QKSTKNGGNSSKKGKKKR

3.3 B-cell Epitope Prediction Results

IEDB database was used to analyse UniProt-retrieved commensal fungus protein sequences in order to anticipate possible B-cell epitopes that can bind to B-cells and stimulate auto-antibodies against self-antigens.

a. C. albicans



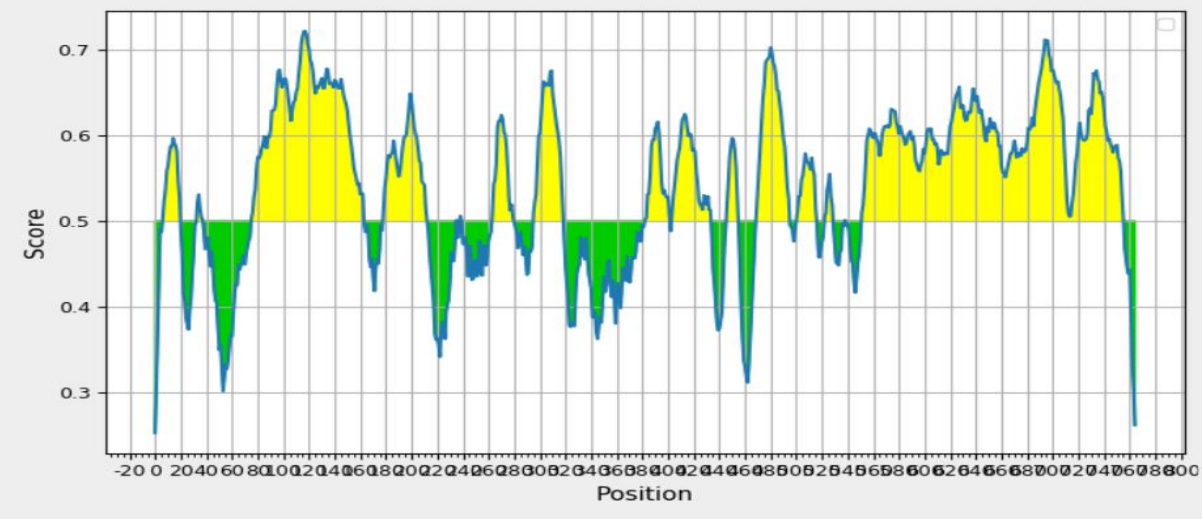
Average: 0.502 **Minimum:** 0.266 **Maximum:** 0.685

Table 3.2 | B-cell epitopes of *C. albicans*

Predicted peptides:

No.	Start	End	Peptide	Length
1	7	77	ILPSVSSLSKLTINDVSKSGFGYNPSIGPISNTITLESSSVLLNKRTISLTPTSSDSIYDRNIITKKPHEI	71
2	98	102	KGIQD	5
3	125	136	REGFKNSKREIR	12
4	157	166	ANELEKSQDL	10
5	186	191	PKEYGD	6
6	222	226	TDANP	5
7	240	245	LIRESL	6

b. C. tropicalis



Average: 0.540 **Minimum:** 0.252 **Maximum:** 0.722

Table 3.3 | B-cell epitopes of *C. tropicalis*

Predicted peptides:

No.	Start	End	Peptide	Length
1	7	20	SFHFEKQTILPSSD	14
2	34	38	TKPID	5
3	76	163	ISAVQFPGFKDLPPKILQDISINNGTNSPKASTPTNELKKTLSNSSVSTVGTTSSDKRPGLFKQKSNGSNTSFLKAANPLHKRNVSLN	88
4	179	213	PTPPQPPVPTNTLPPIPKLEPTSDVRIPETYFPDD	35
5	236	236	K	1
6	239	239	K	1
7	264	280	KTSLKNESFANDDLLKD	17
8	296	319	ELYSKDIPVTKKCLSDGEEIGVLD	24
9	383	402	FLKSKVSASSTQDWKYYISH	20
10	404	434	SCFVNTYESTPENPKRENRSVKSIVLPYTNS	31
11	447	455	KKSKLMKSY	9
12	470	495	FRFDEDVNVASKKSKDKIGGFVGHDD	26
13	501	516	YNLNEYSISCKDSDGF	16
14	523	529	NNNKSSK	7
15	539	539	D	1
16	552	756	KFGNNHYERYSFVQKKVHLKKSYTLPEKRGGFKLELDNLSTPALTGMFTPKIQTPKDSPTEENPFEGHLSDLKVHTASGTTPTETPSKMTPEGSSANLALDAQHRDYLKLQQAFMKQQQEILDLKTKEAQTMELIQKKLENIQEQQSPYLGPARNSSDSLSSFVMPQQTVHAAHQVISNHLQQHSDLPVNFDFGETDGNKTDQSV	205

c. S. cerevisiae

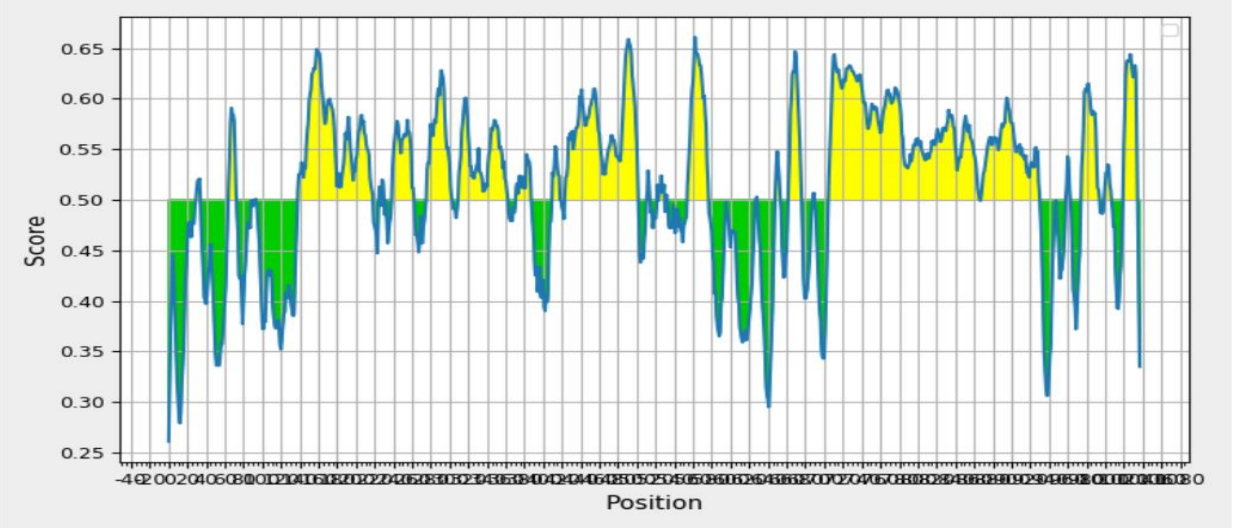


Table 3.4 | B-cell epitopes of *S. cerevisiae*

Predicted peptides:

No.	Start	End	Peptide	Length
1	31	34	NIAD	4
2	65	73	FSRPIPPTP	9
3	91	91	A	1
4	94	94	Q	1
5	139	219	LEHENKVGQIYEMFDESLFTTNNESADESIEKNRKAEYTSELVNEWIKAVQAKYESDITVIKQLNIQGKEGAKKKKVKVPS	81
6	226	226	S	1
7	229	231	PHL	3
8	240	263	LKVFNIDPSESCLNLLEETDSLAE	24
9	276	303	LTTTDRKGYILAKRNENYISEKDTADLE	28
10	310	363	HPFKPYINGGDTDSSCIIEVEGPYNRTLDKFFSTIESSKYALRIQNQESQAQKK	54
11	372	388	DRKIQALLDVQELNERK	17
12	411	418	DQQMDWNT	8
13	424	502	KSEQKKGNRIAQLLNLPLNLKQNKISVKLDLSSKELNTSSDEDNESEGNTTDSSSDSDSEDMESSKERSTKSMKRKSNE	79
14	512	515	LSAY	4
15	523	523	N	1
16	525	530	KKTSAQ	6
17	533	533	K	1
18	555	577	KKKLKDSHSVLKKIRTPYFFEKY	23
19	629	629	E	1
20	649	654	WSKKIS	6
21	663	676	KNVSKFDGSDNSIL	14
22	689	690	QN	2
23	706	867	KVKTSGNEDNGDDDEEEEEEEEEEEEEEEEEEEEEEEEKEEEEKEEEQQQDEDDSNEVNGLEKGGDSNDSTKNNSFEHDNLEKDIEKHCTISSDTDSDSGNAKAKNDNSSTQRILDEPGVPISLIENINSNVRGKRGKLKKIQKKYADQDETERLLRLEALG	162
24	869	931	LKGIEKQQQRKKEEIMKREVREDRKNKREKQRRLQALKFTKKEKARVNYDKHKSELKPSLDKG	63
25	959	963	GSAKK	5
26	976	995	SRPLDGSSTDNEMDWPQEHE	20
27	1000	1007	LKEQDLVL	8
28	1021	1035	QKSTKNGGNSSKKGK	15

The B-cell epitopes of the commensal fungus *C. albicans, C. tropicalis*, and *S. cerevisiae*, which might operate as potential mediators of autoimmunity, were predicted using the Immune Epitope and Database and Analysis Resource (IEDB) and were further analysed for T-cell epitope prediction.

3.4 T-cell epitope Prediction results

Potential B-cell epitope of commensal fungus, which was then utilised to predict T-cell epitopes using the IEDB database to further verify if these T-cell epitopes might bind to MHC and TCR. Possible T-cell epitopes that can bind to TCR and class II MHC have the potential to activate auto-immunity against self-antigens in the host.

a. *C. albicans*

Table 3.5 | T-cell epitopes of *C. albicans*

Seq #	Peptide start	Peptide end	Peptide sequence	Consensus percentile rank	Allele
1	32	46	NTITLESSSVLLNKR	8.5	HLA-DPA1*03:01/DPB1*04:02
1	25	39	PSIGPISNTITLESS	6.6	HLA-DQA1*01:02/DQB1*06:02
1	32	46	NTITLESSSVLLNKR	6.8	HLA-DQA1*01:02/DQB1*06:02
1	39	53	SSVLLNKRTISLTPT	5.4	HLA-DRB1*03:01
1	32	46	NTITLESSSVLLNKR	9.6	HLA-DRB1*03:01
1	1	15	ILPSVSSLSKLTIND	7.8	HLA-DRB1*04:01
1	39	53	SSVLLNKRTISLTPT	6.4	HLA-DRB1*08:02
1	1	15	ILPSVSSLSKLTIND	7.1	HLA-DRB1*11:01
1	39	53	SSVLLNKRTISLTPT	8.7	HLA-DRB1*11:01
1	57	71	SIYDRNIITKKPHEI	9	HLA-DRB1*13:02
1	8	22	LSKLTINDVSKSGFG	2.1	HLA-DRB3*02:02
1	39	53	SSVLLNKRTISLTPT	3.8	HLA-DRB3*02:02
1	18	32	KSGFGYNPSIGPISN	4.7	HLA-DRB3*02:02

b. C. tropicalis

Table 3.6 | T-cell epitopes of *C. tropicalis*

Seq #	Peptide start	Peptide end	Peptide sequence	Consensus percentile rank	Allele
1	2	16	FGNNHYERYSFVQKK	8.7	HLA-DPA1*01:03/DPB1*02:01
1	112	126	QAFMKQQQEILDLKT	5.3	HLA-DPA1*02:01/DPB1*01:01
1	103	117	QHRDYLKLQQAFMKQ	9.8	HLA-DPA1*02:01/DPB1*01:01
1	103	117	QHRDYLKLQQAFMKQ	1.2	HLA-DPA1*02:01/DPB1*05:01
1	112	126	QAFMKQQQEILDLKT	9.6	HLA-DPA1*03:01/DPB1*04:02
1	157	171	SDSLSSFVMPQQTVH	9.8	HLA-DQA1*01:01/DQB1*05:01
1	89	103	MTPEGSSANLALDAQ	5.9	HLA-DQA1*01:02/DQB1*06:02
1	66	80	EGMLSDLKVHTASGT	7.8	HLA-DQA1*01:02/DQB1*06:02
1	89	103	MTPEGSSANLALDAQ	8.2	HLA-DQA1*05:01/DQB1*03:01
1	103	117	QHRDYLKLQQAFMKQ	3.9	HLA-DRB1*01:01
1	31	45	GFKLELDNLSTPALT	7.7	HLA-DRB1*01:01
1	66	80	EGMLSDLKVHTASGT	3.6	HLA-DRB1*03:01
1	31	45	GFKLELDNLSTPALT	2	HLA-DRB1*04:01
1	41	55	TPALTGMFTPKIQTP	9.2	HLA-DRB1*04:01
1	15	29	KKVHLKKSYTLPEKR	5.5	HLA-DRB1*04:05
1	167	181	QQTVHAAHQVISNHL	8.5	HLA-DRB1*07:01
1	41	55	TPALTGMFTPKIQTP	6.8	HLA-DRB1*09:01
1	103	117	QHRDYLKLQQAFMKQ	6.15	HLA-DRB1*12:01
1	15	29	KKVHLKKSYTLPEKR	2.5	HLA-DRB1*15:01
1	103	117	QHRDYLKLQQAFMKQ	9.8	HLA-DRB1*15:01
1	96	110	ANLALDAQHRDYLKL	2.7	HLA-DRB3*01:01
1	136	150	QKKLENIQEQQSPYL	1.9	HLA-DRB4*01:01
1	103	117	QHRDYLKLQQAFMKQ	5.6	HLA-DRB4*01:01
1	112	126	QAFMKQQQEILDLKT	6.2	HLA-DRB4*01:01
1	174	188	HQVISNHLQQHSDLP	6.9	HLA-DRB4*01:01
1	129	143	AQTMELIQKKLENIQ	8.2	HLA-DRB4*01:01
1	103	117	QHRDYLKLQQAFMKQ	0.21	HLA-DRB5*01:01

c. S. cerevisiae

Table 3.7 | T-cell epitopes of *S. cerevisiae*

Seq #	Peptide start	Peptide end	Peptide sequence	Consensus percentile rank	Allele
1	147	161	ADQDETERLLRLEAL	6.9	HLA-DPA1*03:01/DPB1*04:02
1	4	18	TSGNEDNGDDDEEEE	8.4	HLA-DQA1*03:01/DQB1*03:02
1	119	133	GVPISLIENINSNVR	3.7	HLA-DRB1*04:01
1	119	133	GVPISLIENINSNVR	5.9	HLA-DRB1*04:05
1	119	133	GVPISLIENINSNVR	9.5	HLA-DRB1*08:02
1	135	149	KRGKLKKIQKKYADQ	1.3	HLA-DRB1*11:01
1	119	133	GVPISLIENINSNVR	5.7	HLA-DRB1*12:01
1	135	149	KRGKLKKIQKKYADQ	9.8	HLA-DRB1*12:01
1	119	133	GVPISLIENINSNVR	0.37	HLA-DRB1*13:02
1	72	86	KNNSFEHDNLEKDIE	8.1	HLA-DRB3*01:01
1	119	133	GVPISLIENINSNVR	2.1	HLA-DRB3*02:02
1	128	142	INSNVRGKRGKLKKI	4.6	HLA-DRB5*01:01
1	119	133	GVPISLIENINSNVR	8.6	HLA-DRB5*01:01

Above mentioned results indicate that the IEDB database predicted particular T-cell epitopes from the commensal fungus *Candida albicans, Candida tropicalis*, and *Saccharomyces cerevisiae* protein sequence that can cross-react with self-antigens, if similarity with human protein sequences were discovered.

3.5 BLAST Results

T-cell epitope Protein sequences of *C. albicans, C. tropicalis* and *S. cerevisiae* retrieved from IEDB and further were analysed for their homology with human sequences, since these results may give the basis for the cross-reactivity of B and T-cells with human self-antigens

a. C. albicans

Antigen- Trafficking protein particle complex subunit
Role in vesicular transport of proteins

Table 3.8 | BLAST result of *C. albicans*

Peptide Sequence	**Similarity with Human Sequences**	**Percent identity**	**Function**
PSIGPISNTITLESS	lanosterol synthase, partial	88.89%	Lipid biosynthesis, Lipid metabolism, Steroid biosynthesis
SSVLLNKRTISLTPT	metal regulatory transcription factor 1 isoform X2	64.29%	maintain metal homeostasis
SIYDRNIITKKPHEI	KIAA1504 protein, partial	52.94%	Involved in cell structure and cell signalling
LSKLTINDVSKSGFG	immunoglobulin light chain variable region, partial	76.92%	binds to antigen
KSGFGYNPSIGPISN	hCG1790759, isoform CRA_b, partial	100%	Aids in the regulation of the corpus luteum.

b. ***C. tropicalis***

Antigen- PH domain-containing protein

Ability to bind inositol phosphates, and various

Table 3.9 | BLAST result of *C. tropicalis (*Continued)

Peptide Sequence	Similarity with Human Sequences	Percent identity	Function
FGNNHYERYSFVQKK	immunoglobulin light chain junction region	85.71%	makes up a part of antibody light chain
QHRDYLKLQQAFMKQ	DOCK6 protein, partial	55%	regulate GTPases specifically during development of the limbs, skull, and heart, fibers (axons)
SDSLSSFVMPQQTVH	immunoglobulin heavy chain junction region	85.71%	makes up a part of antibody heavy chain
MTPEGSSANLALDAQ	thrombin factor II, partial	75%	maintain blood coagulation
EGMLSDLKVHTASGT	cullin-4A isoform 4	87.5%	Involved in haematopoiesis and DNA repair
GFKLELDNLSTPALT	hypothetical rhabdomyosarcoma antigen MU-RMS-40.3, partial	77.78%	Antigen for tumor cancer (skeletal muscle cell)
TPALTGMFTPKIQTP	Similar to somatostatin receptor 2, partial	87.5%	Produced in hypothalamus, pancreas, digestive system
KKVHLKKSYTLPEKR	Seven transmembrane helix receptor	100%	Transmitt information initiated by signals and binds to epinephrine

QQTVHAAHQVISNHL	Ig kappa light chain (VJ), partial	80%	Makes up a part of antibody light chain
ANLALDAQHRDYLKL	hCG1783598, partial	61.54%	Establish and maintain early pregnancy
QKKLENIQEQQSPYL	ERC1 protein, partial	80%	RIMs are active zone proteins that regulate neurotransmitter release
HQVISNHLQQHSDLP	epididymis tissue protein Li 176	87.5%	regulation of blood coagulation
AQTMELIQKKLENIQ	dynein heavy chain 12, axonemal isoform X8	44%	microtubule-based movement

c. S. cerevisiae

Antigen- Ribosome quality control complex subunit 2

Involved in Ubiquitination of proteins

Table 3.10 | BLAST Results of *S. cerevisiae*

Peptide Sequence	Similarity with Human Sequences	Percent identity	Function
ADQDETERLLRLEAL	Crystal structure of the human glial fibrillary acidic protein 1B domain	75%	Involved in mitosis, and signaling
TSGNEDNGDDDEEEE	sodium/potassium/calcium exchanger 3 precursor	75%	Transport's potassium, calcium and sodium
GVPISLIENINSNVR	golgin subfamily A member 6-like protein 9 isoform X2	76.92%	They may have roles in membrane traffic and Golgi structure
KRGKLKKIQKKYADQ	Chain SI, 40S ribosomal protein S8	75%	structural constituent of ribosome
KNNSFEHDNLEKDIE	caspase activity and apoptosis inhibitor 1 isoform 2	87.5%	Protein that has Anti-apoptotic activity
INSNVRGKRGKLKKI	GPATC2 protein, partial	87.5%	Involved in glycerolipid synthesis

BLAST tool was implied to find similarity between commensal fungus and human sequences, such as in the case of *C.albicans*, only 5 of the 7 T-cell epitopes showed similarity with human auto-antigens. Only 13 of the 18 T-cell epitopes found in *C.tropicalis* shared similarity with human auto-antigens. All T-cell epitopes from *S.cerevisiae* showed similarity with human auto-antigens. These findings shows that antigen-specific memory B and T cells can respond to self-antigens owing to sequence similarity of commensal fungi antigenic epitopes with human sequences, resulting in autoimmune disorders.

3.6 Peptide structure prediction server (Pep-fold 3) Results

T-cell epitope structures of *Candida albicans*, *Candida tropicalis*, and *Saccharomyces cerevisiae* were obtained using PEP-FOLD 3 for molecular docking and to validate if they could bind to MHC Class II as well as with TCR.

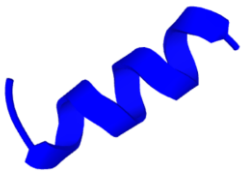
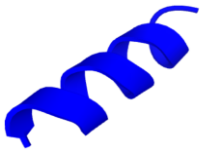
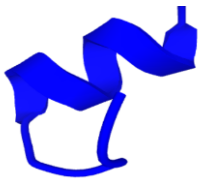
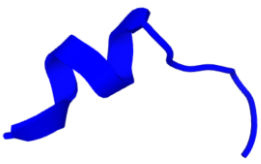
a. C. albicans

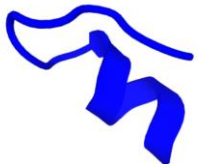
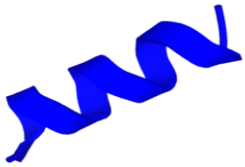
Table 3.11 | Structures of *C. albicans* T-cell epitopes

PEPTIDE SEQUENCE	STRUCTURE
PSIGPISNTITLESS	
SSVLLNKRTISLTPT	
SIYDRNIITKKPHEI	
LSKLTINDVSKSGFG	
KSGFGYNPSIGPISN	

b. C. tropicalis

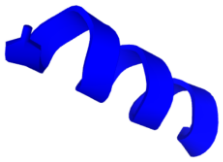
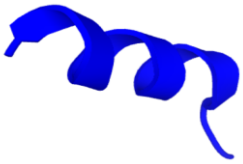
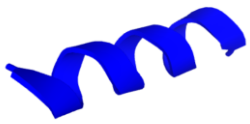
Table 3.12 | Structures of *C. tropicalis* T-cell epitopes (Continued)

PEPTIDE SEQUENCE	STRUCTURE
FGNNHYERYSFVQKK	
QHRDYLKLQQAFMKQ	
SDSLSSFVMPQQTVH	
MTPEGSSANLALDAQ	
EGMLSDLKVHTASGT	
GFKLELDNLSTPALT	

TPALTGMFTPKIQTP	
KKVHLKKSYTLPEKR	
QQTVHAAHQVISNHL	
ANLALDAQHRDYLKL	
QKKLENIQEQQSPYL	
HQVISNHLQQHSDLP	
AQTMELIQKKLENIQ	

c. S. cerevisiae

Table 3.13 | Structures of *S. cerevisiae* T-cell epitopes

PEPTIDE SEQUENCE	STRUCTURE
ADQDETERLLRLEAL	
TSGNEDNGDDDEEEE	
GVPISLIENINSNVR	
KRGKLKKIQKKYADQ	
KNNSFEHDNLEKDIE	
INSNVRGKRGKLKKI	

PEP-fold 3 Server was applied to obtain the structures of commensal fungi T-cell epitopes mentioned above. This was utilised for molecular docking with Class II MHC and TCR in the subsequent steps.

3.7 Structure Retrieval

Structure of MHC class II and TCR Retrieved from Protein Data Bank for molecular docking

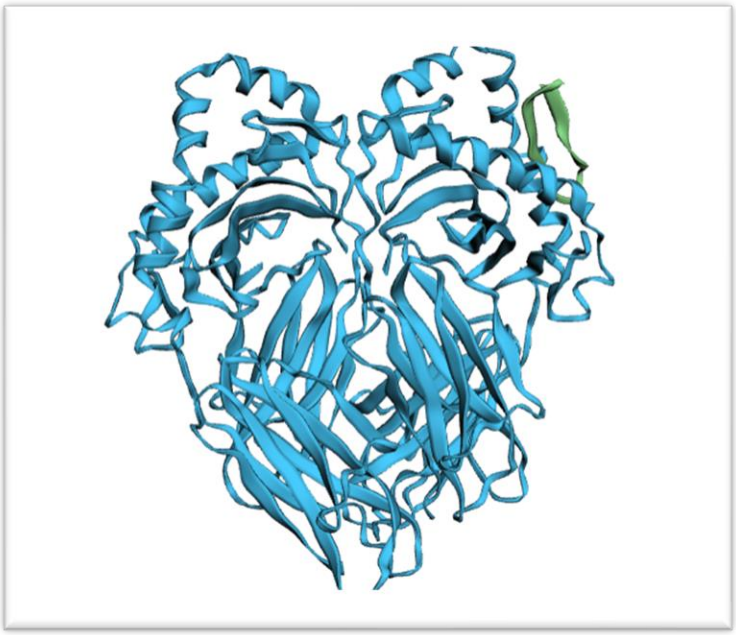


Fig 3.1 | MHC CLASS II Complexed with peptide

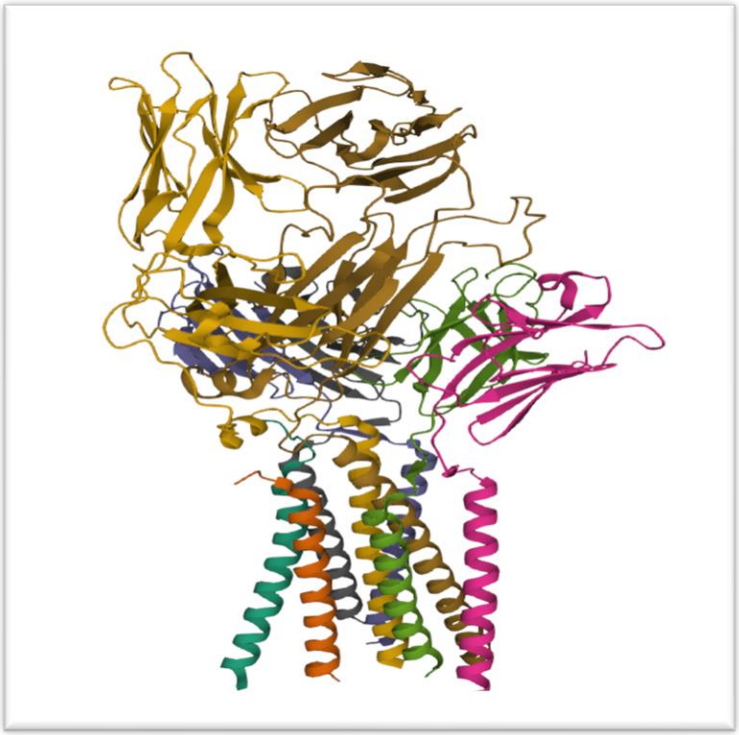


Fig 3.2 | T- CELL RECEPTOR

3.8 Molecular Docking

The HawkDock programme was used to perform molecular docking between commensal fungi T-cell epitopes (antigenic peptides) and class II MHC in order to find possible epitopes that can bind to Class II MHC expressed on B-cells, macrophages, and dendritic cells. Peptides can be only presented to T-cells when they bind to Class II MHC.

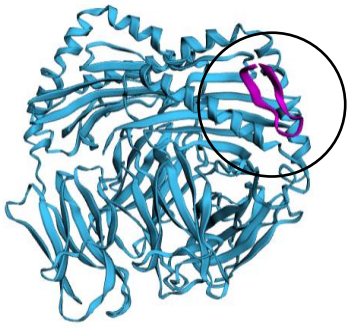
3.8.1 Peptide- CLASS II MHC Docking

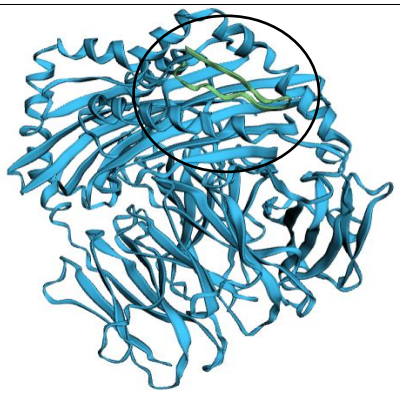
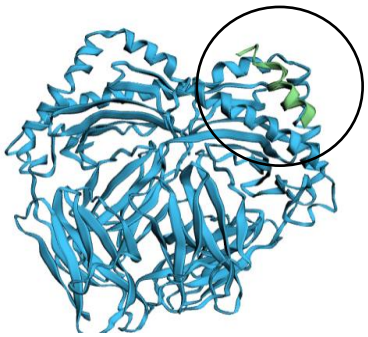
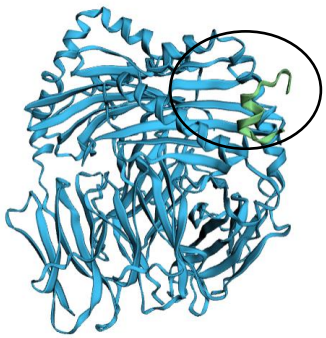
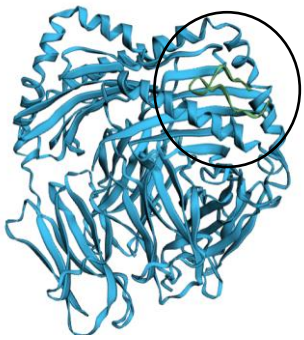
a. C. albicans

Table 3.14 | Peptide- CLASS II MHC Docking of *C. albicans*

S.NO	Amino acid Residue	Peptide Sequence	VDW	ELE	GB	SA	TOTAL (kcal/ mol)
1	15	PSIGPISNTITLESS	-64.02	190.1	-149.49	-9.06	-32.47
2	15	SSVLLNKRTISLTPT	-85.36	687.17	731	-11.61	-53.14
3	15	SIYDRNIITKKPHEI	-65.77	-298.83	340.58	-9.38	-33.41
4	15	LSKLTINDVSKSGFG	-58.64	-379.43	432.03	-8.34	-14.38
5	15	KSGFGYNPSIGPISN	-69.41	-348.48	381.04	-9.69	-46.55

Table 3.15 | *C. albicans* Peptide- CLASS II MHC Docking structures (Continued)

PEPTIDE SEQUENCE	STRUCTURE
1. PSIGPISNTITLESS	

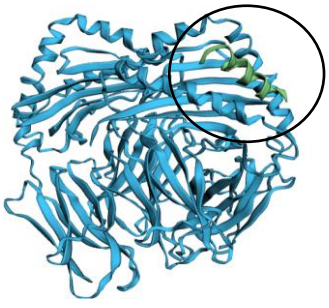
2. SSVLLNKRTISLTPT	
3. SIYDRNIITKKPHEI	
4. LSKLTINDVSKSGFG	
5. KSGFGYNPSIGPISN	

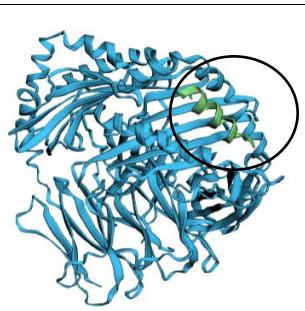
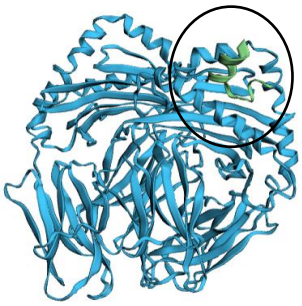
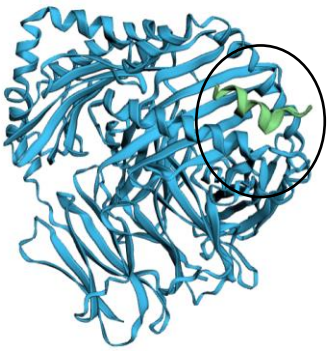
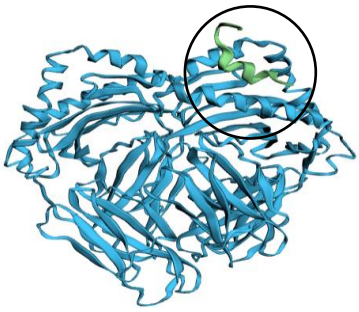
b. C. tropicalis

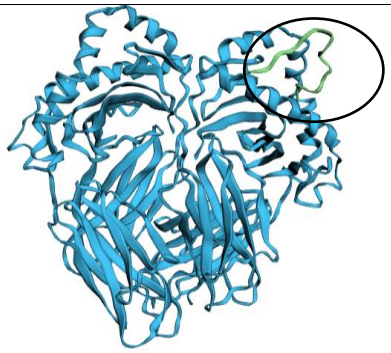
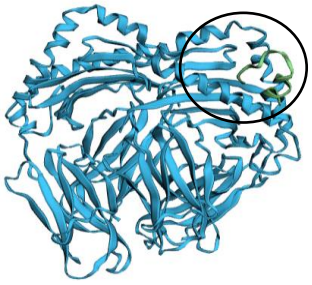
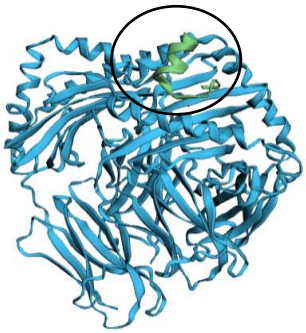
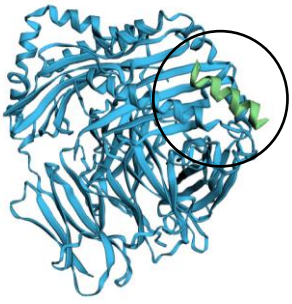
Table 3.16 | Peptide- CLASS II MHC Docking of *C. tropicalis*

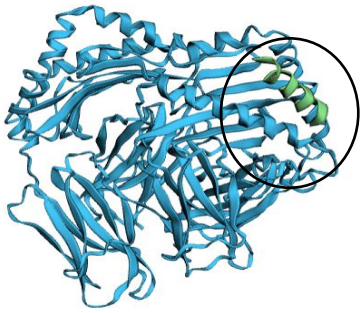
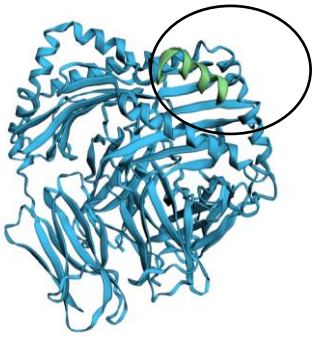
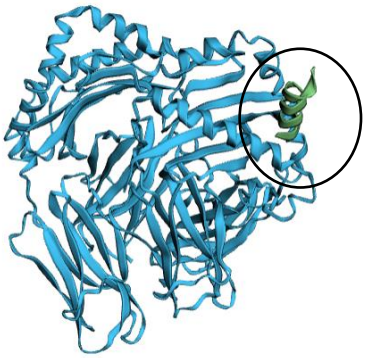
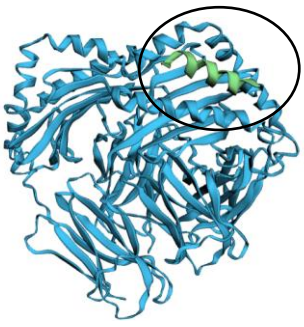
S.NO	Amino acid Residue	Peptide Sequence	VDW	ELE	GB	SA	TOTAL (kcal/ mol)
1	15	FGNNHYERYSFVQKK	-80.33	-484.64	537.96	-10.42	-37.42
2	15	QHRDYLKLQQAFMKQ	-74.59	-390.17	443.39	-10.35	-31.72
3	15	SDSLSSFVMPQQTVH	-72.97	162.04	-127.17	-9.97	-48.06
4	15	MTPEGSSANLALDAQ	-68.64	420.74	-381.44	-9.39	-38.73
5	15	EGMLSDLKVHTASGT	-53.48	-16.33	48.93	-7.61	-28.49
6	15	GFKLELDNLSTPALT	-78.7	109.03	-57.13	-11.7	-38.5
7	15	TPALTGMFTPKIQTP	-66.28	-449.2	459	-10.22	-66.7
8	15	KKVHLKKSYTLPEKR	-70.56	-1369.3	1381.11	-10.64	-69.39
9	15	QQTVHAAHQVISNHL	-65.78	-109.52	147.47	-8.96	-36.79
10	15	ANLALDAQHRDYLKL	-85	-204.11	261.55	-11.64	-39.2
11	15	QKKLENIQEQQSPYL	-44.09	-49.57	78.62	-6.41	-21.45
12	15	HQVISNHLQQHSDLP	-56.41	11.07	37.04	-7.56	-15.87
13	15	AQTMELIQKKLENIQ	-61.95	-251.94	300.34	-8.98	-22.54

Table 3.17 | *C. tropicalis* Peptide- CLASS II MHC Docking structures (Continued)

PEPTIDE SEQUENCE	STRUCTURE
1. FGNNHYERYSFVQKK	

2. QHRDYLKLQQAFMKQ	
3. SDSLSSFVMPQQTVH	
4. MTPEGSSANLALDAQ	
5. EGMLSDLKVHTASGT	

6. GFKLELDNLSTPALT	
7. TPALTGMFTPKIQTP	
8. KKVHLKKSYTLPEKR	
9. QQTVHAAHQVISNHL	

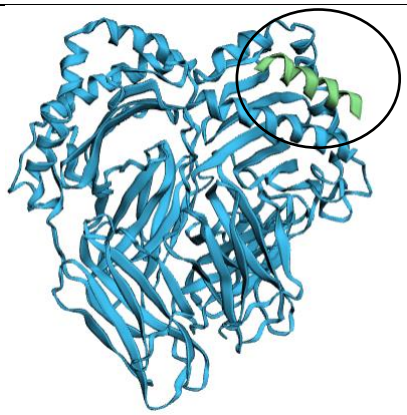
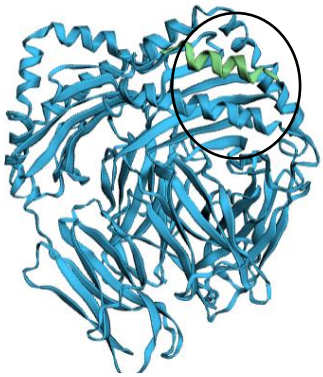
10. ANLALDAQHRDYLKL	
11. QKKLENIQEQQSPYL	
12. HQVISNHLQQHSDLP	
13. AQTMELIQKKLENIQ	

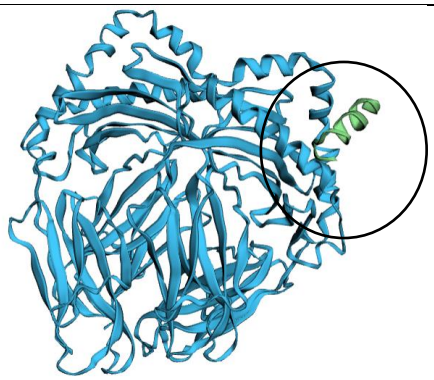
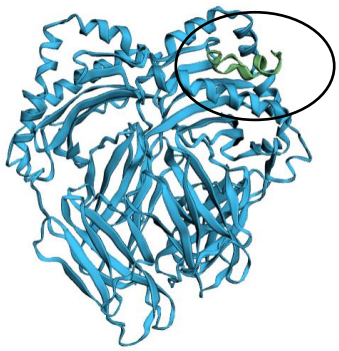
c. S. cerevisiae

Table 3.18 | Peptide- CLASS II MHC Docking of *S. cerevisiae*

S.NO	Amino acid Residue	Peptide Sequence	VDW	ELE	GB	SA	TOTAL (kcal/ mol)
1	15	ADQDETERLLRLEAL	-76.76	516.28	-474.61	-11.67	-46.77
2	15	TSGNEDNGDDDEEEE	-	-	-	-	-
3	15	GVPISLIENINSNVR	-63.51	-101.57	136.01	-9.29	-38.36
4	15	KRGKLKKIQKKYADQ	-	-	-	-	-
5	15	KNNSFEHDNLEKDIE	-50.69	485.49	-450.19	-8.62	-24.01
6	15	INSNVRGKRGKLKKI	-75.35	-1582.85	-1626.59	-10.57	-42.17

Table 3.19 | *S. cerevisiae* Peptide- CLASS II MHC Docking structures (Continued)

PEPTIDE SEQUENCE	STRUCTURE
1. ADQDETERLLRLEAL	
3. GVPISLIENINSNVR	

4. KNNSFEHDNLEKDIE	
6.INSNVRGKRGKLKKI	

The results of molecular docking of T-cell epitope peptides from *Candida albicans, Candida tropicalis,* and *Saccharomyces cerevisiae* with Class II MHC were significant: All 5 of *C. albicans* T-cell epitopes were reported to bind to Class II MHC. *C. tropicalis* T-cell epitopes bind to Class II MHC in all 13 cases. Out of the 6 T-cell epitopes found in S. cerevisiae, only 4 exhibit binding to Class II MHC. Their binding to Class II MHC, can serve potentially for presentation to T-cells and can stem to auto-immune disorders.

3.8.2 Peptide-TCR interactions

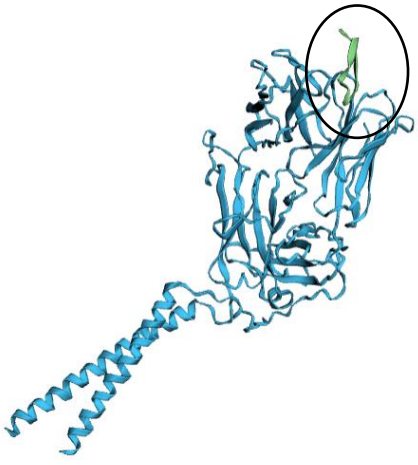
Furthermore, using the HawkDock programme, molecular docking was done between T-cell epitopes (antigenic peptides) of commensal fungus and TCR to discover possible epitopes that can bind to TCR on T cells. Due to molecular mimicry between commensal fungus antigenic epitopes and human sequences, peptide binding to TCR can cause auto-reactivity.

a. C. albicans

Table 3.20 | Peptide- TCR Docking of *C. albicans*

S.NO	Amino acid Residue	Peptide Sequence	VDW	ELE	GB	SA	TOTAL (kcal/ mol)
1	15	PSIGPISNTITLESS	-52.8	-37.77	66.9	-6.54	-30.21
2	15	SSVLLNKRTISLTPT	-	-	-	-	-
3	15	SIYDRNIITKKPHEI	-	-	-	-	-
4	15	LSKLTINDVSKSGFG	-	-	-	-	-
5	15	KSGFGYNPSIGPISN	-	-	-	-	-

Table 3.21 | *C. albicans* Peptide- TCR Docking structures (Continued)

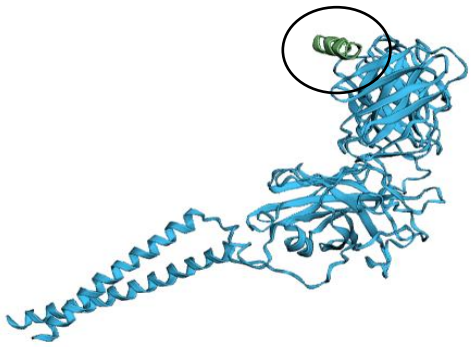
PEPTIDE SEQUENCE	STRUCTURE
1. PSIGPISNTITLESS	

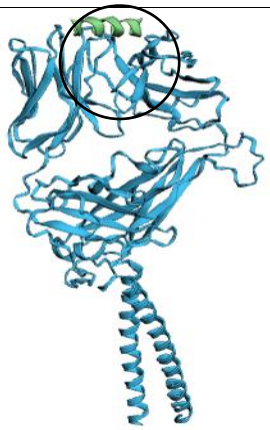
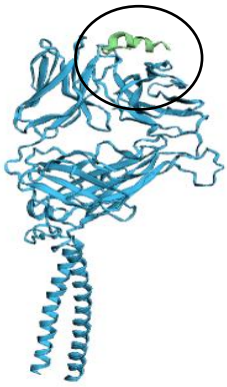
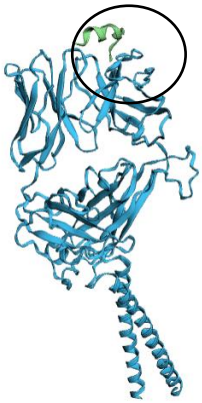
b. C. tropicalis

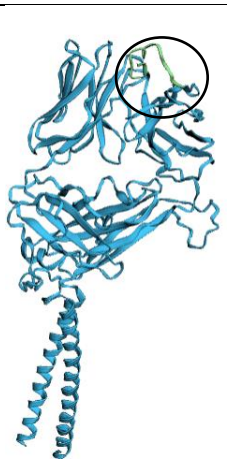
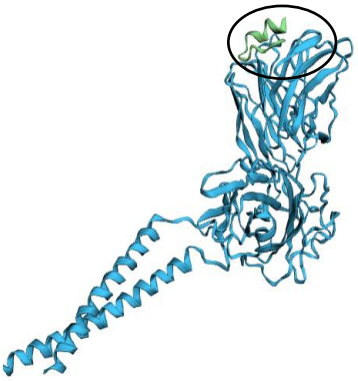
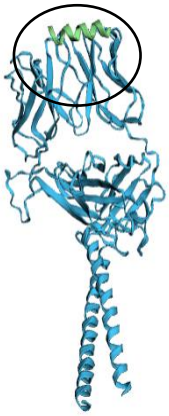
Table 3.22 | Peptide- TCR Docking of *C. tropicalis*

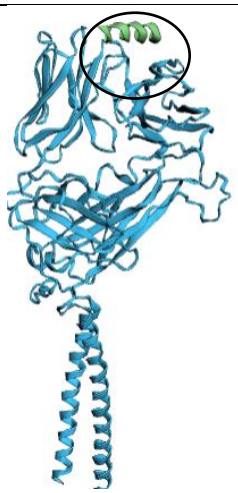
S.NO	Amino acid Residue	Peptide Sequence	VDW	ELE	GB	SA	TOTAL (kcal/ mol)
1	15	FGNNHYERYSFVQKK	-42.72	-124.11	160.95	-6.19	-12.07
2	15	QHRDYLKLQQAFMKQ	-53.12	-199.91	241.26	-7.11	-18.87
3	15	SDSLSSFVMPQQTVH	-	-	-	-	-
4	15	MTPEGSSANLALDAQ	-54.42	20.49	24.01	-7.64	-17.55
5	15	EGMLSDLKVHTASGT	43.3	-164.07	189.73	-7.26	-24.9
6	15	GFKLELDNLSTPALT	-38.06	-164.96	189.41	-6.51	-20.13
7	15	TPALTGMFTPKIQTP	-43.26	-132.79	153.42	-7.45	-30.08
8	15	KKVHLKKSYTLPEKR	-	-	-	-	-
9	15	QQTVHAAHQVISNHL	-46.98	-208.04	233.47	-7.23	-28.78
10	15	ANLALDAQHRDYLKL	-51.42	-84.63	126.94	-7.6	-16.7
11	15	QKKLENIQEQQSPYL	-	-	-	-	-
12	15	HQVISNHLQQHSDLP	-43.24	52.58	-13.52	-5.84	-10.02
13	15	AQTMELIQKKLENIQ	-	-	-	-	-

Table 3.23 | *C. tropicalis* Peptide- TCR Docking structures (Continued)

PEPTIDE SEQUNECE	STRUCTURE
1. FGNNHYERYSFVQKK	

2. QHRDYLKLQQAFMKQ	
4. MTPEGSSANLALDAQ	
5. EGMLSDLKVHTASGT	

6. GFKLELDNLSTPALT	
7. TPALTGMFTPKIQTP	
9. QQTVHAAHQVISNHL	

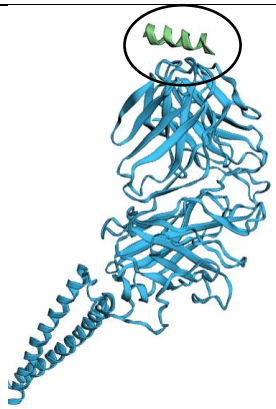
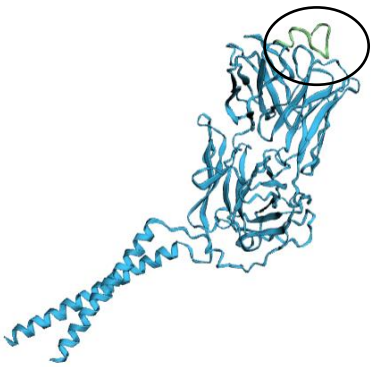
10. ANLALDAQHRDYLKL	
12. HQVISNHLQQHSDLP	

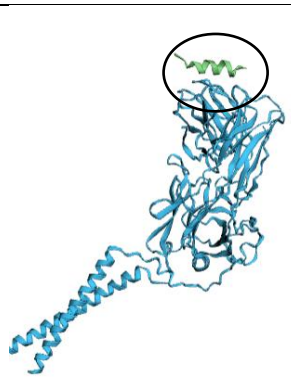
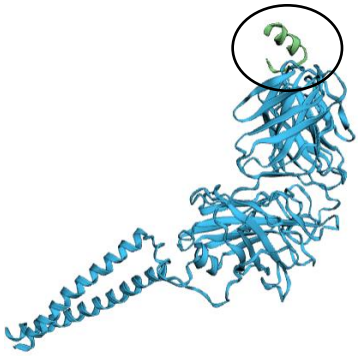
c. Saccharomyces cerevisiae

Table 3.24 | Peptide- TCR Docking of *S. cerevisiae*

S.NO	Amino acid Residue	Peptide Sequence	VDW	ELE	GB	SA	TOTAL (kcal/ mol)
1	15	ADQDETERLLRLEAL	-34.18	-32.63	57.26	-5.38	-14.93
2	15	TSGNEDNGDDDEEEE	-42.63	-286.14	312.4	-7.73	-24.07
3	15	GVPISLIENINSNVR	-36.18	-86	106.9	-5.95	-21.23
4	15	KRGKLKKIQKKYADQ	-	-	-	-	-
5	15	KNNSFEHDNLEKDIE	-36.65	-241.93	248.8	-6.15	-35.92
6	15	INSNVRGKRGKLKKI	-	-	-	-	-

Table 3.25 | *S. cerevisiae* Peptide- TCR Docking structures (Continued)

PEPTIDE SEQUENCE	STRUCTURE
1. ADQDETERLLRLEAL	
2. TSGNEDNGDDDEEEE	

3. GVPISLIENINSNVR	
5. KNNSFEHDNLEKDIE	

The results of molecular docking of *Candida albicans, Candida tropicalis,* and *Saccharomyces cerevisiae* T-cell epitope peptides with TCR demonstrate that: 9 out of 13 T-cell epitopes of *C. tropicalis* have shown the capacity to interact with T-cell receptor, with variable binding affinities, while 1 out of 5 T-cell epitopes of *C. albicans* have shown the capacity to interact with T-cell receptor. *S. cerevisiae* T-cell epitopes have been shown to bind to T-cell receptors in 4 out of 6 cases, with varying binding affinities. This further emphasises the fact that not all commensal fungus epitopes must be able to bind to class II MHC as well as TCR. The common commensal fungal epitopes that display affinity for both Class II MHC and TCR and exhibit high similarity with human sequences can act as potential autoimmune mediators.

3.9 CONCLUSION

Inside the host body, there are several populations of commensal fungus that impact health and illness and vary depending on the body location. Candida usually a dimorphic fungus, can act as commensal as well as opportunistic pathogen. The morphological transition drives their pathogenicity. While S. cerevisiae is a major component of many food, healthy people have more of them, suggesting that having them is protective. Adaptive immunity maintains immune system homeostasis by recognizing antigens from microbial origin. They can generate antigen specific memory B and T-cells that can cross-react with self-antigens due to structural sequential homology and can stem autoimmune diseases**.** A significant proportion of commensal fungus epitopes predicted using the IEDB database can induce humoral and cell-mediated defence against auto-antigens**.** Detected numerous commensal fungus epitopes that have similarity with human sequences using the BLAST database in this work**.** Molecular docking was performed using HawkDock tool to check for perfect binding of antigenic epitopes with MHC Class II and TCR, respectively. Surprisingly only 1 antigenic epitope *PSIGPISNTITLESS* of *C. albicans*, 9 antigenic epitopes FGNNHYERYSFVQKK, QHRDYLKLQQAFMKQ, MTPEGSSANLALDAQ, EGMLSDLKVHTASGT, GFKLELDNLSTPALT, TPALTGMFTPKIQTP, QQTVHAAHQVISNHL, ANLALDAQHRDYLKL, HQVISNHLQQHSDLP of *C. tropicalis*, and 3 antigenic epitopes ADQDETERLLRLEAL, GVPISLIENINSNVR, KNNSFEHDNLEKDIE of *S.cerevisiae* have shown the ability to bind to class II MHC and TCR. These commensal fungi epitopes can act as potential peptides that can bring about autoreactivity due to homology with human auto-antigens. Molecular mimicry by commensal fungi can trigger breakdown of tolerance and induce autoimmunity. Understanding of cross-reactive epitopes in host associated with commensal fungi will provide better devising treatment for auto-immune diseases

CHAPTER 4

4. REFERENCES

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