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In-silico Studies on Virulence Factors of *Cryptococcus* Species: Phylogenetic Analysis and B-cell Epitope Prediction

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Abstract: Virulence proteins ensure the survival of *Cryptococcus* in its host. The epitopes present in these virulence factors can modulate the host's immune system and contribute tocryptococcosis's pathobiology significantly. The amino acid sequences of virulence factors (glucuronoxylomannan (GXM), superoxide dismutase (SOD), mannoprotein (MP), urease, CAP binding protein, galactoxylomannan (GalXM), phospholipase-B, and laccase) of *C. neoformans*, *C. n. grubii*, and *C. gattii* were retrieved from NCBI. Analyses of the phylogenetic relationship between virulence factors were performed by using PhyML software and JMP 13.1 software. Further, ABCpred, BCPred, BcePred web servers were employed for the prediction of linear B-cell epitopes in amino acid sequences of said virulence factors. In all the three *Cryptococcus* species, laccase, CAP binding protein, and mannoprotein were highly conserved compared to GalXM, GXM, and SOD virulence factors. Superoxide dismutase (SOD) with the lowest gamma distribution value is considered to be highly adaptable. Further, the maximum number of B-cell epitopes was observed on the urease of *C. n. grubii.* In due course of time, Cu, Zn Superoxide dismutase (SOD) might play the main role in *Cryptococcus* species' pathogenicity due to its highly variable nature. Additionally, urease could be used to design epitope-based anti-cryptococcal drugs. Nonetheless, the results of this *in-silico* study need wet lab validation.

Keywords: virulence factors; *Cryptococcus*; *in-silico*; phylogenetic analysis; B-cell epitope.

1. Introduction

Amid the emergence and re-emergence of microbial infections, it has become important to re-evaluate their pathogenicity. Like other microbes, fungal pathogens have also been adding new dimensions in their pathogenic potential by gaining novel virulence factors and honing the existing ones. *Cryptococcus* is an opportunistic pathogen of humans and animals. It was considered a 'sleeping giant among fungal diseases [1], but now it has become an 'awakening giant' [2]. *Cryptococcus* can adapt to varying conditions. Regulation and expression of virulence factors facilitate its transition from the environment to mammalian niches [3]. The major virulence factors of *Cryptococcus* species are capsule, glucuronoxylomannan (GXM), mannoprotein (MP), galactoxylomannan (GalXM), laccase, superoxide dismutase (SOD), urease, CAP binding protein, and phospholipase-B.

The mucinous capsule of *Cryptococcus* is primarily composed of polysaccharides having a backbone of α-1, 3-D-mannopyranose units with single residues of ß-D-glucuronopyranosyl and ß-D-xylopyranosyl [4,5]. It is regarded as an important virulence factor and is anti-phagocytic [6]. GXM is the major component of the cryptococcal capsule, which plays a key role in organism's pathogenicity [7]. It is also a superlative target for host antibodies [8]. The capsule also contains transient components MP and GalXM, destined for cellular export [9].

Different enzymes and proteins contribute to the virulence of fungal pathogens. The high urease activity ensures the survival of *Cryptococcus* in avian guano by metabolizing uric acid, xanthines, and creatinine [10]. It helps the fungus to cross the blood-brain barrier, thereby contributing to the pathogenesis of Cryptococcus [11-13] crucially. Laccase is considered a major virulence factor of *Cryptococcus* species. It is responsible for melanin synthesis, which enhances fungal cells' resistance against attack by host effector mechanisms [14,15].

The antioxidant defense of *Cryptococcus* species largely relies on Cu, Zn superoxide dismutase (SOD) [16,17]. SOD catalyzes the conversion of superoxide to hydrogen peroxide and oxygen water and facilitates the growth of *Cryptococcus* species within macrophages by protecting the host's superoxide. Its production increases at 37°C, thus protecting the fungus from the oxidative damage generated by the host. [18,19]. Like other antioxidant enzymes, Cu, Zn superoxide dismutase (SOD) is important for host predilection in *Cryptococcus* species.

Biologically active compounds formed by the cleaving action of phospholipase on phospholipids can alter the microenvironment and facilitate the survival of *C. neoformans* in the host. [20]. The extracellular phospholipase disrupts mammals' cell membranes and helps penetrate yeast cells into host tissues [21].

All epitopes of the pathogen are not able to elicit an immune response in the host. *In-silico* selection using bioinformatics tools can identify potentially immunoprotective epitopes [22-23]. ABCpred, BCPred, and BcePred web servers predict continuous B-cell epitopes based on secondary structure, hydrophilicity, exposed surface area, polarity, flexibility, and charge of amino acids [24-25], whereas DiscoTope, BEpro, and SEPPA use the 3-D structure of protein antigen as input [26]. The predicted epitopes can accelerate the formulation of epitope-based vaccine candidates [21]. Extracellular enzymes like laccase, urease, components of capsule viz. GXM, MP, GalXM, and chitosan of the cell wall are promising targets for antifungal drugs [11, 27-29].

The present study reports phylogenetic analysis and prediction of B cell epitopes of the virulence factors of *C. n. neoformans*, *C. n. grubii*, and *C. gattii* using a bioinformatics approach.

2. Materials and Methods

2.1. Sequences retrieval.

The amino acid sequences of CAP binding proteins, laccase, urease, SOD, phospholipase-B, GalXM, MP, GXM of *C. n. neoformans*, *C. n. grubii*, and *C. gattii* were retrieved from NCBI (National Center of Biotechnology Information, www.ncbi.nlm.nih.gov) in FASTA format. The orthologs were found by performing a BLAST search (https://blast.ncbi.nlm.nih.gov).

2.2. Maximum likelihood test and hierarchical clustering of virulence proteins of Cryptococcus species.

Retrieved sequences of CAP binding proteins, laccase, urease, SOD, phospholipase-B, GalXM, MP, GXM of *C. n. neoformans*, *C. n. grubii* and *C. gattii* were aligned through MUSCLE software (https://www.ebi.ac.uk/Tools/msa/muscle/) [30] in clustal format. Further, these sequences were submitted for testing the likelihood using the PhyML web server (http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=phyml) [31]. Protein datatype and WAG (substitution model) were used in PhyML with default settings for likelihood test. Further**,** clustering was done using software JMP 13.1. (https://www.jmp.com/en_gb/download-jmp-free-trial.html). Clustering was done by the 'ward method'. Data of similar value were grouped to form a cluster.

2.3. B-cell linear epitope prediction in virulence proteins of Cryptococcus species.

Amino acid sequences of CAP binding proteins, laccase, urease, SOD, phospholipase-B, GalXM, MP, GXM of *C. n. neoformans*, *C. n. grubii*,and *C. gattii*. were submitted to ABCpred (http://crdd.osdd.net/raghava/abcpred/), BCPred (http://ailab.ist.psu.edu/bcpred/predict.html) and BcePred webservers (http://crdd.osdd.net/raghava/bcepred/) [32]. These web servers were used to predict B-cell epitopes and the antigenicity of protein sequences. In the ABCpred server, overlapping filters were used. The threshold and window length parameters chosen for prediction were 0.70 and 20, respectively. In the Bcepred server default threshold for all physicochemical properties was selected, and overlapping filters were used. Specificity percentage and epitope length parameters used for prediction were 75 and 20, respectively.

3. Results and Discussion

Its virulence factors determine the pathogenicity of an organism. The gain or loss of virulence is an adaptive mechanism governed by natural selection. Virulence is expressed only in a susceptible host [33]. *Cryptococcus* species can infect and cause disease in humans and a variety of animals. It has been suggested that virulence in *C.neoformans* and *C. gattii* may originate from surviving in its primary niche, the soil or avian guano. The presence of virulence factors in non–virulent strains of *Cryptococcus* indicates selection pressure incurred by environmental predators [34]. Environmental factors also contributed to *Cryptococcus* pathogenicity [35]. The virulence factors inherent to *Cryptococcus* include glucuronoxylomannan (GXM), superoxide dismutase (SOD), mannoprotein (MP), urease, CAP binding protein, galactoxylomannan (GalXM), phospholipase-B, and laccase. Although all these factors are essential for virulence, they could not predict the pathogenicity of the strain.

3.1. Sequence retrieval.

The amino acid sequences of different virulence proteins of *C. n. neoformans*, *C. n. grubii,* and *C. gattii* were retrieved from NCBI in FASTA format (Table 1).

Table 1 List of virulence factors of *Cryptococcus* species retrieved from NCBI.

S.no.	Virulence factors	Accession No.	Sequence in FASTA format
1.	Laccase	ACB05902.1	>ACB05902.1 laccase 1 [*Cryptococcus gattii* VGI] MRGIVNFFFLSCSLILLVSSENTGKLPTATIDPSVFALSNDFEVSDVPTTREYT FDITKALASPDGYEREVYTVNNMFPGPVIEANTGDTIIVHVNNHLDKGQSIH WHGMRQKDTPYMDGVPGITQCPIPPGGSYTYNFTISDQSGTYWWHSHYSN AMADGLWGPLIVHSVHEPIQRGRDYDEDRIVFVSDWLHDDSEIIIAALATPA GYRGSPAPPQGDSILINGRGQTNCTATNSSSCYYPAPPEIHVPVNSRVRLRFI SATAHPMYRISLDNHPLEIVETDGTAVYGPTVHEMSIAPGERYSAIINTSEGK EGDAFWLRTSVALGCMFGGVPQVGLAVVRYTGNEMTTTAEPQSYAWSDL ANATALCAGLDQTYTLSPRERESCRVSRASSQSHIFNSQRGAFVNYLGNTF QGYGFNNISYQNKIFDPILSMVQRGDPYESTLVASTTFPDMGAGAIIINNLDG PIDHPYHLHGNEFQVIGRGTGALSLDNLTNIEFSLDNPVRKDTLWIQGGSWA ALSITTDNPGVWALHCHIGWHLTEGKLAVVVVQPNAVGQIACPESWTNLC ANTDPNAFGPARRSPSPSIQSSKTSAFQRLREVKGNVKRRGAREV
		BAG50325.1	>BAG50325.1 diphenol oxidase [*Cryptococcus neoformans* var. *grubii*] MRGVVKLFFLSCSFVSLVSSEETGKSPTANYDHYMPKATATIDPSVFALSND FEITDVPTTREYTFDITKALASPDGYEREVYVVNNMFPGPVIEANTGDTIIVH VNNHLEEGQSIHWHGLRQLGTAFMDGVPGITQCPIPPGSSFTYQFTVSHQSG TFWWHSHYSNSMADGIWGPLIIHSPNEPLQRGRDYDEDRIVFITDWVHDNS EVVIAALATPEGYKGSPAPPQGDAILINGRGQTNCTATGSSSCTYPPPPEIHV PVNCRVRLRFISATAHPMYRITIDNHPLEVVETDGTAVYGPTVHEISIAPGER YSAIINTSEGKEGDAFWLRTSVALGCMFGGIDQVGLAVVRYTGNGMVSTE EPQTTAWSDLAGATTPCAGLDQTYTLSPRESFSAPREFSQSHVFNSQRGAFV NVYGNTFQGYGFNNISYQNQIFNPLLSIVQRGGSCESTLVASTTFPDLGSGNI IINNLDGVIDHPYHLHGNEFQVIGRGTGALSLDNLTNIDFNLDNPVRKDTLW IQGGSWVVLRITTDNPGVWALHCHIGWHLTEGKLAVVVIQPGAIGHMEGP ESWTNLCANTDPNAFGPARRSPSPSIQSSKTSTFQYLREVKGKVVKRRGAR EA
		BAG50331.1	>BAG50331.1 diphenol oxidase [*Cryptococcusneoformans* var. *neoformans*] MRGLAKLFFLSCSFVSLVSSEKTDESPTAVSDNYMPKATATIDPSVFALSND FEITDVPTTREYTFDIAKAFASPDGYEREVYVVNNMFPGPVIEANTGDTIIVH VNNHLDEGQSLHWHGLRQLGTAFMDGVPGITQCPIPPGGSFTYNFTVSHQS GTFWWHSHYSNSMADGIWGPLIVHSTNEPLQRGRDYDEDRIVFITDWMHD NSEIIIAALATPEGYKGNIAPPQGDAILINGRGQTNCTATGSSSCFYPPPPEIQ VPVNCRVRLRFISATAHPMYRISIDNHPMEVVEADGTAVYGPTVHEISVAPG ERYSAIINTNEGKEGDAFWLRTSVALSCMFGAVSQEGLAVVRYTGNGMVS TEEPQTSAWSDLAGVTVPCTGLDQTYTLSPRDSLSAPREPLQSHFFNSERGA FVNVLGNTFQGYGFNNISYQNQIFNPLLSIVQRGGSCENTLVSSRTFPDFGPG NIIINNLDTVIDHPYHLHGNEFQVIGRGTGALSIDNLTNIDFTLDNPVRKDTL WIQGGSWAVLRITADNPGVWALHCHIGWHLTEGKLAVIVVQPSAIGHMES PESWTNLCANTDPNAFGPARRSSSPSIQSSKTSSFQYLREVKGKVVKRRGAR EA
		KIR41803.1	>KIR41803.1 laccase [*Cryptococcus gattii* VGII Ram5] MRGIVNFFFLSCSLILLVSSENTGKLPTATIDPSVFALSNDFEVSDVPTTREYT FDITKALASPDGYEREVYTVNNMFPGPVIEANTGDTIIVHVNNHLDKGQSIH WHGMRQKDTPYMDGVPGITQCPIPPGGSYTYNFTISDQSGTYWWHSHYSN AMADGLWGPLIVHSVHEPIQRGRDYDEDRIVFVSDWLHDDSEIIIAALATPA GYRGSPAPPQGDSILINGRGQTNCTATNSSSCYYPAPPEIHVPVNSRVRLRFI SATAHPMYRISLDNHPLEIVETDGTAVYGPTVHEMSIAPGERYSAIINTSEGK EGDAFWLRTSVALGCMFGGVPQVGLAVVRYTGNEMTTTAEPQSYAWSDL ANATALCAGLDQTYTLSPRERESCRVSRASSQSHIFNSQRGAFVNYLGNTF QGYGFNNISYQNKIFDPILSMVQRGDPYESTLVASTTFPDMGPGAIIINNLDG PIDHPYHLHGNEFQVIGRGTGALSLDNLTNIEFSLDNPVRKDTLWIQGGSWA ALGITTDNPGVWALHCHIGWHLTEGKLAVVVVQPNAVGQIACPESWTNLC ANTDPNAFGPARRSPSPSIQSSKTSAFQRLREVKGNVKRRGAREV
		BAG50349.1	>BAG50349.1 diphenol oxidase [*Cryptococcus neoformans*] MRGIVNFFFLSCSLILLVSSENTGKLPTATIDPSVFALSNDFEVSDVPTTREYT FDITKALASPDGYEREVYTVNNMFPGPVIEANTGDTIIVHVNNHLDKGQSIH WHGMRQKDTPYMDGVPGITQCPIPPGGSYTYNFTISDQSGTYWWHSHYSN AMADGLWGPLIVHSVHEPIQRGRDYDEDRIVFVSDWLHDDSEIIIAALATPA GYRGSPAPPQGDSILINGRGQTNCTATNSSSCYYPAPPEIHVPVNSRVRLRFI SATAHPMYRISLDNHPLEIVETDGTAVYGPTVHEMSIAPGERYSAIINTSEGK EGDAFWLRTSVALGCMFGGVPQVGLAVVRYTGNEMTTAEPQSYAWSDLA NATALCAGLDQTYTLSPRERESCRVSRASSQSHIFNSQRGAFVNYLGNTFQ GYGFNNISYQNKIFDPILSMVQRGDPYESTLVASTTFPDMGAGAIIINNLDGP IDHPYHLHGNEFQVIGRGTGALSLDNLTNIEFSLDNPVRKDTLWIQGGSWA ALSITTDNPGVWALHCHIGWHLTEGKLAVVVVQPNAVGQIACPESWTNLC ANTDPNSFGPARRSPSPSIQSSKTSAFQRLREVKGNVKRRGAREV

S.no.	Virulence factors	Accession No.	Sequence in FASTA format
		KIR33454.1	>KIR33454.1 laccase [*Cryptococcus gattii* VGII MMRL2647] MRGIVNFFFLSCSLILLVSSENTGKLPTATIDPSVFALSNDFEVSDVPTTREYT FDITKALASPDGYEREVYTVNNMFPGPVIEANTGDTIIVHVNNHLDKGQSIH WHGMRQKDTPYMDGVPGITQCPIPPGGSYTYNFTISDQSGTYWWHSHYSN AMADGLWGPLIVHSVHEPIQRGRDYDEDRIVFVSDWLHDDSEIIIAALATPA GYRGSPAPPQGDSILINGRGQTNCTATNSSSCYYPAPPEIHVPVNSRVRLRFI SATAHPMYRISLDNHPLEIVETDGTAVYGPTVHEMSIAPGERYSAIINTSEGK EGDAFWLRTSVALGCMFGGVPQVGLAVVRYTGNEMTTTAEPQSYAWSDL ANVTALCAGLDQTYTLSPRERESCRVSRASSQSHIFNSQRGAFVNYLGNTF QGYGFNNISYQNKIFDPILSMVQRGDPYESTLVASTTFPDMGAGAIIINNLDG PIDHPYHLHGNEFQVIGRGTGALSLDNLTNIEFSLDNPVRKDTLWIQGGSWA ALSITTDNPGVWALHCHIGWHLTEGKLAVVVVQPNAVGQIACPESWTNLC ANTDPNAFGPARRSPSPSIQSSKTSAFQRLREVKGNVKRRGAREV
2.	Urease	AAC62257.1	>AAC62257.1 urease [*Cryptococcus neoformans* var. *grubii*] MHLLPRETDKLILTTLGTLAQRRLARGLILNRAETIALISSQLQEFVRDGRHS VAELMDLGKKMLGRRHVRKGVPESIHTIQVEGTFPDGVFLVTVDDPISSDD GDLNNAFYGSFLPIPSADVFPAAPEPADTLLGALICRKETVKINASRRRFRLE VKNAGDRPVQVGSHYHFLETNPALIFDRLLSYGYHLDIPAGTAVRFEPGEK KTVTMVEFGGKKIFHGGSGLGNGSFDENLRETKVKEMVEKVGFGHKEQEK IEEGPVTEMNREVYASMFGPTTGDKIKLADMDLWIEVEKDYTVYGDECKF GGGKVIRDGGGQASGRHDHEVLDLVITNALIVDWTGIYKADIGVKNGIIVGI GKAGNPDMMDGVTDGMIVGSSTEVISGEKLITTAGRLDVHVHYISPQLMTE ALASGITTVIGGGTGPADGSNATTCTSSSFYMQNMIKATDTIPLNFGFTGKG SDSGTNAMRDIIEAGACGLKVHEDWGATAEVIDRALSMADEYDVQINLHS DTLNESGYVESTLAAIKGRTIHSYHTEGAGGGHAPDIIVVCEYENVLPSSTN PTRPYAVNTLDEHLDMLMICHGLDKSIPEDIAFADSRIRSETVAAEDVLQDT GAISMISSDCQAMGRIGEVVTRTWRTAAKMKQFRGPLEGDEPTRDNNRVK RYVAKYTINPAITHGMSHLIGQVAVGCLADLVLLDGESFGARPEMILKGGV IAWAAVGDANASIPTVQPVLGRPMWALSLRPLHSIQLFVSQASLDKDLVKR YRLRKRAEAVKNCRSIGKKDMKWNDTMPKMTVDPETYDVRADGVLCDV PPADKLPLTRRYFVY
		XP_012053644.1	>XP_012053644.1 urease [*Cryptococcusneoformans* var. *grubii* H99] MSTDGTGWETKPHKGTQLPHPLRPVFDSLLLFFIFCFPFLPPHHTKNIMHLLP RETDKLILTTLGTLAQRRLARGLILNRAETIALISSQLQEFVRDGRHSVAELM DLGKKMLGRRHVRKGVPESIHTIQVEGTFPDGVFLVTVDDPISSDDGDLNN AFYGSFLPIPSADVFPAAPEPADTLLGALICRKETVKINASRRRFRLEVKNAG DRPVQVGSHYHFLETNPALIFDRLLSYGYHLDIPAGTAVRFEPGEKKTVTM VEFGGKKIFHGGSGLGNGSFDENLRETKVKEMVEKGGFGHKEQEKIEEGPV TEMNREVYASMFGPTTGDKIKLADMDLWIEVEKDYTVYGDECKFGGGKVI RDGGGQASGRHDHEVLDLVITNALIVDWTGIYKADIGVKNGIIVGIGKAGN PDMMDGVTDGMIVGSSTEVISGEKLITTAGALDVHVHYISPQLMTEALASGI TTVIGGGTGPADGSNATTCTSSSFYMQNMIKATDTIPLNFGFTGKGSDSGTN AMRDIIEAGACGLKVHEDWGATPEVIDRALSMADEYDVQINLHSDTLNESG YVESTLAAIKGRTIHSYHTEGAGGGHAPDIIVVCEYENVLPSSTNPTRPYAV NTLDEHLDMLMICHGLDKSIPEDIAFADSRIRSETVAAEDVLQDTGAISMISS DCQAMGRIGEVVTRTWRTAAKMKQFRGPLEGDEPTRDNNRVKRYVAKYT INPAITHGMSHLIGQVAVGCLADLVFWTAESFGARPEMILKGGVIAWAAVG DANASIPTVQPVLGRPMWGSQPEAAALNSIVWVSQASLDKDLVKRFNIKKR AEAVKNCRSIGKKDMKWNDTMPKMTVDPETYDVRADGVLCDVPPADKLP LTRRYFVY
		XP_572365.1	>XP_572365.1 urease [*Cryptococcus neoformans* var. *neoformans* JEC21] MHLLPRETDKLIVTTLGTLAQRRLARGLILNRAETIALISSQLQEFIRDGRHS VAELMDLGKKMLGRRHVRKGVPESIHTIQVEGTFPDGVFLVTVDDPISSDD GDLNNAFYGSFLPIPSADVFPAAPEPADTLLGALICRKEPIKINASRRRFKLE VKNAGDRPIQVGSHYHFLETNPALIFDRLLSYGYHLDIPAGTAVRFEPGEKK TVTMVEFGGKKIFHGGSGLASGSFDENLRETKVKEMVEKGGFGHKDQEKV EEGPTTEMNREVYASMFGPTTGDKIKLADMDLWIEVEKDYTVYGEECKFG GGKVLRDGGGQASGRHEHEVLDLVITNALIVDWNGIYKADIGVKNGIIVGI GKAGNPDMMDGVTDGMIVGSSTEVIAGEKLIITAGALDVHVHYICPQLMTE ALASGITTVVGGGTGPADGSNATTCTSSSFYMQNMIKATDTVPLNFGFTGK GNDSGTNALRDVIEAGACGLKVHEDWGATPEVIDRALSIADEYDVQVNLH SDTLNESGYVESTLAAIKGRTIHSYHTEGAGGGHAPDIIVVCEYENVLPSST NPTRPYAVNTLDEHLDMLMVCHHLDKSIPEDIAFADSRIRSETVAAEDVLQ DTGAISMISSDCQAMGRIGEVITRTWRTAAKMKQFRGPLEGDEPTRDNNRV KRYVAKYTINPAITHGMSHLIGQVAVGCLADLVFWTAESFGARPEMILKGG VIAWAAMGDANASIPTVQPVIGRPMWGSQPEAAALNSIVWVSQASLDKDL VKRFNIKKRAEAVKNCRAIGKKDMKWNDSMPKMTVDPETYDVHADGVL CDVPPADKLPLTKRYFVY

S.no.	Virulence factors	Accession No.	Sequence in FASTA format
		KGB78493.1	>KGB78493.1 urease [*Cryptococcus gattii* VGII R265] MHLLPRETDKLILTTLGTLAQRRLARGLILNRAETIALISSQLQEFIRDGRHS VAELMDMGKKMLGRRHVRKGVPESIHSIQVEGTFPDGVFLVTVDDPISSDD GDLNNAFYGSFLPIPSADVFPAAPEPKDTLLGALICREEPIKINVSRRRFRLEV KNAGDRPIQVGSHYHFLETNPALVFDRLLSYGYHLDIPAGTAVRFEPGEKK TVTMVEFGGKKIFHGGSGLGSGPFNENLRETTIKAMVEKGGFSHKEQEKVE EGPVTEMNREVYASMFGPTTGDKIKLADMDLWIEIEKDYTVYGEECKFGG GKVLRDGGGQASGRYDHEVLDLVITNALIVDWNGIYKADIGVKNGIIVGIG KAGNPDMMDGVTDGMIVGSNSEVIAGEKLIVTAGALDVHVHYICPQLMTE ALASGITTVVGGGTGPADGSNATTCTSSPFYMQNMIKATDTMPLNFGFTGK GNDSGTNSLRDIIEAGACGLKVHEDWGATPEVIDRALTIADEYDVQVNLHS DTLNESGYVESTLAAIKGRTIHSYHTEGAGGGHAPDIIVVCEHENVLPSSTN PTRPYAVNTLDEHLDMLMVCHHLDKSIPEDIAFADSRIRSETVAAEDVLQD TGAISMISSDSQAMGRIGEVITRTWRTAAKMKQYRGPLEGDEPTRDNNRVK RYVAKYTINPAITHGMSHLIGHVAVGCLADLVFWTAESFGARPEMVLKGG VIAWAAIGEANAAIPTVQPVIGRPMWGAQPAAAALNSIVWVSQASLDKDL VKRFDIKKRAEAVKNCRAIGKKDMKWNDTMPKMTVDPETYDVRADGVL CDVPPADKLPLTKRYFVY
		KIR84016.1	>KIR84016.1 urease [*Cryptococcus gattii* VGIV IND107] MHLLPRETDKLILTTLGTLAQRRLARGLILNRAETIALISSQLQEFIRDGRHS VAELMDMGKKMLGRRHVRKGVPESIHSIQVEGTFPDGVFLVTVDDPISSDD GDLNNAFYGSFLPIPSADVFPAAPEPKDTLLGALICRKEPIKINVSRRRFRLE VKNAGDRPIQVGSHYHFLETNPALIFDRLLSYGYHLDIPAGTAVRFEPGEKK TVTMVEFGGKKIFHGGSGLGSGPFNENLRDTKIKEMVEKGGFSHKEQEKVE EGPVTEMNREVYASMFGPTTGDKIKLADMDLWIEIEKDYTVYGEECKFGG GKVLRDGGGQASGRYDHEVLDLVITNALIVDWNGIYKADIGVKNGIIVGIG KAGNPDMMDGVTDGMIVGSNSEVIAGEKLIVTAGALDVHVHYICPQLMTE ALASGITTVVGGGTGPADGSNATTCTSSPFYMQNMIKATDTMPLNFGFTGK GNDSGTNSLRDIIEAGACGLKVHEDWGATPEVIDRALTIADEYDVQVNLHS DTLNESGYVESTLAAIKGRTIHSYHTEGAGGGHAPDIIVVCEHENVLPSSTN PTRPYAVNTLDEHLDMLMVCHHLDKSIPEDIAFADSRIRSETVAAEDVLQD TGAISMISSDSQAMGRIGEVITRTWRTAAKMKQYRGPLEGDEPTRDNNRVK RYVAKYTINPAITHGMSHLIGHVAVGCLADLVFWTAESFGARPEMVLKGG VIAWAAIGEANAAIPTVQPVIGRPMWGAQPAAAALNSIVWVSQASLDKDL VKRFDIKKRAEAVKNCRAIGKKDMKWNDTMPKMTVDPETYDVRADGVL CDVPPADKLPLTKRYFVY
		XP_003197080.1	>XP_003197080.1 Urease (Urea amidohydrolase), putative [*Cryptococcus gattii* WM276] MHLLPRETDKLILTTLGTLAQRRLARGLILNRAETIALISSQLQEFIRDGRHS VAELMDMGKKMLGRRHVRKGVPESIHSIQVEGTFPDGVFLVTVDDPISSDD GDLNNALYGSFLPIPSADVFPAAPEPTDTLLGALICRKEPIKINVSRRRFRLEV KNAGDRPIQVGSHYHFLETNPALIFDRLLSYGYHLDIPAGTAVRFEPGEKKT VTMVEFGGKKIFHGGSGLGSGPFNENLRNTKIKEMVEKGGFSHKEQEKIEE GPVTEMNREVYASMFGPTTGDKIKLADMDLWIEIEKDYTVYGEECKFGGG KVLRDGGGQASGRYDHEVLDLVITNALIVDWNGIYKADIGVKNGIIVGIGK AGNPDMMDGVTDGMIVGSNTEVIAGEKLIVTAGALDVHVHYICPQLMTEA LASGITTVVGGGTGPADGSNATTCTSSPFYMQNMIKATDTMPLNFGFTGKG NDSGTNSLRDIIEAGACGLKVHEDWGATPEVIDRALTIADEYDVQVNLHSD TLNESGYVESTLAAIKGRTIHSYHTEGAGGGHAPDIIVVCEHENVLPSSTNPT RPYAVNTLDEHLDMLMVCHHLDKSIPEDIAFADSRIRSETVAAEDVLQDTG AISMISSDSQAMGRIGEVITRTWRTAAKMKQYRGPLEGDEPTRDNNRVKRY VAKYTINPAITHGMSHLIGHVAVGCLADLVFWTAESFGARPEMVLKGGVIA WAAIGEANAAIPTVQPIIGRPMWGAQPAAAALNSIVWVSQASLDKDLVKRF DIKKRAEAVKNCRAIGKKDMKWNDTMPKMTVDPETYDVRADGVLCDVPP ADKLPLTKRYFVY
3.	SOD	XP_570285.1	>XP_570285.1 copper-zinc superoxide dismutase [*Cryptococcus neoformans* var. *neoformans* JEC21] MVKAVAVLKGDSHVYGTITFTQDSEGAPVCVSGEIKNLDADAKRGFHVHE FGDNTNGCTSAGPHYNPFHKNHGGPTAAERHVGDLGNVQTNGCGVAMVD ISDKVISLFGPHSIIGRSMVVHAGTDDLGKGGNEESLKTGNAGARLACGVIG IAA
		KIR50011.1	>KIR50011.1 superoxide dismutase [Cu-Zn] [*Cryptococcus gattii* CA1280] MLAVAVLKGDSPVTGVITFTQEKEGAPVTVSGDIKNLDANAERGFHVHEFG DNTNGCTSAGPHFNPHGKNHGAPSDSERHVGDLGNVKTDSNGVASVNISD KSLSLFGPYSIIGRTIVVHAGTDDFGKGGNAESLKTGNAGARAACGVIGISS

S.no.	Virulence factors	Accession No.	Sequence in FASTA format
		KGB74761.1	>KGB74761.1 superoxide dismutase [Cu-Zn] [*Cryptococcus gattii* VGII R265] MPAVAVLKGDSPVTGVITFTQEKEGAPVTVSGDIKNLDANAERGFHVHEFG DNTNGCTSAGPHFNPHGKNHGAPSDSERHVGDLGNVKTDGNGVASVNISD KSLSLFGPYSIIGRTIVVHAGTDDFGKGGNPESLKTGNAGARAACGVIGISN
		AAK01665.1	>AAK01665.1 Cu/Zn superoxide dismutase [*Cryptococcus neoformans* var. *grubii*] MVKAVVVLKGESYAHGIVCFTQESENAPVCITGEIKDMDADAKRGMHVHE FGDNTNGCTSAAPHYNPFKKHHGAPTDSERHVGDLGNIQTNSCGAAQLDF SDKIISLYGPHSIIGGSFVVHASTDDLGKGGNEESLKTGNAGARLACGVIGIS TCQCYHSKLIVFAAVFLPKRTVTTYSWLNK
		XP_003193504.1	>XP_003193504.1 copper zinc superoxide dismutase [*Cryptococcus gattii* WM276] MRAVAVLKGDSPVTGVITFTQEKEGAPVTVSGDIKNLDANAERGFHVHEF GDNTNGCTSAGPHFNPHGKNHGAPSDSERHVGDLGNVKTDGNGVASVNIS DKSLSLFGPYSIIGRTIVVHAGTDDFGKGGNAESLKTGNAGARAACGVIGIS N
		KIR29693.1	>KIR29693.1 superoxide dismutase [Cu-Zn] [*Cryptococcus gattii* VGII LA55] MLAVAVLKGDSPVTGVITFTQEKEGAPVTVSGDIKNLDANAERGFHVHEFG DNTNGCTSAGPHFNPHGKNHGAPSDSERHVGDLGNVKTDGNGVASVNISD KSLSLFGPYSIIGRTIVVHAGTDDFGKGGNPESLKTGNAGARAACGVIGISN
4.	GXM	XP_568628.1	>XP_568628.1 O-acetyltransferase [*Cryptococcus neoformans* var. *neoformans* JEC21] MPNSSKPRSQASAAKLNPLWYTYACATLVAAVVLGNILRWAFLELPDSYH CSALLNTGKWLDPGTWTNWQPEGCFQLPLSAQSWQKCLASPTVNTHQAL HSSYYDKRTALFVGDSTVRQLYFAAARKVGKTSKAWELEGEKHTDRSLLV SDPLGGPSLELEFWWDPYLNSSKTIGLLSGQSSVPSSLLVMGSGLWYLRNPS SGGLASWGAMIYDTFELVKKNQGSPQTALINPWDNMLLGPGITLPGLLPNQ PPKFVDHSREVEARSLFSRASSISHRPTDFSISDAIVFLPISTPVREKLSPSRAE TIFHTDVEAMNADLYARLTHPDPPPVVIPSVLNQLLVDDETEDGLHFSDKIM NKQAELLLSWRCNDVMRHEGATGTCCKRYDWVTPIQGLILAVLILWAPLG TFITPRLPPNSPILDYLPATSIAPALSTFGLAMGYLFLADRTHVFQKEQKDYD AVIFGMITLAAFVAGLLTIKNSGKDLGFLNRDITDEWKGWMQIAILIYHFFG ASKISGIYNPIRVLVASYLFMTGYGHFFFYYKKADFGFQRVVMVLVRLNLL SVVLPYTMNTDYAFYYFAPLVSWWYLIIYATMAIGSKYNDRPAFLLTKLFT CAGLVTLFMHFPWLMEDVFKVLNTVFNIQWSAKEWSFRVTLDLFIVWVG MLCAYGFVKFNEHQISDRPWFPVMRTATLVGSVLGMIWYFWFELHLASKF VYNEYHAVVCIVPIMSFVFLRNASPVLRSSTSKIFCFIGQCSLETFILQFHGW LASDTKAILLAVPSTQWRPVNLVISTICFIWLSYRVSGATGEITEWLVGKKK ALPLPATSANSSTSPGRQATSPTLTSASAMQAVVVGPQDGAKGGIPESIPMM NQADKDIGGLTPMEDETLERRDSWPTWMASTAASLTGRTVEGYAPLTRRW KDQTVLSVIQNLGDLMKKHNSVKIAVILLGLWALNWIY
		XP_012053554.1	>XP_012053554.1 O-acetyltransferase [*Cryptococcus neoformans* var. *grubii* H99] MPNSSNSRSQATAAKLNPLWYTYACATLVAAVVLGNILRWAFLELPDSYH CSALLNTGKWLDPGTWTNWQPEGCFQLPLSAQSWQRCLASPTVNTHQAL HSYYDKRTALFVGDSTVRQLYFATARKVGKASKAWESEGEKHTDRSLLVS DPLGGPSLELEFWWDPYLNSSKTIGLLSGRGLAPSSLLVMGSGLWYLRNPS SGGLASWGAMIHDTFEFIKKNQGSPQAALINPWDNMLLGSGLTLPGLLPQQ SPKFVDSSREVEARSLFSRASSASHRPADFSISDAIVYLPISTPVHEKLSSSRA ETIFHTDVEAMNADLYARLTHPDPPPVVIPSVFNQLLVDDETEDGLHFSDKI MDKQAELLLSWRCNDVMRHEGATGACCKRYDWVTPIQGLILAVLILWAPL GTFITPRLPPNSPIHDYLPSPSIAPALSTFGLAVGYLFLADRTHVFQKEQKDY DAVVFGVITFAAFVAGLLTIKNSGKDLGFLNRDITDEWKGWMQIAILIYHFF GASKISGIYNPIRVLVASYLFMTGYGHFFFYYKKADFGFQRVVMVLVRLNL LSVVLPYTMNTDYAFYYFAPLVSWWYLIIYATMAFGSKYNDRPAFLLAKL FTCAGLVTLFMHFPWLMEDVFKVLNTVFNIQWSAKEWSFRVTLDLFIVWA GMLCAYGFVKFKEYQISDRPWFPTMHTATLIGSVLGMIWYFWFELHLANK FVYNEYHAVVCIVPIISFIFLRNASPVLRSSTSKIFCFIGQCSLETFILQFHGWL ASDTKAVLLAVPSTQWRPVNLVISTICFIWLSYRVSGATGEITEWLVGKKK ALPLPATSAGPSTSTSRQATSPTLTTASAMQAVVEGPQDGAKGGIPESIPMM NQADKDIGGLTPMEDETLERRDSWPTWMASTAASLTGRTAEGYAPLTRQ WKDQTVLSVIQNLGDLMKKHTSVKIAVILFGLWVLNWIY
		KIR84277.1	>KIR84277.1 O-acetyltransferase [*Cryptococcus gattii* VGIV IND107] MPNSSKRRSQATAAKLNPLWYTYACATLLAAVVLGNFLRWAFLELPDSYH CSALLNTGKWLDPGTWTNWQPEGCFQLPLPAQSWQKCLASPTVNTHQSLH SSYFDKRTALFVGDSTVRQLYFAAARKVGKTSKAWESEGEKHTDRSLLVN DPLGGPSLELEFWWDPYLNSSKTVGLLSGHSPVPSSLLVMGSGLWYLRNPS SGGLASWGAMIYDTFELFKKNQGSPQTALINPWDNMLLGPGVTLPGLLPD QPPKSVDYSPEVEARSFLSRASSISRRPTDFSISDAIVFLPISTPVPEKLSPSRAE TILHTDVEAMNADLYARLTHPDPPPVIMPSVFNQLLVDDETEDGLHFSDKI MNKQAELLLSWRCNDVMRHEGATGTCCKRYDWVTPVQGLILAILVLWAP

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			LGTLIAPRLRPKSPVHDYLPSPSIAPALSTFGLAMGYLFLADRTHIFQKEQKD YDSIIFGMITLAAFVAGLLTVRNSGKDLGFLNRDITDEWKGWMQIAILIYHF FGASKISGIYNPIRVMVASYLFMTGCEYIAFYYKKADFSFQRVIMVLVRLNL LSVVLPYTMNTDYAFYYFAPLVSWWYLIIYATMAIGSRYNDRPAFLLPKLFI CAGLVTLFMHFPWLMADVFKVLNTVFNIQWSAKEWSFRVTLDLFIVWAG MLCAYGFVKFKEHQISDRPWFPVMRTSTLVGSVLGMIWYLWFELHLPSKF VYNEYHAVVCVVPIMSFVFLRNASPALRSSTSKIFCFIGQCSLETFILQFHGW LASDTKAILLAVPSTRWRPVNLVISTICFIWLSYRVSGATGEITEWLIGKKKA LPPPATSTGPSTSSSRQATTPMFTTASAMQAVVEGPQDGAKGGVPESIPLMN QADKEVGGLTPVEDETLERRDSWPTWMASTAASFTGRTAEGYAPLTRRW KDQTVLSVIQNLGDLMKKHNSVKIAVILLGLWILNWIY
		KGB79193.1	>KGB79193.1 O-acetyltransferase [Cryptococcus gattii VGII R265] MPNSSKPRSQATAAKLNPLWYTYACATLLAAVVLGNFLRWAFLELPDSYH CSALLNTGKWLDPGTWTNWQPEGCFQLPLPAQSWQRCLALPTVNTHQSLH SSYFDKRTALFVGDSTVRQLYFAAARKVGKTSKAWESEGEKHTDRSLLVN DPLGGPSLELEFWWDPYLNSSKTVGLLSGHSPVPSSLLVMGSGLWYLRNPS SGGLASWGAMIYDTFELIKKNQGSPQTALINPWDNMLLGPGVTLPGLLPDQ PLKSDDYSLEVEARSFLSRASSISRRPTDFSISDAIVFLPISTPVPEKLSPSRAET ILHTDVEAMNADLYARLTHPDPPPVIMPSVFNQLLVDDETEDGLHFSDKIM NKQAELLLSWRCNDVMRHEGATGTCCKRYDWVTPVQGLILAVLVLWAPF GTLIAPRLPPKSPVHNYLPSTSIAPALSTFGLAMGYLFLADRTHVFQKEQKD YDAIIFGTITLAAFVAGLLTVRNSGKDLGFLNRDITDEWKGWMQIAILIYHF FGASKISGIYNPIRVMVASYLFMTGYGHFFFYYKKADFSFQRVIMVLVRLNL LSVVLPYTMNTDYAFYYFAPLVSWWYLIIYATMAIGSRYNDRPAFLLPKLFI CAGLVTLFMHFPWLMADVFKVLNTVFNIQWSAKEWSFRVTLDLFIVWAG MLCAYGFVKFKEHQISDRPWFPVMRTSALVGSVLGMIWYLWFELHLPSKF VYNEYHAVVCVVPIMSFVFLRNASPALRSSTSKIFCFIGQCSLETFILQFHGW LASDTKAILLAVPSTRWRPVNLVISTICFIWLSYRVSGATGEITEWLVGKKK ALPPPATSTGPSTSSSRQATSPTFTTASAMQAVIEGPQDGAKGGVPESIPLMN QADKEVGGLTPVEDETLERRDSWPTWMASTAASFTGRTAEGYAPLTRRW KDQTVLSVIQNLGDLMKKHNSVKIAVILLGLWILNWIY
		KIR27425.1	>KIR27425.1 O-acetyltransferase [*Cryptococcus gattii* VGII LA55] MPNSSKPRSQATAAKLNPLWYTYACATLLAAVVLGNFLRWAFLELPDSYH CSALLNTGKWLDPGTWTNWQPEGCFQLPLPAQSWQRCLALPTVNTHQSLH SSYFDKRTALFVGDSTVRQLYFAAARKVGKTSKAWESEGEKHTDRSLLVN DPLGGPSLELEFWWDPYLNSSKTVGLLSGHSPVPSSLLVMGSGLWYLRNPS SGGLASWGAMIYDTFELIKKNQGSPQTALINPWDNMLLGPGVTLPGLLPDQ PLKSDDYSPEVEARSFLSRASSISRRPTDFSISDAIVFLPISTPVPEKLSPSRAET ILHTDVEAMNADLYARLTHPDPPPVIMPSVFNQLLVDDETEDGLHFSDKIM NKQAELLLSWRCNDVMRHEGATGTCCKRYDWVTPVQGLILAVLVLWAPF GTLIAPRLPPKSPVHNYLPSTSIAPALSTFGLAMGYLFLADRTHVFQKEQKD YDAIIFGTITLAAFVAGLLTVRNSGKDLGFLNRDITDEWKGWMQIAILIYHF FGASKISGIYNPIRVMVASYLFMTGYGHFFFYYKKADFSFQRVIMVLVRLNL LSVVLPYTMNTDYAFYYFAPLVSWWYLIIYATMAIGSRYNDRPAFLLPKLFI CAGLVTLFMHFPWLMADVFKVLNTVFNIQWSAKEWSFRVTLDLFIVWAG MLCAYGFVKFKEHQISDRSWFPVMRTSALVGSVLGMIWYLWFELHLPSKF VYNEYHAVVCVVPIMSFVFLRNASPALRSSTSKIFCFIGQCSLETFILQFHGW LASDTKAILLAVPSTRWRPVNLVISTICFIWLSYRVSGATGEITEWLVGKKK ALPPPATSTGPSTSSSRQATSPTFTTASAMQAVVEGPQDGAKGGVPESIPLM NQADKEVGGLTPVEDETLERRDSWPTWMASTAASFTGRTAEGYAPLTRR WKDQTVLSVIQNLGDLMKKHNSVKIAVILLGLWILNWIY
		KIR53077.1	>KIR53077.1 O-acetyltransferase [Cryptococcus gattii Ru294] MPNSSKPRSQATAAKLNPLWYTYACATLLAAVVLGNFLRWAFLELPDSYH CSALLNTGKWLDPGTWTNWQPEGCFQLPLPAQSWQKCLASPTVNTHQSLH SSYFDKRTALFVGDSTVRQLYFAAARKVGKTSKAWESEGEKHTDRSLLVN DPLGGPSLELEFWWDPYLNSSKTVGLLSGHSPVPSSLLVMGSGLWYLRNPS SGGLASWGAMIYDTFELLKKNQGSPQTALIDPWDNMLLGPGVTLPGLLPD QPPKSVDYSPEVEARSFLSRASSISRRSTDFSISDAIVFLPISTPVPEKLSPSRAE TIFHTDVEAMNADLYARLTHPDPPPVIMPSVFNQLLVDDETEDGLHFSDKI MNKQAELLLSWRCNDVMRHEGATGTCCKRYDWVTPVQGLILAILILWAPL GTLIAPRLPPKSPVHDYLPSPSIAPALSTFGLAMGYLFLADRTHVFQKEQKD YDAIIFGTITLAAFVAGLLTVRNSGKDLGFLNRDITDEWKGWMQIAILIYHF FGASKISGIYNPIRVMVASYLFMTGCEYIAFYYKKADFSFQRVIMVLVRLNL LSVVLPYTMNTDYAFYYFAPLVSWWYLIIYATMAIGSRYNDRPAFLLPKLFI CAGLVTLFMHFPWLMADVFKVLNTVFNIQWSAKEWSFRVTLDLFIVWAG MLCAYGFVKFKEHQISDRPWFPVMRTSTLVGSVLGMIWYLWFELHLPSKF VYNEYHAVVCVVPIMSFVFLRNASPALRSSTSKIFCFIGQCSLETFILQFHGW LASDTKAILLAVPSTRWRPVNLVISTICFIWLSYRVSGATGEITEWLVGKKK

S.no.	Virulence factors	Accession No.	Sequence in FASTA format
			ALPPPATSTGPSTSSSRQATSPTFTTASAMQAVVEGPQDGAKGGVPESIPLM NQADKEVGGLTPVEDETLERRDSWPTWMASTAASFTGRTAEGYAPLTRR WKDQTVLSVIQNLGDLMKKHNSVKIAVILLGLWILNWIY
5.	CAP binding proteins	XP_569639.1	>XP_569639.1 cap binding protein, putative [*Cryptococcus neoformans* var. *neoformans* JEC21] MTSTAIPPAAVAANNNTLNSALAAEQISSPASPVDKPVDEKKQLEEGEIEEN PSEGDSQTKTIFDDASKFNVKHPLFSTWTLYFDSPQSKSLPKTPQTTPAMPQ GSHGWMADIRKVVSFDSVEEFWGLYNNIIPPSQLPGKANYYLFKNGIIPAW EDPQNKNGGKWSIQVPKNSESKSSIDRMWLYTMLAAIGETFETASTDSENA PSPTQSDLITGVIVSPRPAFYRISIWTREASDVNVLDTDAIKARLLNIGKHFKT SVLGYELEQKLTEGGFQTEL TFDAHKDSEKKVNKNKFTV
		XP_003192844.1	>XP_003192844.1 cap binding protein, putative [*Cryptococcus gattii* WM276] MSSTAIPPAAVSANNNALNSALAAEQISSPASPIDKPEDEKKQLEEGEIEENS SEGDSQAKTIFDDASKFNVKHPLFSTWTLYFDSPQSKSLPKTPQTTPAMPQG SHGWMADIRKVVSFDSVEEFWGLYNNIIPPSQLPGKANYYLFKNGIIPAWE DPQNKNGGKWSIQVPKNSESKGSIDRMWLYTMLAAIGETFETPSTDSENAP SPIQSDLITGVIVSPRPAFYRISIWTREASDVNVPDTDAIKARLLNIGKHFKTS VLGYELEQKLTEGGFQTEL TFDAHKDSEKKVNKNKFTV
		XP_012052793.1	>XP_012052793.1 translation initiation factor 4E [*Cryptococcus neoformans* var. *grubii* H99] MSSTTIPPAAVTANSNTLNSALAAEQISSPASPVGKPVDEKKQLEEGEIEENP SESDSQTKTIFDDASKFNVKHPLFSTWTLYFDSPQSKSLPKTPQTTPAMPQG SHGWMADIRKVVSFDSVEEFWGLYNNIIPPSQLPGKANYYLFKNGIIPAWE DPQNKNGGKWSIQVPKNSESKGSIDRMWLYTMLAAIGETFETPSTESETAPS PTQSDLITGVIVSPRPAFYRISIWTREASDINIPDTDAIKARLLNIGKHFKTSVL GYELEQKLTEGGFQTEL TFDAHKDSEKKVNKNKFTV
		KIR87178.1	>KIR87178.1 translation initiation factor 4E [*Cryptococcus gattii* VGIV IND107] MSSTAIPPAAVSANNNALNSALAAEQISSPASPIDKPEDEKKQLEEGEIEENP SEGDSQTKTIFDDASKFNVKHPLFSTWTLYFDSPQSKSLPKTPQTTPAMPQG SHGWMADIRKVVSFDSVEEFWGLYNNIIPPSQLPGKANYYLFKNGIIPAWE DSQNKNGGKWSIQVPKNSESKGSIDRMWLYTMLAAIGETFETPSTDSENAP SPTQSDLITGVIVSPRPAFYRISIWTREASDVNVPDTDAIKARLLNIGKHFKTS VLGYELEQKLTEGGFQTEL TFDAHKDSEKKVNKNKFTV
		KGB77518.1	>KGB77518.1 translation initiation factor 4E [Cryptococcus gattii VGII R265] MSSAAIPPAAVSANNNALNSALAAEQISSPASPIDKPEDEKKQLEEGEIEENP SEGDSQTKTIFDDASKFNVKHPLFSTWTLYFDSPQSKSLPKTPQTTPAMPQG SHGWMADIRKVVSFDSVEEFWGLYNNIIPPSQLPGKANYYLFKNGIIPAWE DPQNKNGGKWSIQVPKNSESKGSIDRMWLYTMLAAIGETFETPSTDSENAP SPTQSDLITGVIVSPRPAFYRISIWTREASDVNVPDTDAIKARLLNIGKHFKTS VLGYELEQKLTEGGFQTEL TFDAHKDSEKKVNKNKFTV
		KIR48974.1	>KIR48974.1 translation initiation factor 4E [Cryptococcus gattii CA1280] MSSTAIPPAAVSANNNALNSALAAEQISSPASPIDKPEDEKKQLEEGEIEENP SEGDSQTKTIFDDASKFNVKHPLFSTWTLYFDSPQSKSLPKTPQTTPAMPQG SHGWMADIRKVVSFDSVEEFWGLYNNIIPPSQLPGKANYYLFKNGIIPAWE DPQNKNGGKWSIQVPKNSESKGSIDRMWLYTMLAAIGETFETPSTDSENAP SPTQSDLITGVIVSPRPAFYRISIWTREASDVNVPDTDAIKARLLNIGKHFKTS VLGYELEQKLTEGGFQTELTFDAHKDSEKKVNKNKFTV
6.	Phospholipase-B	XP_012052996.1	>XP_012052996.1 phospholipase B [Cryptococcus neoformans var. grubii H99] MSIATATFAFSLFATIAFAVPPETPRIELQAERGLGDKSYAPWQVDCPSNVT WIRNATTGLGSGERAYIEAREKLVQPVIEQMMAARGLETPPRTPNIGVALS GGGYRAMLTGLGGIMGMMNESTEASESETGGWLDGVSYWAGLSGGSWA TGTFMSNGGQLPTNLLENLWNIDSNLVFPDDDKLSFYTELYTETNAKSDLG FPIQITDVWGLAIGSHVLPERYQLSNTPNLTFSSLPSVVSALGNASLPMPIIIA AEREAGELVIAENATVWEFTPYEFGSWAFGSQYKSPGAFTPIEYLGTSVDD GSPNGTCWKGFDQLSFVMGTSATLFNGAFLELNGTDSGLLTNLITAFLADL GEDQADISRIPNTFSNYNSGENPIYNLTYITLVDAGETNQNIPLEPLLVPTRD VDAIVAFDSSYDTDYIWPNGTALRTTYERAKVLAEHENTRVLMPEVPSMN GFVNGGYNSRPTFFGCNDTTTPLIIYVPSYPWSFAANTSTYQLSYENDEANE MLLNGMRSLTLNHSVPTWPTCFACALTDRSFMYTSENRSTTCQKCFDTWC WAGDDNTTEPATYEPVINSVPPWLVANNLSIGVADAPASNESTAGTASSGA AKADVSMGMVALAAGLGLML
		AAF61964.1	>AAF61964.1 phospholipase B [*Cryptococcus neoformans* var. *neoformans*]

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			MSIITTAFALSLLATTAFAVPPETPRIELQAERGLGDQSYAPWQVDCPSNVT WIRNATTGLGTGERAYIEAREKLVQPAIEQMMAARGLETPPRTPVIGVALA GGGYRAMLTGLGGIMGMMNESTEASQSETGGWLDGVSYWSGLSGGSWA TGSFMSNGGQLPTTLLENLWNIDSNLVFPDDGKLSFYTNLYTETNAKSDLG FPVQITDIWGLAIGSHVLPEPYQLSNTPNLTFSSLPSVVAALGNASLPMPIIVA AEREAGELVIAENATVWEFTPYEFGSWAFGSQYKSPGAFTPIEYLGTSVDD GSPNGTCWKGFDQLSFVMGTSATLFNGAFLELNGTDSGLLTNLITAFLADL GEDQADISRIPNSFSNYNSGENPIYNLTYITLVDAGETNQNIPLEPLLVPTRD VDAIVAFDSSYDSDYIWPNGTALRTTYERAKILAEHENTRVLMPEVPSMNG FVNGGYNSRPTFFGCNDTTTPVIIYIPSYPWSFAANTSTYQLSYENNEANEM LLNGMRSLTLNHSVPTWPTCFACALTDRSFMYTSENRSTTCQECFDTWCW AGDDNTTEPANYEPVINSVPPWLIANNLSIGMADAPGSNESTAGTASSGAA KMGVGMGMVALTAGLGLML
		KIR59014.1	>KIR59014.1 phospholipase B [*Cryptococcus gattii* CA1873] MSVITTTFALSLFAATALAIPPETPRIELHAERGLGDKSYAPWQVDCPTNVT WIRNATTGLGSGERAYIEAREKLVQPAIEHMMAARGLETPPRTPVIGVALA GGGYRAMLTGLGGIMSMMNESTEASESETGGWLEGVSYWSGLSGGSWAT GTFMSNGGQLPTSLLENLWNIDSNLIFPDDDKVSFYAELYIETNAKSDLGFP TQITDLWGLAIGSHVLPEQYQLSNNPNLTFSSLPSVVASLGNASLPMPIIIAA EREAGELIIAENATVWEFTPYEFGSWAFGSQYKSPGAFTPIEYLGTSVNDGS PNGTCWKGFDQLSFVMGTSATLFNGAFLELNGTDSGLLTSLITAFLAELGE DQVDISRIPNTFSNYNSGENPIYNLTYITLVDAGETNQNVPLEPLLIPARAVD AIVAFDASYDTNYIWPNGTALRTTYERARVLAEHENTRVLMPEVPSMNGF VNGGYNSRPTFFGCNDTTTPLIIYVPSYPWSFAANTSTYQLSYETDEANQML LNGMRSLTLNHSVPTWPTCFACALTDRSFMYTSENRSTTCQECFDTWCWA GDDNTTQPAEYEPVINSVPPWLIANNLSLGVADAPASNESTPGTASSGAAKI GVSVGMVALAAGLGLMF
		KIR38674.1	>KIR38674.1 phospholipase B [*Cryptococcus gattii* VGII Ram5] MSVITTTFALSLFAVTALAIPPETPRIELQAERGLGDKSYAPWQVDCPTNVT WIRNATSGLGSGERAYIEAREKLVQPAIEDMMAARGLETPPRTPVIGVALA GGGYRAMLTGLGGIMSMMNESTEASESETGGWLEGVSYWSGLSGGSWAT GTFMSNGGQLPTSLLENLWNIDSNLIFPDDDKVSFYTELYTETNAKSDLGFP TQITDLWGLAIGSHVLPEQYQLSNNPNLTFSSLPSVVAALGNASLPMPIIIAA EREAGELIIAENATVWEFTPYEFGSWAFGSQYKSPGAFTPIEYLGTSVNDGS PNGTCWKGFDQLSFVMGTSATLFNGAFLELNGTDSGLLTSLITAFLAELGE DQADISRIPNTFSNYNSGENPIYNLTYITLVDAGETNQNVPLEPLLVPARVVD AIVAFDASYDTDYIWPNGTALRTTYERARVLAEHENTRVLMPEVPSMNGF VNGGYNSRPTFFGCNDTTTPLIIYVPSYPWSFAANTSTYQLSYETDEANQILL NGMRSLTLNHSVPTWPTCFACALTDRSFMYTSENRSTTCQECFDTWCWAG DDNTTQPAKYEPVINSVPPWLIANNLSLGVANAPASNESTPGTASSDAAKIG VSVGIVALAAGLGLMF
		KIR84691.1	>KIR84691.1 phospholipase B [*Cryptococcus gattii* VGIV IND107] MSVIATTFALSIFAATALAIPPETPPIELQAERGLGDKSYAPWQVDCPTNVT WIRNATTGLGSGERAYIEAREKLVQPAIEHMMAARGLKTPPRTPVIGVALA GGGYRAMLTGLGGIMSMMNESTEASESETGGWLEGVSYWSGLSGGSWAT GTFMSNGGQLPTSLLENLWNIDSNLIFPDDDKVSFYTELYTETNAKSDLGFP TQITDLWGLAIGSHVLPEQYQLSNNPNLTFSSLPSVVAALGNASLPMPIIIAA EREAGELIIAENATVWEFTPYEFGSWAFGSQYKSPGAFTPIEYLGTSVNDGS PNGTCWKGFDQLSFVMGTSATLFNGAFLELNGTDSGLLTSLITAFLAELGE DQVDISRIPNTFSNYNSGENPIYNLTYITLVDAGETNQNVPLEPLLVPARAVD AIVAFDASYDTDYIWPNGTALRTTYERARVLAEHENTRVLMPEVPSMNGF VNGGYNSRPTFFGCNDTTTPLIIYVPSYPWSFAANTSTYQLSYETDEANQILL NGMRSLTLNHSVPTWPTCFACALTDRSFMYTSENRSTICQECFDTWCWAG DDNTTKPAEYEPVINSVPPWLIANNLSLGVADAPASNGSIPGTASSGAAKIG VSVGMVALAAGLGLMF
		KIR52753.1	>KIR52753.1 phospholipase B [*Cryptococcus gattii* Ru294] MSVITTTFALSLFAATALAIPPETPRIELQAERGLGDKSYAPWQVNCPTNVT WIRNATTGLGSGERAYIEAREKLVQPAIEHMMAARGLETPPRTPVIGVALA GGGYRAMLTGLGGIMSMMNESTEASESETGGWLEGVSYWSGLSGGSWAT GTFMSNGGQLPTSLLENLWNIDSNLIFPDDDKVSFYTELYTETNAKSDLGFP TQITDLWGLAIGSHVLPQQYQLSNNPNLTFSSLPSVVAALGNASLPMPIIIAA EREAGELIIAENATVWEFTPYEFGSWAFGSQYKSPGAFTPIEYLGTSVNDGS PNGTCWKGFDQLSFVMGTSATLFNGAFLELNGTDSGLLTSL ITAFLAELGEDQADISRIPNTFSNYNSGENPIYNLTYITLVDAGETNQNVPLE PLLIPARAVDAIVAFDASYDTDYIWPNGTALRTTYERARVLAEHENTRVLM PEVPSMNGFVNGGYNSRPTFFGCNDTTTPLIIYVPSYPWSFAANTSTYQLSY ETDEANQILLNGMRSLTLNHSVPTWPTCFACALTDRSFMYTSENRSTICQEC

S.no.	Virulence factors	Accession No.	Sequence in FASTA format
			FDTWCWAGDDNTTQPAEYEPVINSVPPWLIANNLSLGVADAPASNESTPGT ASSGAAKIGVSVGMVALAAGLGLMF
7.	GalXM	XP_568018.1	>XP_568018.1 cryptococcal xylosyltransferase 1 [*Cryptococcus neoformans* var. *neoformans* JEC21] MPLNLPFSSPLKLPLPRRFIILILSASILILFLHTFAPSTLPPVLTPNLPHHEPDA SYFSPSKWLPPILNPNAPTRPLEFDEDGQCLFLSPFDALSAAEKARARVLSLD EISPGIVRADAPPAEGTDADPDFDDEFSELSNATRKMPAGLTHPILGLLRDG EAKWNSMVTMQSQTLEQAVDVYMDRWGRRPPKGFDEWWHFAKANNVL LPDEYDPIMNSLLPFYALPIDTLKERLVEAEKIPETFTLIVHDGKVELKWND DYSRDTWWASRPRADSQINLMEPFIKHIGTFRATFTIHDQPSILLDHERQEEL LTAARHGKISTHPNELDRAEQNWRKACPPDSPLNKGEEELEAPDSFISSHLA AMDICQHPSYMENHGMLLEEKNSDSHPKPHTKLYPILVPSKTALNGDIPVTP IGKDGRRDDIGHDPEWNRKSGKLYWRGLATGLQHNKKAGAKWRQSHRER LHFLANDKSDTYTEVLSPVGSSGEAELARMPLRELGQYYMDVKLAGGNW QCDWGDGTCEEMEKEIDFAPKDSSERSNDFKYVFDTDGNAWSSRFPRLMA SNNVVIKSTVFPEWNTNSLPEWYAYVPSKMDYSDLFSIMTFFRGTPSGRGA HDEVARRIALNGQCWVERTWRREDLQAYMFRLYLEYARLTSPDRDNGKM DYVSTQKKASKEADDVPAAADIETPVIDQ
		KIR46264.1	>KIR46264.1 beta-1,2-xylosyltransferase 1 [*Cryptococcus gattii* CA1280] MPLNLPFSSALKLPLPRRFIILILSASILILFLHTFAPSTLPPVLTPNLQHHEPDA SYFSPSKWLPPILNPNAPTRPLEFDEEGQCLFLSPFDALSAAEKARAQVLSLN EISPGIVRAEAPPAEGTDADPDFDDEFSELSNATRKMPTGLTHPILGLLRDGE AKWNSMLARQSQTLEEAVNVYIERWGRKPPKGFDEWWHFAKSNNVLLPD EYDAIMNSLLPFYALPIETLKERLAEAEKIPETFTLIVHDGKVELQWNDDYS RDTWWASRPRADSQINLMEPFIKHIGTFRATFTIHDQPSVLLDYERQKELLT AARQGKISTHPNEIDRAEQNWKKACAPDSPLNKGEEELEASDSFISSHLAA MDICQHPSYLENHGMLLEEKNSDTHPKPHTKLYPILVPSKTALNGDIPVTPI GKDGRRDDVGHDPEWSRKSGKLYWRGLATGLQHNKKAGAKWRQSHRER LHFLANDKSDAYTEVLSPVGSSGEAELAQMPLKELGQYYMDVKLAGGNW QCDWGDGTCEEMEKEIDFAPKDSSERSNDFKYVFDTDGNAWSSRFPRLMA SNNVVIKSTVFPEWNTNSLPEWYAYVPSKMDYSDLFSIMTFFRGTPSGRGA HDEVARRIALNGQCWVERTWRREDLQAYMFRLYLEYARLTSPDRDNGKM DYVPTPKKISNVAHGVHVAADVEPPVDQ
		KIR75920.1	>KIR75920.1 beta-1,2-xylosyltransferase 1 [*Cryptococcus gattii* VGII CA1014] MPLNLPFSSALKLPLPRRFIILILSASILILFLHTFAPSTLPPVLTPNLQHHEPDA SYFSPSKWLPPILNPNAPTRPLEFDEEGQCLFLSPFDALSAAEKARAQVLSLN EISPGIVRAEAPPAEGTDADPDFDDEFSELSNATRKMPPGLTHPILGLLRDGE AKWNSMLARQSQTLEEAVNVYIERWGRKPPKGFDEWWHFAKANNVLLPD EYDAIMNSLLPFYALPIETLKERLTEAEKIPETFTLIVHDGKVELQWNDDYS RDTWWASRPRADSQINLMEPFIKHIGTFRATFTIHDQPSVLLDYERQKELLT AARQGKISTHPNEIDRAEQNWKKACAPDSPLNKGEEELEASDSFISSHLAA MDICQHPSYLENHGMLLEEKNSDTHPKPHTKLYPILVPSKTALNGDIPVTPI GKDGRRDDVGHDPEWSRKSGKLYWRGLATGLQHNKKAGAKWRQSHRER LHFLANDKSDAYTEVLSPVGSSGEAELAQMPLKELACRWKLAM
		KIR85290.1	>KIR85290.1 beta-1,2-xylosyltransferase 1 [*Cryptococcus gattii* VGIV IND107] MPLNLPFSSALKLPLPRRFIILILSASILILFLHTFAPSTLPPVLTPNLQHHEPDA SYFSPSKWLPPILNPNAPTRPLEFDEEGQCLFLSPFDALSAAEKARAQVLSLN EISPGIVRAEAPPAEGTDADPDFDDEFSELSNATRKMPTGLTHPILGLLRDGE AKWNSMLARQSQTLEEAVNVYIERWGRKPPKGFDEWWHFAKANNVLLPD EYDAIMNSLLPFYALPIETLKERLTEAEKIPETFTLIVHDGKVELQWNDDYS RDTWWASRPRADSQINLMEPFIKHIGTFRATFTIHDQPSVLLDYERQKELLT AARQGKISTHRNEIDRAEQNWKKACAPDSPLNKGEEELEASDSFISSHLAA MDICQHPSYLENHGMLLEEKNADTHPKPHTKLYPILVPSKTALNGDIPVTPI GKDGRRDDVGHDPEWSRKSGKLYWRGLATGLQHNKKAGAKWRQSHRER LHFLANDKSDAYTEVLSPVGSSGEAELAQMPLKELGQYYMDVKLAGGNW QCDWGDGTCEEMEKEIDFAAKDSSERSNDFKYVFDTDGNAWSSRFPRLMA SNNVVIKSTVFPEWNTNSLPEWYAYVPSKMDYSDLFSIMTFFRGTPSGRGA HDEVARRIALNGQCWVERTWRREDLQAYMFRLYLEYARLTSPDRDNGKM DYVPTPKKISNVAHGVPVAADVEPPVDQ
		XP_012048740.1	>XP_012048740.1 beta-1,2-xylosyltransferase 1 [*Cryptococcus neoformans* var. *grubii* H99] MPLNLPFSSPLKLPLPRRFIILILSASILILFLHTFAPSTLPPVFTPSLPHHEPDAS YFSPSKWLPPILNPNAPTRPLEFDENGQCLFLSPFDALSAAEKARAQVLSLD EVSPGIVRADAPPAEGTDADPDFDDEFSELSNATRKMPAGLTHPILGLLRDG EAKWNSMLARQSQTLEQAVDVYIDRWGRKPPKGFDEWWHFAKANNVLL PDEYDPIMNSLLPFYALPIDTLEERLIEAEKIPETFTLIVHDGKVELKWNDDY SRDTWWASRPRADSQINLMEPFIKHIGTFRATFTIHDQPSILLDHERHEELLT AARHGKVSTHPNELDRAEQNWKKACPPDSPLNKGEVELEAPDSFISSHLAA

S.no.	Virulence factors	Accession No.	Sequence in FASTA format
			MDICQHPSYMENHGMLLEEKNSDTHPKPHTKLYPILVPSKTALNGDIPVTPI GKDGRRDDIGHDPEWSRKSGKLYWRGLATGLQHNKKAGAKWRQSHRER LHFLANDKSDTYTEVLSPVGSTGEAELAQMPLRELGQYYMDVKLAGGNW QCDWGDGTCDEMEKEIDFAPKDSSERSNDFKYVFDTDGNAWSSRFPRLMA SNNVVIKSTVFPEWNTNSLPEWYAYVPSKMDYSDLFSIMTFFRGTPSGRGA HDEVARRIALNGQCWVERTWRREDLQAYMFRLYLEYARLTSPDRDNGKM DYIPSQERP
		KIR54007.1	>KIR54007.1 beta-1,2-xylosyltransferase 1 [*Cryptococcus gattii* Ru294] MPLNLPFSSALKLPLPRRFIILILSASILILFLHTFAPSTLPPVLTPNLQHHEPDA SYFSPSKWLPPILNPNAPTRPLEFDEEGQCLFLSPFDALSAAEKARAQVLSLN EISPGIVRAEAPPAEGTDADPDFDDEFSELSNATRKMPTGLTHPILGLLRDGE AKWNSMLARQSQTLEEAVNVYIERWGRKPPKGFDEWWHFAKSNNVLLPD EYDAIMNSLLPFYALPIETLKARLAEAEKIPETFTLIVHDGKVELQWNDDYS RDTWWASRPRADSQINLMEPFIKHIGTFRATFTIHDQPSVLLDYERQKELLT AARQGKISTHPNEIDRAEQNWKKACAPDSPLNKGEEELEASDSFISSHLAA MDICQHPSYLENHGMLLEEKNSDTHPKPHTKLYPILVPSKTALNGDIPVTPI GKDGRRDDVGHDPEWSRKSGKLYWRGLATGLQHNKKAGAKWRQSHRER LHFLANDKSDAYTEVLSPVGSSGEAELAQMPLKELGQYYMDVKLAGGNW QCDWGDGSCEEMEKEIDFAPKDSSERSNDFKYVFDTDGNAWSSRFPRLMA SNNVVIKSTVFPEWNTNSLPEWYAYVPSKMDYSDLFSIMTFFRGTPSGRGA HDEVARRIALNGQCWVERTWRREDLQAYMFRLYLEYARMTSPDRDNGK MDYVPTPKKASNVAHGVPVAADVEPPVNQ
8.	MP	XP_567104.1	>XP_567104.1 88 kDa immunoreactive mannoprotein MP88, putative [*Cryptococcus neoformans* var. *neoformans* JEC21] MISKVALGAAAALMAGVANVNAQVTATGTMGPTNPSEPTLGTAINQTSYA RLLSLNAIDDFCLFAPPEPDSVIGDTEAEEVAWCVQPRNNARVIPDGVLTAV HFVKTPLYWQIQGFGDFTHLNIQDGDEGGELDPHGATGLGNPVGGNVTTN ATGSDVSYEEWMNYMAYDQFCLRICISENSTYSAANECQHTLDEMGCSWV MPGDYTNNSFTECDGDSAYPPGWYILANGSTSTFQQRYTGTYTNGDGSLG TWTQGETVTPQTAYSTPATSNCKTYTSVGNGIASLALSNAGSVNSTAAATN SSSGGASAAATGSSSSGSTAGSSAGSSAGSGSGSAAAGSTAAASSSGDSSSS TSAAMSNGINYGTAMAGVISVVALVAGAGSFLL
		CAC78985.1	>CAC78985.1 macrofage activating glycoprotein [*Cryptococcus neoformans* var. *neoformans*] MGPTNPPEPTLGTPINQTSYARLLSLNAIDDFCLFAPPEPDSVIGDTEAEEVA WCVQPRNNARVIPDGVLTAVHFVKTPLYWQIQGFGDFTHLNIQDGDEGGE LDPHGATGLGNPVGGNVTTNATGSDVSYEEWMNYMAYDQFCLRICISENS TYSAANECQHTLDEMGCSWVMPGDYTNNSFTECDGDSAYPPGWYILANG STSTFQQRYTGTYTNGDGSLGTWTQGETVTPQTAYSTPATSNCKTYTSVGN GIASLALSNAGSVNSTAAATNSSSGGASAAATGSSSSGSTAGSSAGSSAGSG SGSAAAGSTAAASSSGDSSSSTSAAMSNGINYGTAMAGVISVVALVAGAGS FLL
		XP_012046953.1	>XP_012046953.1 immunoreactive mannoprotein MP88 [*Cryptococcus neoformans* var. *grubii* H99] MISKVAVGAAAALMAGVANVNAQVTATGTMGPTNPPEPTLGTPINQTSYA RLLSLNAIDDFCLFAPPVPNSVIGETEAEEVAWCVQPRNNARVIPDGVLTAV HFVKTPLYWQIQGFGDFTHLNIQSGDEGGELDPHGATGLGNPVGGNVTTN ATGSDVSYEEWMNYMAADQFCLRICISENSTYSAANECQHTLDEMGCSWV MPGDYTADSFTECDGDSAYPPGWYILGNGSTSTFQQRYTGTFTGADGSLGT WTQGETVTPQSAYSTPASSNCKTYTSVGNGIASLALSNAGSVNSTASATNSS SGGSSAAATRSSSSGSSAGSGSGSAVAGSTAAASSSGESSSSTSAAMSSFNGI SYGTAMAVVALVAGAGSFLL
		KGB75317.1	>KGB75317.1 immunoreactive mannoprotein MP88 [*Cryptococcus gattii* VGII R265] MGCAPDGKWQSSPHVSFHHPSNVAIGAAAALMAGVANVNAQVTATGTM GPTNPPAATLGTAINQTSYARLLSLNAIDDFCLFAPPVPNSVIGETEAEEVA WCVQPRNDARVIPDGVLTAVHFVKTPLYWQIQGFGDFTHLNIQSGDEGGEL DPHGATGLGNPVGGNVTTNATGSDVSYEEWMNYMAFDQFCLRICISENDT YSAANECQHTLDEMGCSWVMPGDYTNNSFTECDGDSAYPPGWYPEANGS TSTFQQRYTGTFTNADGSLGTWTQGETVTPQSAYSIPATSNCKTYTSVGNGI SSLALSNAGSVNSTAASTGSSSGSSPAAATGSSSSGGSGASGSANAGSTAAA SASGSSSKSAAMSSFSGVNYGSAVAGAISVVALVAGAGSFLL
		KIR51504.1	>KIR51504.1 immunoreactive mannoprotein MP88 [*Cryptococcus gattii* Ru294] MISKVAIGAAAALMAGVANVNAQVTATGTMGPTNPPAATLGTAINQTSYA RLLSLNAIDDFCLFAPPVPNSVIGETEAEEVAWCVQPRNDARVIPDGVLTAV HFVKTPLYWQIQGFGDFTHLNIQSGDEGGELDPHGATGLGNPVGGNVTTNS TGSDVSYEEWMNYMAFDQFCLRICISENDTYSAANECQHTLDEMGCSWV MPGDYTNNSFTECDGDSAYPPGWYPEANGSTSTFQQRYTGTFTNADGSLG

S.no.	Virulence factors	Accession No.	Sequence in FASTA format
			TWTQGQTVTPQSAYSVPATSNCKTYTSVGNGIASLALSNAGSVNSTGASSG SSSSNSPAAATGSSSSSGGSGASGSANAGSTAAASASGSSSNSGAMSSFSGV NYGSAMAGAISVVALVAGAGSFLL
		KIR83819.1	>KIR83819.1 immunoreactive mannoprotein MP88 [*Cryptococcus gattii* VGIV IND107] MISKVAIGAAAALMAGVANVNAQVTATGTMGPTNPPAATLGTAINQTSYA RLLSLNAIDDFCLFAPPVPNSVIGETEAEEVAWCVQPRNDARVIPDGVLTAV HFVKTPLYWQIQGFGDFTHLNIQNGDEGGELDPHGATGLGNPVGGNVTTN STGSDVSYEEWMNYMSFNQFCLRICISENDTYSAANECQHTLDEMGCSWV MPGDYTNNSFTECDGDSAYPPGWYPEANGSTSTFQQRYTGTFTNADGSLG TWTQGQTVTPQSAYSVPATSNCKTYTSVGNGISSLALSNAGSVNSTAASSG SSSGNSPAAATGSSSSGGSGASGSANAGSTAAASASGSSTKSGAMSSFSGVN YGSAMAGAISVVALVAGAGSFLL

The pathogenic potential of virulence factors of *Cryptococcus* was evaluated *in-silico* using orthologs. We retrieved 48amino acid sequences of different virulence factors of *Cryptococcus* species. In 2019, Elhassan *et al*. retrieved 38 amino acid sequences of heat shock 70 KDa protein of *C. n.grubii* from the NCBI protein database and used them for *in-silico* epitopes prediction and vaccine development [36]. In another study, Sati *et al.* retrieved 28 amino acid sequences of pyruvate kinase protein of *C. albicans* from NCBI and were subjected to multiple sequence alignment and epitope prediction [37]. In another study, Schneider *et al.* (2015) retrieved sequences of four zinc transporter genes of *C. gattii* R265 for phylogenetic analysis [38].

3.2. Maximum likelihood test and hierarchical clustering of virulence proteins of Cryptococcus species.

The shape of a gamma distribution is a statistical numerical parameter showing variation. Its higher value indicates the low variation of substitution rates among sites. Amino acid sequences of laccase, CAP binding protein, and mannoprotein (MP) of different *Cryptococcus* species with gamma distribution values 97.967, 97.898, and 97.759, respectively, were found to be highly conserved (Table 2). In contrast, GalXM, GXM, and SOD's low gamma distribution values represent moderate and high rates of variation, respectively. Amongst all virulence factors, superoxide dismutase (SOD) with the lowest gamma distribution value (0.907) was considered as highly adaptable (Table 2) and susceptible to selection pressure.

Table 2. Gamma distribution parameter for virulence factors of *Cryptococcus* species.

S.No.	Virulence Factors	Gamma distribution parameter
1	SOD	0.907
2	GalXM	1.088
3	GXM	1.312
4	Phospholipase B	8.300
5	Urease	37.894
6	MP	97.759
7	CAP binding protein	97.898
8	Laccase	97.967

Hierarchical clustering grouped the virulence factors of *C. n. neoformans, C. n. grubii,* and *C. gattii* into 8 clusters and 48 cluster orders. It also demonstrated the formation of homogenous groups of variables and phylogenetic evolutionary relationship among different virulence factors of *Cryptococcus* species (Figure 1). Laccase, CAP binding protein, and MP

showed maximum similarity as compared to other virulence factors. SOD proteins, however, were observed to be distantly located in the dendrogram.

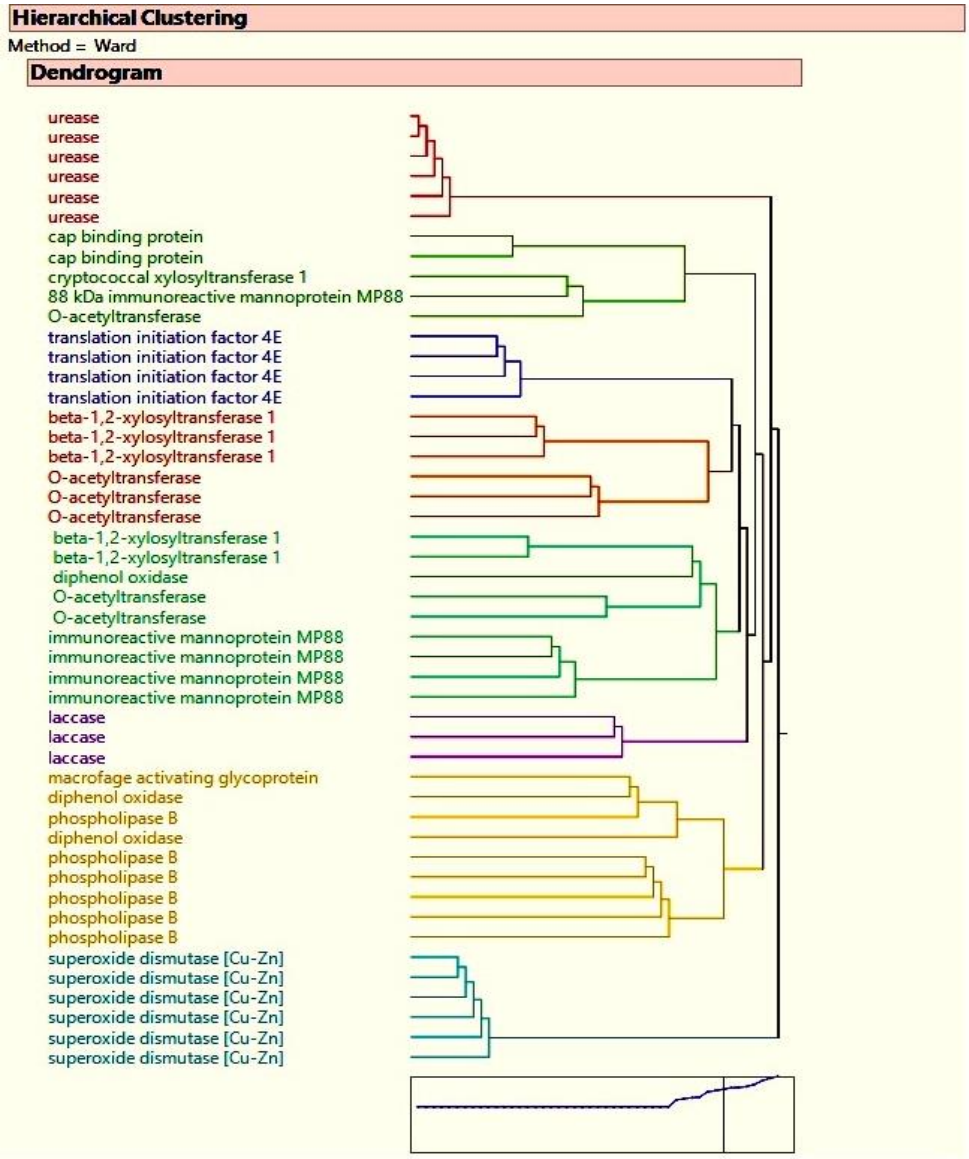

Figure 1. Phylogenetic relationship and hierarchical clustering of virulence factors of *Cryptococcus* species

Phylogenetic analysis in the present study recognized a core set of virulence proteins in the pathogenic species of *Cryptococcus*. The orthologs were used to detect the evolution rate, which was further clustered hierarchically to assess the relationship and variation amongst the virulence proteins of *Cryptococcus* species. All virulence proteins except SOD were grouped separately in one cluster.

Cu, Zn superoxide dismutase encoded by the SOD1 gene is crucial for the pathogenesis of *Cryptococcus*[16]. The low estimated value of the gamma distribution parameter symbolizes a high rate of variation in substitution. Unlike its highly conserved mammalian counterparts [39], Cu, Zn SODof *Cryptococcus* was highly variable and can be governed by natural selection pressure. The resultant adaptability might enhance its importance in cryptococcal pathogenesis.

3.3. B-cell linear epitope prediction in virulence proteins of Cryptococcus species.

The consensuses epitopes were obtained from three web servers ABCpred, BCPred, and BcePred (Table 1). The virulence factors of *C. n. grubii* were found to have more B-cell

epitopes than those of *C. n. neoformans* and *C. gattii*. The maximum number of predicted epitopes was observed in the urease of *C. n. grubii.*

Table 1. Consensuses of linear epitopes of virulence factors in *Cryptococcus* sps. predicted by online web servers.

Species of *Cryptococcus*	Virulence factors	No. of epitopes	Start Position	Predicted epitope residues
C. n. neoformans	Cap Binding Protein	1	89	SKSLPKTPQT
		2	41	KKQLEEGEIEENP
		3	200	STDSENAPSPT
		4	174	SESKSS
	GalXM	1	73	APTRPLEFD
		2	344	SPLNKGEEELE
		3	673	KASKEAD
		4	635	TWRREDL
		5	523	TCEEMEKEID
		6	258	WNDDYSRD
	GXM	1	327	HPDPPPVVIPSVL
		2	831	SSTSPGRQAT
	Laccase	1	230	TNCTATNSS
		2	570	NTDPNSFGPARRSPSPSIQ
		3	176	QRGRDYDEDR
		4	48	PTTREYT
	MP	1	317	ASSSGDSSSST
		2	277	SGGASAAATGSSSSG
		3	206	TFQQRYTGT
		4	96	QDGDEGG
	Phospholipase-B	1	600	PGSNESTAGTAS
		2	484	VIIYIP
		3	569	NTTEPAN
		4	127	ASQSE
		5	371	SFSNYNSG
	SOD	1	53	DNTNGCT
		2	128	GKGGNEESLK
		3	78	AERHV
	Urease	1	253	HKDQEKVEEGPT
		2	427	PADGSNATTCT
		3	656	GDEPTRDNNRVKR
		4	460	TGKGNDSG
C. n. grubii	Cap Binding Protein	1	89	SKSLPKTPQT
		2	41	KKQLEEGEIEENPSESDS
		3	199	PSTESETAPSPT
		4	160	NKNGG
	GalXM	1	73	APTRPLEF
		2	634	TWRREDL
		3	523	TCDEMEKEI
		4	331	RAEQNWK
		5	419	GKDGRRDD
	GXM	1	835	SRQAT
		2	856	PQDGA
		3	245	PGLLPQQSP
		4	134	WESEGE
		5	889	LERRDSW
	Laccase	1	219	EGYKGSP
		2	320	NTSEGKE
		3	183	NEPLQRGRDYDEDR
		4	362	STEEPQT
	MP	1	338	ASSSGESSS

Species of *Cryptococcus*	Virulence factors	No. of epitopes	Start Position	Predicted epitope residues
		2	299	ASATNSSSGGSS
		3	270	ASSNCKT
	Phospholipase B	1	600	PASNESTAGTAS
		2	90	ETPPRTP
		3	569	NTTEPAT
		4	485	IIYVPS
		5	123	STEASES
		6	504	LSYENDEA
	SOD	1	69	KKHHG
		2	75	PTDSER
		3	126	DLGKGGNEESLK
	Urease	1	253	HKEQEKIE
		2	427	PADGSNATTCT
		3	656	GDEPTRDNNRVKR
		4	318	GGQASGRHD
		5	460	TGKGSDSG
		6	204	EPGEKKT
		7	784	SIGKKDMK
C. gattii	Cap Binding Protein	1	35	DKPEDEKKQLEEGEIEE
		2	89	SKSLPKTPQT
		3	197	ETPSTDSENA
		4	160	NKNGG
		5	171	PKNSESKGS
	GalXM	1	73	APTRPLEFDE
		2	345	PLNKGEEELEA
		3	658	PDRDNGK
		4	419	GKDGRRDDVGH
		5	634	RTWRREDL
		6	270	SRPRADS
	GXM	1	373	HEGAT
		2	133	KAWESEGEKHTD
	Laccase	1	176	RGRDYDEDR
		2	309	NTSEGKE
		3	48	PTTREYT
	MP	1	313	ATGSSSSSGGSGASGS
		2	296	NSTGASSGSSSS
		3	149	VTTNSTGS
	Phospholipase B	1	600	PASNESTPGTAS
		2	568	NTTQPAEY
		3	485	NTTQPAEY
		4	127	ASESE
		5	372	FSNYNSG
	SOD	1	20	TQEKE
		2	125	DFGKGG
		3	88	KTDGNG
	Urease	1	253	HKEQEKVE
		2	427	PADGSNATTC
		3	656	GDEPTRDNNRVKR
		4	460	TGKGNDSGT
		5	204	EPGEKKT
		6	582	LMVCHHLD

Acquaintance about virulence is also essential to identify potent antifungal targets. In 2018, Khalil and co-workers predicted 11 conserved antigenic B-cell epitopes in MP88 (mannoprotein) of *C. neoformans* and designed a peptide vaccine against it using *in-silico* simulations [40]. However, the current study utilized sequences of virulence proteins of *C. n.*

neoformans, *C. n. grubii*, and *C. gattii* for linear B cell-binding epitope prediction. Silva *et al.* (2019) highlighted new methodologies using bioinformatic tools and servers that have led to the development of new vaccines, like peptide-based vaccines [41].

As humans lack urease, a nickel-containing enzyme [42], therefore, the B-cell epitopes of *C. n. grubii* urease viz HKEQEKIE, PADGSNATTCT, GDEPTRDNNRVKR, GGQASGRHD, TGKGSDSG, EPGEKKT, and SIGKKDMK predicted in this study could be useful in designing and developing of the epitope-based vaccine against cryptococcal infections.

4. Conclusions

Induction of adaptation and production of true virulence factors are two important processes in cryptococcal virulence. The study reveals the phylogenetic relationship among orthologs of virulence proteins of *C. n. neoformans*, *C. n. grubii*,and *C. gattii*. Superoxide dismutase (SOD) proteins of *Cryptococcus* exhibited independent evolvement and high variability. Thus could contribute significantly to the pathogenicity of *Cryptococcus.* This study further performed B-cell epitope prediction using a bioinformatics approach and reports urease as a target for epitope-based anti-cryptococcal drugs. However, the results should be corroborated by experimental studies.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Ajello, L. The medical mycological iceberg, p. In Proc: Xth Intern. Symposium Mycoses, PAHO *Scient. Publ*., Washington DC, **1970.**
2. Munivenkataswamy, R.; Gopi, A.; Shaik Mohammed Usman, J. Human immunodefeciency virus associated cryptococcal meningitis at a tertiary care centre: diagnostic tools and antifungal susceptibility testing. *Journal of clinical and diagnostic research: JCDR* **2013**, *7*, 1623, https://doi.org/10.7860/JCDR/2013/6147.3271.
3. Kronstad, J.; Saikia, S.; Nielson, E.D.; Kretschmer, M.; Jung, W.; Hu, G.; Geddes, J.M.H.; Griffiths, E.J.; Choi, J.; Cadieux, B.; Caza, M.; Attarian, R. Adaptation of*Cryptococcus neoformans* to Mammalian Hosts: Integrated Regulation of Metabolism and Virulence. *Eukaryot. Cell* **2012**, *11*, 109, https://doi.org/10.1128/EC.05273-11.
4. Cherniak, R.; Sundstrom, J.B. Polysaccharide antigens of the capsule of Cryptococcus neoformans. *Infect. Immun.* **1994**, *62*, 1507-1512, https://doi.org/10.1128/IAI.62.5.1507-1512.1994.
5. Casadevall, A.; Coelho, C.; Cordero, R.J.B.; Dragotakes, Q.; Jung, E.; Vij, R.; Wear, M.P. The capsule of Cryptococcus neoformans. *Virulence* **2019**, *10*, 822-831,https://doi.org/10.1080/21505594.2018.1431087.
6. de Oliveira, H.C.; Trevijano-Contador, N.; Garcia-Rodas, R. Cryptococcal Pathogenicity and Morphogenesis. *Current Fungal Infection Reports* **2019**, *13*, 67-76,https://doi.org/10.1007/s12281-019-00340-y.
7. Kozel, T.R.; Levitz, S.M.; Dromer, F.; Gates, M.A.; Thorkildson, P.; Janbon, G. Antigenic and Biological Characteristics of Mutant Strains of *Cryptococcus neoformans*Lacking Capsular O Acetylation or Xylosyl Side Chains. *Infect. Immun.* **2003**, *71*, 2868, https://doi.org/10.1128/IAI.71.5.2868-2875.2003.

8. Probert, M.; Zhou, X.; Goodall, M.; Johnston, S.A.; Bielska, E.; Ballou, E.R.; May, R.C. A Glucuronoxylomannan Epitope Exhibits Serotype-Specific Accessibility and Redistributes towards the Capsule Surface during Titanization of the Fungal PathogenCryptococcus neoformans. *Infect. Immun.* **2019**, *87*, e00731-00718, https://dx.doi.org/10.1128%2FIAI.00731-18.
9. De Jesus, M.; Moraes Nicola, A.; Chow, S.-K.; Lee, I.R.; Nong, S.; Specht, C.A.; Levitz, S.M.; Casadevall, A. Glucuronoxylomannan, galactoxylomannan, and mannoprotein occupy spatially separate and discrete regions in the capsule of Cryptococcus neoformans. *Virulence* **2010**, *1*, 500-508,https://doi.org/10.4161/viru.1.6.13451.
10. Toplis, B.; Bosch, C.; Schwartz, I.S.; Kenyon, C.; Boekhout, T.; Perfect, J.R.; Botha, A. The virulence factor urease and its unexplored role in the metabolism of Cryptococcus neoformans. *FEMS Yeast Res.* **2020**, *20*,https://doi.org/10.1093/femsyr/foaa031.
11. Olszewski, M.A.; Noverr, M.C.; Chen, G.-H.; Toews, G.B.; Cox, G.M.; Perfect, J.R.; Huffnagle, G.B. Urease expression by Cryptococcus neoformans promotes microvascular sequestration, thereby enhancing central nervous system invasion. *The American journal of pathology* **2004**, *164*, 1761-1771, https://doi.org/10.1016/S0002-9440(10)63734-0.
12. Almeida, F.; Wolf, J.M.; Casadevall, A. Virulence-Associated Enzymes of *Cryptococcus neoformans*. *Eukaryot. Cell* **2015**, *14*, 1173,https://doi.org/10.1128/EC.00103-15.
13. Zaragoza, O. Basic principles of the virulence of Cryptococcus. *Virulence* **2019**, *10*, 490-501, https://doi.org/10.1080/21505594.2019.1614383.
14. Williamson, P.R. Laccase and melanin in the pathogenesis of Cryptococcus neoformans. *Front. Biosci.* **1997**, *2*, e99-107, https://doi.org/10.2741/a231.
15. Park, Y.-D.; Chen, S.H.; Camacho, E.; Casadevall, A.; Williamson, P.R. Role of the ESCRT Pathway in Laccase Trafficking and Virulence of*Cryptococcus neoformans*. *Infect. Immun.* **2020**, *88*, e00954-00919,https://doi.org/10.1128/IAI.00954-19.
16. Narasipura, S.D.; Ault, J.G.; Behr, M.J.; Chaturvedi, V.; Chaturvedi, S. Characterization of Cu,Zn superoxide dismutase (SOD1) gene knock-out mutant of Cryptococcus neoformans var. gattii: role in biology and virulence. *Mol. Microbiol.* **2003**, *47*, 1681-1694,https://doi.org/10.1046/j.1365-2958.2003.03393.x.
17. Smith, A.D.; Garcia-Santamarina, S.; Ralle, M.; Loiselle, D.R.; Haystead, T.A.; Thiele, D.J. Transcription factor-driven alternative localization of Cryptococcus neoformans superoxide dismutase. *J. Biol. Chem.* **2021**, 100391, https://doi.org/10.1016/j.jbc.2021.100391.
18. Cox, G.M.; Harrison, T.S.; McDade, H.C.; Taborda, C.P.; Heinrich, G.; Casadevall, A.; Perfect, J.R. Superoxide Dismutase Influences the Virulence of Cryptococcus neoformans by Affecting Growth within Macrophages. *Infect. Immun.* **2003**, *71*, 173,https://doi.org/10.1128/IAI.71.1.173-180.2003.
19. Narasipura, S.D.; Chaturvedi, V.; Chaturvedi, S. Characterization of Cryptococcus neoformans variety gattii SOD2 reveals distinct roles of the two superoxide dismutases in fungal biology and virulence. *Mol. Microbiol.* **2005**, *55*, 1782-1800,https://doi.org/10.1111/j.1365-2958.2005.04503.x.
20. Santangelo, R.; Zoellner, H.; Sorrell, T.; Wilson, C.; Donald, C.; Djordjevic, J.; Shounan, Y.; Wright, L. Role of Extracellular Phospholipases and Mononuclear Phagocytes in Dissemination of Cryptococcosis in a Murine Model. *Infect. Immun.* **2004**, *72*, 2229,https://doi.org/10.1128/IAI.72.4.2229-2239.2004.
21. Buchanan, K.L.; Murphy, J.W. What makes Cryptococcus neoformans a pathogen? *Emerging Infect. Dis.* **1998**, *4*, 71-83,https://doi.org/10.3201/eid0401.980109.
22. Soria-Guerra, R.E.; Nieto-Gomez, R.; Govea-Alonso, D.O.; Rosales-Mendoza, S. An overview of bioinformatics tools for epitope prediction: Implications on vaccine development. *J. Biomed. Inf.* **2015**, *53*, 405-414,https://doi.org/10.1016/j.jbi.2014.11.003.
23. Potocnakova, L.; Bhide, M.; Pulzova, L.B. An Introduction to B-Cell Epitope Mapping and In Silico Epitope Prediction. *Journal of Immunology Research* **2016**, *2016*, 6760830,https://doi.org/10.1155/2016/6760830.
24. Larsen, J.E.P.; Lund, O.; Nielsen, M. Improved method for predicting linear B-cell epitopes. *Immunome Res.* **2006**, *2*, 1-7, https://doi.org/10.1186/1745-7580-2-2.
25. Sun, P.; Ju, H.; Liu, Z.; Ning, Q.; Zhang, J.; Zhao, X.; Huang, Y.; Ma, Z.; Li, Y. Bioinformatics Resources and Tools for Conformational B-Cell Epitope Prediction. *Comput. Math. Methods Med.* **2013**, *2013*, 943636, https://doi.org/10.1155/2013/943636.
26. Raoufi, E.; Hemmati, M.; Eftekhari, S.; Khaksaran, K.; Mahmodi, Z.; Farajollahi, M.M.; Mohsenzadegan, M. Epitope Prediction by Novel Immunoinformatics Approach: A State-of-the-art Review. *Int. J. Pept. Res. Ther.* **2020**, *26*, 1155-1163,https://doi.org/10.1007/s10989-019-09918-z.

27. Casadevall, A.; Coelho, C.; Alanio, A. Mechanisms of Cryptococcus neoformans-Mediated Host Damage. *Front. Immunol.* **2018**, *9*, 855, https://doi.org/10.3389/fimmu.2018.00855.
28. Hole, C.R.; Lam, W.C.; Upadhya, R.; Lodge, J.K. *Cryptococcus neoformans*Chitin Synthase 3 Plays a Critical Role in Dampening Host Inflammatory Responses. *mBio* **2020**, *11*, e03373-03319,https://doi.org/10.1128/mBio.03373-19.
29. Han, L.-T.; Wu, L.; Liu, T.-B. A Predicted Mannoprotein Cmp1 Regulates Fungal Virulence in Cryptococcus neoformans. *Pathogens* **2020**, *9*,https://doi.org/10.3390/pathogens9110881.
30. Edgar, R.C. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **2004**, *5*, 113, https://doi.org/10.1186/1471-2105-5-113.
31. Guindon, S.; Lethiec, F.; Duroux, P.; Gascuel, O. PHYML Online—a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res.* **2005**, *33*, W557-W559,https://doi.org/10.1093/nar/gki352.
32. Galanis, K.A.; Nastou, K.C.; Papandreou, N.C.; Petichakis, G.N.; Iconomidou, V.A. Linear B-cell epitope prediction: a performance review of currently available methods. *bioRxiv* **2019**,https://doi.org/10.1101/833418.
33. Coelho, C.; Bocca, A.L.; Casadevall, A. Chapter One - The Tools for Virulence of Cryptococcus neoformans. In *Adv. Appl. Microbiol.*, Sariaslani, S., Gadd, G.M., Eds. Academic Press: 2014; 87, 1-41, https://doi.org/10.1016/b978-0-12-800261-2.00001-3.
34. Ma, H.; May, R.C. Chapter 5 Virulence in Cryptococcus Species. In *Adv. Appl. Microbiol.*, Academic Press: 2009; 67, 131-190, https://doi.org/10.1016/S0065-2164(08)01005-8.
35. Maliehe, M.; Ntoi, M.A.; Lahiri, S.; Folorunso, O.S.; Ogundeji, A.O.; Pohl, C.H.; Sebolai, O.M. Environmental Factors That Contribute to the Maintenance of Cryptococcus neoformans Pathogenesis. *Microorganisms* **2020**, *8*,https://dx.doi.org/10.3390%2Fmicroorganisms8020180.
36. Elhassan, R.M.; Alsony, N.M.; Othman, K.M.; Izz-Aldin, D.T.; Alhaj, T.A.; Ali, A.A.; Abashir, L.A.; Ahmed, O.H.; Hassan, M.A. Computational vaccinology approach: Designing an efficient multi-epitope peptide vaccine against Cryptococcus neoformans var. grubii's heat shock 70KDa protein. *bioRxiv* **2019**,https://doi.org/10.1101/534008.
37. Sati, A.O.M.; Abdelmoneim Hamza, A.H.; Dawoud, E.D.K.; Abbas, T.A.A.; Abdelrahman, F.A.B.; Saad, K.A.H.; Abduallah, R.B.A.; Mustafa, M.M.E.S.; Sidahmed, T.a.; Elhassan, R.M.; Hassan, M.A. Multi-epitope Peptide Vaccine Prediction for Candida albicans targeting Pyruvate Kinase Protein; an Immunoinformatics Approach. *bioRxiv* **2019**,https://doi.org/10.1101/758920.
38. Schneider, R.d.O.; Diehl, C.; dos Santos, F.M.; Piffer, A.C.; Garcia, A.W.A.; Kulmann, M.I.R.; Schrank, A.; Kmetzsch, L.; Vainstein, M.H.; Staats, C.C. Effects of zinc transporters on Cryptococcus gattii virulence. *Sci. Rep.* **2015**, *5*, 10104,https://doi.org/10.1038/srep10104.
39. Robinett, N.G.; Peterson, R.L.; Culotta, V.C. Eukaryotic copper-only superoxide dismutases (SODs): A new class of SOD enzymes and SOD-like protein domains. *J. Biol. Chem.* **2018**, *293*, 4636-4643, https://doi.org/10.1074/jbc.TM117.000182.
40. Khalil, I.; Omer, I.; Farh, I.Z.A.; Mohamed, H.A.; Elsharif, H.A.; Mohamed, A.A.H.; Awad-Elkareem, M.A.-E.; Salih, M.A. Design of an epitope-based peptide vaccine against*Cryptococcus neoformans*. *BioRxiv***2018**, *1*, 434779, https://doi.org/10.1101/434779.
41. B. R. Da Silva, L.; P. Taborda, C.; D. Nosanchuk, J. Advances in Fungal Peptide Vaccines. *Journal of Fungi* **2020**, *6*,https://doi.org/10.3390/jof6030119.
42. Rutherford, J.C. The Emerging Role of Urease as a General Microbial Virulence Factor. *PLoS Path.* **2014**, *10*, e1004062, https://doi.org/10.1371/journal.ppat.1004062.