**Supplementary 1.** Important adaptive transcription factors of *C. neoformans* and the phenotypic defects/advantages associated with their deletion*.* Some of these transcription factors are homologues of yeasts, such as *Saccharomyces* and *Fusarium* species.

| **Mutants** | **Phenotypic defects/advantages** |
| --- | --- |
| *Δabp1, Δcrn1, Δsla2* | Poor cellular endocytosis but normal growth under hypoxic conditions (1% O2) [1] |
| *Δacs1* | Growth defect in ethanol, acetate, and glycerol but utilised sucrose, arabinose, galactose, and lactate; *wt* thermotolerance, melanin, and capsule formation but delayed progressive infection in murine inhalation model due to partial production of acetyl-CoA from various metabolic routes [2] |
| *Δade2* | Adenine auxotroph mutants showed normal growth at 37oC, capsular size, and melanin formation; attenuated virulent in *C. elegans* [3]; avirulent in corticosteroid-treated rabbits when injected intrathecally in immunocompromised rabbit meningitis model with effective CSF clearance by day 7 post-infection [4] |
| *Δalg3, Δalg9, Δalg12* | Defective lipid-linked *N*-glycans assembly; moderate-normal *in vitro* virulence phenotype, but the mutant was avirulence in a mouse model of systemic cryptococcosis [5] |
| *Δaox1* | Sensitive to oxidative stress that impaired mitochondria oxidative process/cellular process; less virulence and defective growth in the macrophage-killing experiment; functionally redundant with *Ccp1* [6] |
| *Δaph1* | Attenuated virulence in *G. mellonella* and mice model of infection despite a *wt* level of thermotolerance, melanin and capsule production; impaired canonical secretion of catalytically active acid phosphatase under phosphate starvation [7] |
| *Δatg8* | Loss of virulence in an MIMC; impaired lipidation (lipid-binding) for vesicular transport [8] |
| *Δbck1, Δmkk2, Δmpk1* | None of the mutants was sensitive to as much as 1 mM oxidative and nitrosative stressors in YNB at pH 4.0; no detectable Mpk1 phosphorylation in the presence of stressors [9] |
| *Δbwc1, Δbwc2* | Either bilateral or unilateral mating and haploid hyphal formation significantly remained higher than the *wt* in the light/dark, but the mutants are hypersensitive to UV-light with attenuated infection rate [10] |
| *Δcac1* | Impaired capsule formation, even in the presence of 5% CO2 [11] |
| *Δcan2* | Growth defect in ambient air; impaired decarboxylation process in lipid, amino acids, and pyrimidine metabolism; upregulation of *Fur1* expression for pyrimidine salvage pathway [12] |
| *Δcna1* | Avirulent strain with complete growth defect at 37oC that cannot be restored with 1 M sorbitol; unviable recovery fungal cells from the brain and lung of the infected murine; serotype A mutant was sensitive to osmotic stress [13,14] |
| *Δcap, Δplp* | Defective capsule formation (acapsular) and lipolysis, mutants could not survive amoeba and macrophage phagocytosis [15-17] |
| *Δcch1* | This slightly attenuated virulence mutant in the MIMC showed severe growth defect from 25 to 39oC in media with 50 – 100 nM Ca2+ with added 1 mM BAPTA (a Ca2+ chelator) like the *S. cerevisiae* mutant but could be rescued at all temperatures with higher Ca2+ concentration (~140 nM without BAPTA); however, the *S. cerevisiae* mutant remained drastically more defective at temperature ≥ 37oC. The presence of 1.0 M sorbitol in the media with Ca2+ and BAPTA showed no rescuing of growth defects in the two fungi mutants and probably the *Δmid1* mutant as well [18] |
| *Δccp1* | Impaired oxidative stress response against exogenous oxidant but no effect on the virulence [19] |
| *Δccr4* | Encapsulated but defective cell wall integrity characterised by increased absorption of trypan blue and hypersensitivity to lysing enzymes; evidence of gradual restoration of cell wall integrity in the presence of 1 M sorbitol when cultured in asparagine salts agar containing 2% dextrose and 1 mg/mL YNB; effective mannan-binding lectin (MBL – an innate immune mediator) just like *Δcap60* mutants; sensitive to CpF and other cell wall stressors with reduced virulence in MIMC [20] |
| *Δcdc3, Δcdc10, Δcdc11, Δcdc12* | Mutants lacked septin production with a tendency of changes in cell ploidy (*Δcdc10* showed haploid while others showed diploid-like morphology); displayed *wt* growth at 24oC but are defective at 37oC; defective bilateral mating characterised with aberrant clamp cells, poor spore formation, and defective nuclear distribution and multinucleation in the abnormal hyphae; poor aggregation of septin-like proteins at the bud-neck of the actively dividing cells.  *Δcdc3*, *Δcdc12,* and *Δcdc3Δcdc12* showed reduced virulence in the *G. mellonella* model of infection [21] |
| *Δcdc24* | Cdc24 is a downstream effector of Ras1-GTP, and the mutants are hypersensitive at ≥37oC but could be partially resolved by the expression of Rho-GEF [22] or Ste20 but not Ras1or Pak1; overexpression of *Cdc42* could restore high-temperature growth defect in *Δcdc24,* and even in *Δras1* mutant*;* enlarged and aggregated cell morphology as temperature progresses from 30 to 39oC; normal growth rate at 30oC and karyokinesis even at 39oC; *in vivo*  survival yet completely avirulent in MIMC [23] |
| *Δcdc43* | Hypersensitive mutants to temperature ≥37oC; survival defect in macrophage; attenuated virulence due to failure to grow at physiological temperature and the mislocalisation of Cdc42p though Ras1p and Rac2p membrane-localisation remained unaffected; normal growth at 30oC but defective cytokinesis at 37oC; normal unilateral mating but delayed bilateral mating process characterised with poor basidiospore chain formation and one-fifth of the cell showed abnormal morphology in the clam cells; highly susceptible to manumycin A than tipifarnib (two potent farnesyltransferase inhibitors) [24,25]. These phenotypic defects are similar to *Δcdc42, Δcdc420, Δcdc42Δcdc420, Δcdc24,* and *Δras1* mutants, which also lacked actin cables leading to actin repolarisation of the cytoskeleton after thermal stress [24,26] |
| *Δcdc42, Δcdc420* | Each mutant is viable at 30oC with no defect in the capsule and melanin formation, but the growth decreased at ≥37oC and became thermosensitive at 39oC just like *Δras1* and *Δcdc24* mutants; complementation by *Cdc42* or overexpression of *Cdc420* could restore the growth defect at 39oC; *Δcdc42Δcdc420* mutants displayed a more severe growth defect even at 30oC. Surprisingly, only the *Δcdc42* mutant was avirulent, just like *Δcdc24* and *Δras1* mutants, while *Δcdc420* mutants remained virulence as much as the *wt* [24] |
| *Δcdk8* | Mutants displayed impaired mitochondrial functions with increased susceptibility to oxidative stress, poor proliferation in the macrophage (phagocytes), decreased tissue burden, and attenuated virulence. Normal growth in CSF media but impaired BBB [27] |
| *Δcdk8, Δssn801* | Reduced mating filaments in *Δcdk8* and *Δssn801* (H99 strain) but not in *Δssn801* (KN99 strain). Reduced resistance to Congo red and CFW but not with SDS and ethanol [27] |
| *Δcfo1* | Attenuated virulence, increased sensitivity to FCZ and AmpB, and impaired ergosterol synthesis due to reduced intracellular iron content [28] |
| *Δcft1, Δcft2* | *Δcft1* showed significant attenuation of virulence, growth reduction, and tissue burden reduction due to inadequate *in vivo* iron acquisition from transferrin, but this strain could utilise iron from siderophore and heme; sensitive to azole and AmpB due to impaired ergosterol synthesis; *Δcft1Δcft2* mutant showed further attenuation of virulence [29] |
| *Δcgl1* | Failed to survive [30] |
| *Δcgp1* | Highly susceptible to FCZ and AmpB with severe growth defect in YNB fortified with creatinine, Leu, Ile, Lys, Gly, Met, Try, Val, Thr, and His [31] |
| *Δcig1* | Impaired growth with defective capsule size in the iron-limiting media; failed to trigger cytokine production in immune cells; iron supplement restored the defect with a larger capsule than the *wt* at ≥24 hours post-incubation [32] |
| *Δcin1, Δrho-GEF, Δsh3-rho-GEF* | Defective endocytosis (partially restored by promoting autoactivation of Wsp1p); defective actin polymerisation and cytokinesis (that could be restored by constitutive expression of Cdc42); fused Cdc42-GFP-Wsp1p could partially restore growth defect and poor actin organisation; non-melanised and acapsular with severe cell deformation; could not produce urease and phospholipase B; thermosensitive; sterile and unable to produce conjugation tubes for the mating specific dikaryotic filament in unilateral crossing; no hyphal formation in the bilateral crossing.  No significant defects were observed with *Δrho-GEF* and *Δsh3-Rho-GEF* mutants [22,26] |
| *Δcir1, Δtup1* | Impaired regulation of *Hap* genes, poor production of all the major virulence factors and poor growth at 37oC [33-35]  *Δtup1* mutant displayed high expression of *Cqs1, Ctr1* and *Mf* in both mating types; high expression of *Fdh1* and *Mp88* in *MAT****a*** but both repressed in *MATα*; *Cel1* was induced in *MATα* but slightly repressed in *MAT****a***; decreased mating frequencies; temperature-dependent growth reduction from 37 to 25oC [36,37] |
| *Δcir1* | Complete loss of virulence regulatory genes; downregulation of high-affinity iron uptake proteins encoded by *Cft1* and *Cfo1* but promotes intracellular iron transporters encoded by *Cft2* and *Cfo2* as well as iron-dependent enzymes, such as laccase encoded by *Lac1* [28,29,38]; no effect on the major high-affinity phosphate uptake proteins and polyphosphate polymerase except Pho89 (which performs a pH-dependent sodium-coupled phosphate transport under phosphate-limiting condition) [39]; better growth in YPD at low pH and 30oC; growth restored even at a low phosphate supplement; slight growth defect in the presence of calcium but failed to grow in the presence of zinc, CsA, and CsA+calcium; susceptible to heavy metal, sodium, and manganese [40] |
| *Δclc-A* | Defective laccase activity (which could be restored by exogenous copper supply), a significant reduction in capsule production and poor growth in a slightly alkaline medium (just like *Δvph1* mutants)attenuate the virulence of the mutant [41] |
| *Δcmt1, Δcmt2* | Double deletion caused defective Cu2+-detoxifying metallothionein; severely attenuated virulence and reduced pulmonary colonisation [42] |
| *Δcna1* | Thermosensitive and hypersensitive to cell stressors – oxidative, nitrosative, ionic, and osmotic [43-45]; sharing similar Ca2+ perturbation as *Δgrx4* in the LIM [46] |
| *Δcnn1* | Produced *ts* mutant to the optimum of 35oC with short hyphae; readily caused subcutaneous lesions but failed to cause systemic infection [47] |
| *Δcph1 (Ste12α homologue)* | Low capsule production, a severe defect in filamentation and haploid fruiting/sporulation in nitrogen shortage media [48]; apparent reduction in the ***a****-α* mutant unilateral mating compared to the *wt*; more reduced filamentation in *MAT****a*** x *MATαΔste12Δznf2;* most reduced filamentation in *MAT****a*** *Δznf2 x MATαΔste12;* no mating between *MAT****a*** *Δznf2 x MATαΔste12Δznf2* [49] |
| *Δcpr1 (Δste3)* | Attenuated mating but not completely sterile; normal hyphae production (haploid fruiting) but reduced pheromone production with basal activity [50] |
| *Δcps1* | Poor endothelial cell-fungal adhesion due to lack of hyaluronate; reduced virulence due to poor capsule integrity [51] |
| *Δcrk1* | Increased mating efficiency by promoting dikaryotic filamentation, basidia, and basidiospores, but its effect on unisexual mating is ambiguous [52] |
| *Δcrg1* | Increased virulence, enhanced pheromone production (mating) and melanin synthesis [53] |
| *Δctr1/Δctr2, Δctr4* | Poor copper assimilation, severe growth retardation in copper-deficient media, defective capsule, and melanin production, prone to phagocytosis under copper-deficient [54,55]. The *Δctr4* is highly susceptible to FCZ with severe growth defects in YNB fortified with NH4+, creatinine, and urea [31] |
| *Δcrz1/Δsp1, Δcna1* | Prone to cell wall fragility due to poor expression of *Chs6* encoding chitin synthase, poor thermotolerance, susceptible to low oxygen and antifungal [56]. Attenuated virulence for *Δcrz1* mutant, but the *Δcna1* mutants remained avirulence [43] |
| *Δcrz1, Δhad1* | Reduced growth at ≥37oC, increased sensitivity to cell wall perturbation and ER stressor, and attenuated virulence in MIMC. Double deletion *Δcrz1Δhad1* increased thermosensitivity, cell wall distress and further impaired virulence. Increased sensitivity of *Δcrz1Δhad1* mutants to cell wall perturbators, temperature, and higher attenuated virulence coupled with a more reduced fungal burden as compared to *Δcrz1* or *Δhad1* mutant suggests synergistic downstream regulation of Crz1p and Had1p in Cna1/Cnb1 cascade event to regulate cell wall integrity [57] |
| *Δcuf1/Δmac1* | Reduced melanisation (impaired laccase function), filamentation, and growth at 37oC; sensitivity to low and high-copper-containing media; normal capsule and urease production; poor CSF dissemination but normal growth in the lung; attenuated virulence in a mouse model [55,58-60] |
| *Δczc1* and *Δcpp2* | Normal growth in YPD/synthetic media with melanin and capsule production, whether *in vivo/in vitro* [30] |
| *Δena1, Δcna1* | The *Δena1* mutants displayed attenuated virulence, impaired resistance to high salt concentration (osmotic stress), especially in the glucose-limited medium, and sensitivity to alkaline pH but not acidic medium [61]. *Δcna1* mutants are sensitive to alkaline pH, and higher sensitivity to higher pH was observed with *Δcna1Δena1* [62]. |
| *Δena1, Δrub1,* and *Δpik1* | None survived hCSF medium; hyper-reduction of CFU recovered from macrophage intracellular milieus after 48 hours at 37oC; normal capsulation but only *Δrub1* remained albino; attenuated virulence in a rabbit model of infection; *wt* osmoresistant; CSF metabolites <3kDa inhibited their growth; proteinase K-treated or peroxide-treated CSF failed to restore their growth defect; however, heat-treated CSF may probably slightly restore their growth [63,64]. |
| *Δerg6* | Impaired thermotolerance, susceptibility to osmotic stress, oxidative stress, and different antifungals, avirulent, and decreased membrane ergosterol [65] |
| *Δfbp1* | The mutant displayed a *wt* melanin formation, capsule (even in physiological 10%CO2 condition), phagocytic index and survival, and thermotolerance yet avirulent in a murine inhalation infection model, which was attributed to impaired formation SCF E3 ubiquitin ligase-mediated proteolysis and poor membrane integrity; lack of tissue dissemination and invasion with a very minimal tissue lesion in the lung; normal unilateral mating; normal dikaryotic hyphae formation but failed to produce basidiospores (for sporulation) during bilateral mating of the mutants due to impaired meiosis and nuclear division; hypersensitive to SDS but not CFW/Congo red. The *Fbp1* expression is under glucose repression and negatively represses *Hxt3* expression in glucose-rich medium [66] |
| *Δfhb1, Δgno1, Δsod1* | The *Δfhb1* mutants are susceptible to nitrosative stress but not *Δgno1*; *Δfhb1* mutants showed attenuated virulence in a murine model and reduced survival in activated macrophages; *Δfhb1Δgno1* or *Δfhb1Δsod1* mutants displayed cumulative attenuated virulence [67] |
| *Δgat1/Δare1* | Impaired nitrogen uptake and metabolism from different nitrogen sources, including the nitrogen sources from the ecological niche of *C. neoformans* such as NH4+, urate, urea, and creatinine; impaired NCR but improved mating (filamentation and basidiospore formations); impaired expression of *Amt1, Amt2,* and *Gdh1;* a high dose inoculum is required to induce virulence in mouse model [68] but Lee et al. concluded a slightly more virulence of this mutant in MIMCthan the *wt* with a claim that the mutant was thermotolerance at 37oC with increased melanin production [69]; infection in *C. elegans* is the same as the *wt,* but in mice, the infection progresses to morbidity slightly earlier than the *wt* infected mice [69] |
| *Δgat201* and *Δcap* | Impaired capsule induction, poor growth, and highly prone to phagocytosis [70] |
| *Δgcn5* | Defective nucleus localised histone acetyltransferase for chromatin remodelling, thermosensitive due to poor expression of *Cna1* gene, and delayed growth due to suspected repression of G-protein regulators; decreased capsule size and defective capsular-cell wall attachment (poor cell wall integrity) due to poor expression of glycan-associated Skn1/Kre61 family protein and *Cap64* gene product; normal melanin production; susceptible to FK506 inhibition because of the purported increased in the *Fkbp12* expression; sensitive to oxidative stress because of the *CatA* repression, and the mutant is avirulent [71] |
| *Δgcs1* | Mutants displayed *wt* capsule and melanin production with unaffected growth within the macrophage internalised compartment. Nevertheless, the mutants are highly attenuated for virulence with a significant impairment of tissue invasion, drastic tissue burden CFU reduction, and a rapid immunological response squad within the lungs in an intranasally inoculated MIMC. Intravenous inoculation, however, leads to less attenuation with a high degree of dissemination, characterised by a higher tissue burden in the brain than any other internal organs. A neutral-alkaline solution of pH 7.4 and CO2 level >0.04% will not support the growth of this mutant, which is perpetually locked in the S and G2/M growth phase, unlike in the acidic pH 4.0 medium where growth is supported. The growth defect at pH 7.4/5% CO2 appeared restored when transferred to pH 4.0 [72]. These growth defects, attenuated virulence, and immunological arrest of *Δgcs1* mutants in the intranasal inoculation are very similar to the *Δsmt1* mutants, which accumulate demethylated ceramide and demethylated glucosylceramide (GlcCer) unfit for Gcs1p to maintain the membrane integrity and permeability [73] |
| *Δgpa1* | cAMP not produced, leading to defective mating, impaired melanin and capsule production, and susceptibility to macrophage killing [30,74] |
| *Δgpa1, Δcac1, Δpka1* | Individually and collectively showed impaired capsule formation in *Δhog1* background serotype Amutant [75] |
| *Δgpa1*, *Δpka1* | Impaired sugar metabolism, attenuated virulence, and prone to phagocytosis [30] |
| *Δgpp1, Δgpp2, Δdog1, Δdog2* | Normally capsulated but hypovirulent and unable to produce melanin, phospholipase, and urease. The mutants are sensitive to cold shock, membrane and cell wall destabiliser, alkaline conditions, and osmotic stress. There is an unregulated sulphur-containing amino acid biosynthesis due to a perpetual expression of *Cys3* orchestrated by the Cna1 pathway. Growth defect in SD fortified with various nitrogen sources/amino acids except with Pro, Gln, Asn, Asp, and Met at 30 or 37oC [76,77] |
| *Δgpx1, Δgpx2* | The single and double mutants are sensitive to oxidative-induced macrophage killing and show no impact on the survival and virulence in the MIMC[78] |
| *Δgrasp* | Poor morphology of Golgi apparatus; attenuated virulence (hypocapsulation due to poor secretion of GXM); poor phagoresistance but normal pigmentation/melanisation and urease activity [79] |
| *Δgrx4* | The avirulent mutant showed impaired growth at 37 and 39oC with poor chitin-chitosan production at 39oC, decolourised (in *L-*DOPA medium) and hypocapsulated (in defined LIM) colonies at 30oC, hypersensitive to SDS, CFW, caffeine, phleomycin, CoCl2, FK506, and CsA. Mutant displayed partial-to-complete nuclear delocalisation of Crz1p (in the presence of glucosamine, calcium, iron limitation, and elevated temperature), impaired Cna1p function, and poor iron homeostasis due to impaired functional Cir1p coupled with dysregulation of iron-dependent metabolisms [80-82]. The mutant shared similar phenotypic defects along with *Δcrz1* and *Δcna1,* showing parallel regulation and coordination of each factor by the calcineurin-responsive proteins [46] |
| *Δgrx4Δcna1* | *Δgrx4Δcna1* displayed more severe growth defects to the ionic, osmotic, membrane, and cytosolic stress than either of the single mutants; however, similar sensitivity to *Δcna1* was observed in SDS, caffeine, Congo red, CpF, MNS, and calcium; more severely deformed cell morphology in the LIM characterised by unseparated enlarged cell size [46] |
| *Δgrx4Δcrz1* | Generally displayed *Δgrx4*-like phenotypic defects caused by CFW, CR, and stress by ions, salts, and unfolded proteins. Again, *Δgrx4Δcrz1* and *Δgrx4* shared common cell deformation, unsegregated cell division, and highly attenuated capsule formation [46] |
| *Δgsk3, Δkic1* | Impaired *Lac1* and *Bzp4* induction, perturbation of Bzp4 nuclear translocation, poor regulation of Hob1, Usv101, and Mbs1 [83] |
| *Δhap3, Δhap5, ΔhapX, Δcfo1* | *Δhap3,* *Δhap3Δcfo1,* *Δhap5,* and *Δhap5Δcfo1* produced smaller capsule sizes compared to the *wt* or *ΔhapX* but no influence on the *Lac1* expression; *Δhap3Δcfo1* and *Δcfo1* showed similarly delayed virulence; all the mutants including *ΔhapX* and *ΔhapXΔcfo1* grew better than the *wt* at37oC, but the addition of hemin enhances all the growths including *Δcfo1* mutant; there is no significant growth difference in all the mutant strains and *wt* at 30oC in YPD [28,84] |
| *ΔhapX, Δhap3* | Under iron starvation, the mutants failed to utilise iron-rich heme and transferrin in the media and repressed the expression of mitochondria genes for respiration and TCA but regulated the expression of *Erg* genes for ergosterol synthesis; either of the mutations failed to further attenuate virulence in *Δcfo1* background mutant [84] |
| *ΔhapX* | Impaired environmental iron uptake by siderophore iron transporter, apparent loss of virulence in *Δcir1* background mutant, poor regulation of *Sit1* and *Cir1* expression [84] |
| *Δhog1* | Stimulate melanin and capsule production and restore melanin production in *Δgpa1, Δcac1,* and *Δpka1* background mutants; the mutant showed attenuated virulence [75]. Enhance mating, agar adhesion, and invasion; mutant is FDX and FCZ resistant. Unlike *Δcna1* mutant*, Δhog1* displayednormal cell morphology (failed to accumulate glycerol) and cytokinesis in the presence of FDX, similar to serotype D *wt* [85] |
| *Δhom3, Δthr1* | Under a repressible *Pctr4,* both mutants showed temperature-dependent growth and were hypersensitive to 37oC either in the repressed or induced state. Under SD repressible media, the presence of Met, Thr, Ala-Thr, Met-Leu, and homoserine improved *Δhom3* growth better than *Δthr1,* but Ala-Thr appeared better with *Δthr1* mutant than *Δhom3*. In all, no *wt* growth level could be achieved in all the mutants [86] |
| *Δhxk1, Δhxk2,* and *Δpyk1* | Impaired glucose utilisation but active glucose catabolite repression; severely attenuated virulence though with no apparent effect on the melanin and capsule formation; mutants failed to survive in the rabbit CSF but survived in the mice lungs [87]. Reduced growth with *Δhxk2* in the presence of glucose [88] |
| *Δhxl1, Δire1* | Attenuated virulence [89] |
| *Δhxt1* | Decreased intracellular activated glucose; early melanin formation with normal capsule synthesis hence unaffected virulence [2] |
| *Δicl, Δmls1* | Each mutant showed full virulence in animal models but failed to grow in acetate medium [2,88,90] |
| *Δilv2* | An avirulent mutant that failed to survive *in vivo* but was highly resistant to sulfonylurea sulfometuron methyl irrespective of the strain/serotype due to transferable and dominant *ilv2* gene. In Ile-Val or Val-Ile dipeptide supplemented medium, no growth at 37oC; melanin production remained unaltered, but capsule production was significantly lower than the *wt*/reconstituted mutant [91] |
| *Δima1* | Poor growth in isomaltose medium but overexpression of Ima2p, which ordinarily will not catalyse isomaltose, restored the growth [92] |
| *Δipk1, Δkcs1* | Attenuation of phosphate sensing pathway in phosphate-depleted minimal media despite the accumulation of inositol pyrophosphate intermediates [93]; profound attenuation of virulence accompanied with a more reduced lung burden in *Δipk1Δkcs1* than *Δipk1* mutant; *Δipk1Δkcs1* and *Δkcs1* but not *Δipk1* mutants failed to disseminate into the brain in a mouse model infection perhaps because of the more accumulation of IP5 and PP-IP4 in the *Δipk1* due to functional Kcs1p (IP6 kinase)*,* which may provide an alternative route of generating PP-IP5/IP7 that is strongly needed for virulence; the two mutants are attenuated for laccase and urease activities with a poor growth under oxidative/nitrosative stress; *Δkcs1* mutants but not *Δipk1* are unable to utilise alternative carbon sources effectively; *Δkcs1, Δipk1,* and *Δipk1Δkcs1* lacked PP-IP5/IP7 so also *Δipk1* and *Δipk1Δkcs1* lacked IP6; *Δkcs1* and *Δipk1Δkcs1* mutantsshowed a mild growth defect in YPD as temperature increased from 30 to 37oC; all the mutants are slightly susceptible to Congo red and much more to Caffein [94,95] |
| *Δkcs1, Δarg1* | The *Δkcs1* mutants compromised growth in the low-glucose environment of the lungs in the mice infection model with impaired virulence in *Galleria mellonella* and mice. Both are more susceptible to NTC, PCZ, VCZ, ICZ, FCZ, 5-FC, and AmpB than the *wt*. Both displayed a *wt* susceptibility to AdF, McF, CpF, and ECC. The *Δkcs1* is hyper-resistant to neomycin, but the *wt* is hypersensitive. Though *Δkcs1* budded cells were observed in the infected animal lungs with proof of enlarged *in vitro* capsular production but a defective melanisation. Apparent monocyte recognition due to reduced expression of cell surface mannoprotein and impaired filament production when unilaterally cross-mated with KN99 *MAT* ***a*** [95] |
| *Δkre, Δskn1* | *Δkre5* and *Δkre6Δskn1* mutants showed an impaired formation of β-1,6-glucan – leading to reduced formation, retention, and cell wall-associated GPI-anchored Plb1p but rather increased secretion due to poor GPI-anchor; poor formation of cell wall mannoproteins; formation of chitosan but improperly positioned and mislocalised within the cell wall (loss of cell wall integrity), and sensitive to the cell wall and membrane stressors. The mutants further showed poor cell morphology characterised with enlarged aggregated cells having incomplete budding; enlarged capsules just like *Δchs3* and *Δcda1Δcda2Δcda3* mutants but poor exopolysaccharide formation characterised with rough edges and highly diffused and porous; reduced/delayed melanin production unlike *Δkre6*, *Δskn1,* and *Δkre6Δkre61* and completely avirulent in mouse inhalation infection model [96] |
| *Δlac1, Δlac2* | Reduced virulence; no melanin production; susceptible to oxidants and macrophage killing; impaired tissue invasion/dissemination but retained tissue persistence/proliferation [30,97-99] |
| *Δleu1* | Leu autotroph mutants with upregulated mitochondrial Fe-S protein levels such as Nfu1p and Aco1p (indication of mitochondrial dysfunction) but the cytoplasmic Fe-S protein such as Fra2p remained unaffected under LIM; increased activity of mitochondrial Mn-Sod2; increased sensitivity to oxidative stress and cell wall/membrane disruptors; though melanin formation remained unaffected, the capsule formation is greatly reduced (acapsulation) leading to a significantly attenuated virulence in MIMC with a drastic reduction in the CFU recovered from the systemic organs [100] |
| *Δlhp1, Δcrz1* | *Δlhp1* mutants remained virulent, *Δcrz1* mutants are attenuated in virulence while *Δcrz1Δlhp1* are more attenuated than *Δcrz1* [43] |
| *Δlys4* | Impaired mitochondrial function characterised by increased accumulation of ROS and reduced activity of Aco1p due to partial iron homeostasis linked to Lys4 deletion because Lys4p is a Fe-S containing protein; increased sensitivity to oxidative stress and antifungals such as MCZ and FCZ; reduced ergosterol level; failed to grow at 37oC; hypersensitive to 10 mM SHAM and 50 μM DPI; attenuated virulence in MIMC [101] |
| *Δman1* | Total loss of phosphomannose isomerase enzyme activity, poor capsule formation, reduced polysaccharide secretion, defective cell morphology (wrinkled and irregular), impaired cytokinesis and cell budding, complete loss of virulence, effective clearance of the mutant from the infected host system [102] |
| *MAT* locus (located on chromosome 4) | A >100 kb gene encoding ≈20 transcription factors with various functions, however, a mutation in any of the following mating-type specific genes (*MATα: Cap1α, Cid1α, Etf1α, Gef1α, Lpd1α, Mfα1, Mfα2, Mfα3, Myo2α, Ncm1, Prt1α, Rpl22α, Rpl39α, Rpo41α, Rum1α, Spo14α, Ste11α, Ste12α, Ste3α, Ste20α,* Sxi1*α, Tcn21, Znf1α* or *MAT****a***: *Cap1****a****, Cid1****a****, Etf1****a****, Gef1****a****, Lpd1****a****, Mf****a****1, Mf****a****2, Mf****a****3, Myo2****a****, Ncp1****a****, Prt1****a****, Rpl22****a****, Rpo41****a****, Rum1****a****, Spo14****a****, Ste11****a****, Ste12****a****, Ste20****a****, Ste3****a****, Znf1****a***) leads to defective or total disruption of pheromone production, pheromone response (formation of shmoos cells with conjugation tubes), cell fusion, mating, dikaryotic filamentation, basidia, basidiospores, and cell viability at any stage of *C. neoformans* life cycle and in few cases attenuation of the virulence or avirulent may occur [103-106]. The *Mat2* and *Znf2* (encoding Zn-finger protein) are non-mating type locus. |
| *Δmat2* (located on chromosome 13) | Severely impaired filamentation with no cell fusion either in unilateral/bilateral mating just like in *Δcpk1* mutant, but evidence of delayed unilateral mating is apparent [49]; promote pheromone-independent unisexual development in conjunction with the filamentation/primary hyphal regulator (Znf2), calcium-regulated and temperature-induced calcineurin (Cna1/Cnb1). Filamentation was induced at 37oC, high calcium level (2.0 mM) and high copper level (400 μM) [107]. Like *Δznf2*, *Δmat2* mutant displayed severely impaired dimorphic filamentation during *α-α* bisexual mating in the filament-inducing media at 22oC in the dark; like *Δste7, Δmat2* mutant displayed a *wt* virulence in MIMC [49] |
| *Δmay1* | Attenuated virulence; sensitive to acidic pH; low replication and reduced cell density; reduced tissue burden and significant low accumulation within the macrophage after phagocytosis [108] |
| *Δmbs1* | Increased resistance to azole but decreased resistance to polyene because Mbs1 is a negative regulator of *Erg11* [109] |
| *Δmet3* | Initial loss of melanin formation but reappeared on incubation after 36 hours; reduced growth rate but increased thermotolerance; normal capsule production yet avirulent with poor *in vivo* survival [110] |
| *Δmfe2, Δhad1,* and *Δhad2* | Poor growth in fatty-acid enriched media, poor production of polysaccharide capsule and melanin, and poor brain colonisation in animal infection model [111] |
| *Δmga2* | Extremely sensitive to FCZ and fenpropimorph (sterol biosynthesis inhibitors) but not cycloheximide (a protein biosynthesis inhibitor); poor thermotolerance due to impaired membrane remodelling and poor biosynthetic pathway leading to amino acid and pyrimidine formation; impaired cell morphology [112,113] |
| *Δmip1, Δmip2* | No visible growth defect in phosphate-limiting condition [40] |
| *Δmpd1* | Susceptible to oxidative and nitrosative attack; reduced PMN survival [2,114] |
| *Δmpf3* | A glucose-repression transcript whose deletion appeared not to affect the growth under glucose, glycerol, or human serum; growth defect in NaCl/sorbitol medium due to defective cell wall similar to *Δvad1;* attenuated virulence and survival in mice when inoculated at CFU ≤103 but a *wt* virulence when the CFU is ≥106 [115] |
| *Δmpk1* | Attenuated virulence; defective cell wall integrity; susceptible to antifungal; defective *in vitro* growth at 37oC but can be rescued with 1 M sorbitol in osmotic cushion [116] |
| *Δmrs3/4* | Showed no effect on the mitochondria function or cellular viability, but overexpression causes temperature-dependent phenotypic defects [117]; *Δmrs3/Δmrs4* mutants are allelic to *Δfrr1* and *Δoxy1* mutants possessing a high level of *Frr1* transcript and iron uptake but sensitive to H2O2 due to the high iron content and slow to grow in LIM [118] |
| *Δncs1, Δcna1, Δcam1* | Mutants showed impaired Ca2+ homeostasis, sensitivity to temperature >30oC and high Ca2+ level. The *wt* expression is induced by Ca2+, inhibited by FK506, and impaired in the *Δcrz1* background mutant. Reduced growth, delayed budding, poor cell division, and impaired virulence are the features of *Δncs1* mutants [119] |
| *Δnmt1* | Mutants are *ts* myristate auxotrophs, entirely non-viable at 37oC, and completely cleared from the subarachnoid cavity of the brain within 12 hours post-infection; impaired functional Arf1p, Arf2p, and Gpa1p [120,121]. |
| *Δnrg1* | Delayed cAMP-dependent capsule formation and mating; impaired Pka-dependent carbohydrate metabolism and substrate oxidation; impaired *Udg1* gene expression for nucleotide sugar capsular components and cell wall integrity; attenuated virulence with impaired cryptococcal tissues dissemination; impaired siderophore transporter, Sit1[122] |
| *Δoxy1, Δoxy2* | Albino colonies (no melanin); delayed growth after oxygen exposure; hypovirulence; sensitivity to oxidants generated from Fenton reaction and failure to metabolise catecholamine; normal activities of catalase and membrane raft concentrated superoxide dismutase (Sod) [123,124] |
| *Δova1* | Enhanced Pka1 activity leading to hypercapsulation, hypermelanisation, and hypervirulence [125] |
| *Δpak1* | Normal growth and cytokinesis, but mating and haploid differentiation are defective. Mutants of serotypes A and D are generally attenuated for virulence due to lack of capsule formation but could be fully restored when complemented with the *wt Pak1* gene. Like *Δste13*, *Δpak1* mutants displayed no melanin defect [112] |
| *Δpbx1, Δpbx2* | Low shedding of capsular GXM slightly enriched in xylose but lacks glucose; poor polysaccharide fibril formation (low density) with poor cell wall attachment; atypical cell morphology (dry colonies, defective cell wall formation and integrity); attenuated mating filamentation; attenuated virulence; increased macrophage phagocytosis; metabolic imbalance; *Δpbx1Δpbx2* exhibited worse cell wall deformation and integrity [126] |
| *Δpbp1, Δcrz1* | Attenuated virulent mutants and *Δcrz1Δpbp1* mutants even remained avirulent in the MIMC [43] |
| *Δpck1* | Survive in the rabbit CSF [87] but showed severely attenuated virulence in murine despite unaffected laccase activity; the mutant failed to grow in lactate medium (**Table 3**); the mutant displayed a *wt* growth in a broth of 2% glucose at 37oC [115] (**Table 3**) |
| *Δpdk1, Δsin1,* and *Δypk1* | Impaired growth at 37oC, reduced virulence due to an impaired capsule and melanin production, and sensitivity to FCZ [127] |
| *Δpex* | Mutants like *Δpex1* and *Δpex6* showed impaired peroxisomal protein localisation with growth defect in the presence of glucose but unconnected to the virulence factor formation; intriguingly, however, deletion of *Hxk2* in the *Δpex1* background mutants restored the growth defect[88] |
| *Δpho4, Δkcs1, Δarg1, Δasp1* | Displayed a drastic defective/delayed growth in phosphate-depleted minimal media, but *Δasp1* mutant remained unaffected. Impaired biosynthesis of inositol polyphosphate due to inability to upregulate phosphate acquisition/sensing genes (PHO genes) [93]; however, *Δkcs1* mutant of *S. cerevisiae* displayed hyperactivation of PHO pathway [128]. The *Δarg1* and *Δkcs1* failed to produce PP-IP5/IP7, *Δkcs1* failed to produce PP-IP4, and *Δasp1* failed to produce (PP)2-IP4/IP8; therefore, *Δarg1Δkcs1* mutants displayed attenuated growth, poor cell wall integrity, poor melanin production, a reduced urease activity, and impaired mating filament [94]. Again, *Δasp1* mutant showed similar virulence with the *wt* – an indication that IP8 is dispensable for virulence, unlike IP7 [95] |
| *Δpho81* | Failure of IP7 to interact with mutated Pho81 (a CDK inhibitor); dramatic loss of virulence in a mouse infection model with drastic tissue burden CFU reduction in the lung and brain of the infected mouse [93] |
| *Δpho84, Δpho89,* and *Δpho840* | *ΔΔΔpho* mutants are less susceptibility to zinc better than single/double deleted mutants with better growth at 30oC than 37oC; however, mutants involving *Pho840* deletion and any other are susceptible to CsA or CsA+calcium but not calcium only (because high-affinity phosphate transporter Pho80-Pho85 complex, excluding Pho840, inhibits Crz1*-*Cna1activation) – this shows the inherent preference of Pho840 for CsA. The *ΔΔΔpho* mutants are slightly susceptible to calcium only. Irrespective of the carbon sources and temperature, the single/double deleted mutants showed no growth defects. However, the *ΔΔΔpho* mutants showed better growth at 30oC, characterised by a lower percentage of irregularly enlarged cell sizes than at 37oC. These mutants showed increased resistance to heavy metals but were susceptible to sodium and manganese [40] |
| *Δpka1, Δpkr1* | There was no significant growth defect in a phosphate-limiting condition, irrespective of the carbon source. The *Δpka1* mutant is characterised by Cfo1p mislocalisation. In the presence of zinc, 30oC favours growth almost equally in both mutants but 37oC favours growth in *Δpka1* while *Δpkr1* failed to grow. From 30 to 37oC, *Δpkr1* mutants failed to grow in the presence of CsA, but *Δpka1* was resistant. Likewise, the *Δpka1* mutant was resistant to heavy metals, manganese, calcium, and CsA+calcium because Pka1 negatively regulates Crz1-Cna1 activation, but *Δpkr1* was susceptible to CsA+calcium in the same way with CsA, heavy metals, and manganese. Each mutant showed no growth defect in the presence of sodium. Each mutant showed no growth defect in YPD, but *Δpka1* mutant is susceptible to macrophage killing [30,40] |
| *Δpkc1* | Loss of C1 domain of Pkc1 that binds DAG leading to improper localisation of active laccase hence reduction in the cell wall integrity [129]; mutant is sensitive to both nitrosative and oxidative attacks with osmotic instability; *ts* mutant that can be rescued by osmotic stabiliser (1 M sorbitol) between 25 – 30oC; hypersensitive to cell wall disruptors; impaired capsule and melanin formation even in the presence of 1 M sorbitol. The melanin formation in this mutant is better than the *Δlac1Δlac2,* less than the *Δcku80Δpkc1,* and far less than *Δcku80* mutant; normal chitin and chitosan formation but highly mislocalised [9] |
| *Δplc1, Δplc2* | The *Δplc1* mutant displayed a reduced expression of phosphatidylinositol‐specific phospholipase C (PI-PLC); hence no release/secretion of Plb1 from the GPI anchor; unreleased Plb1 accumulated largely within the membrane and cytoplasm, and to some extent, the cell wall; hence, no secreted phospholipase activities (LPL and LPTA); unable to produce melanin due to unexpressed laccase, and failed to grow at 37oC; defective cell wall and cell morphology characterised with cell aggregation, incomplete bud separation, membrane penetration of the CFW, hypersensitive to SDS and caffeine and sensitive to cell wall disruptors and hyperosmotic conditions; slower growth and unable to activate MAPK in the presence of the cell wall disruptors; avirulent in MIMC with no trace of fungal burden; attenuated virulence in *C. elegans* at 25oC despite unaffected capsule formation; hypersensitive to ICZ, VCZ, 5-FC, AmpB but not CpF. The *Δplc2* mutant displayed the *wt* phenotypic traits and was redundantly complementary to *Plc1* [130] |
| *Δpmc1, Δvcx1* | The *Δpmc1* is an avirulent mutant that failed to survive in the brain parenchyma or penetrate the CNS in MIMC due to an appreciable decrease in urease activity and poor Ca2+ homeostasis [131,132]. The *Δvcx1* mutants are highly virulent, but the *Δpmc1Δvcx1* mutants are avirulent and are more susceptible to macrophage phagocytosis like *Δpmc1* mutant [132]. The *Δvcx1* mutants are hypersensitive to CsA at temperatures >30oC even the presence of Ca2+ or Mn2+ cannot rescue the growth defect in CsA. The mutant showed no flaw in the cell wall integrity and capsule size; however, contrary to Squizani et al.,2018, it was previously reported that *Δvcx1* is attenuated in virulence [133] |
| *Δpmt4* | *Δpmt4* mutants showed abnormal growth morphology characterised by excessive cell aggregation, an overall decrease in the cell wall-associated glycoprotein, and defective mother-daughter cell separation during cytokinesis (conjoined cells); decreased level of *Fks1* expression in *Δpmt4,* making this mutant compromising the cell wall integrity and become sensitive to SDS at 30oC and AmpB but not echinocandin CpF; attenuated for virulence and pathogenesis in murine inhalation/intravenous injection model of cryptococcosis; similar melanin production with the *wt* but capsule production seems to collapse due to unseparated aggregated colonies; morphology of the mutants remained the same as the *wt* in the lungs and brain of infected murine but displayed conjoined morphology in the spleen as compared to the *wt* [134] |
| *Δptp1* | Impaired assimilation of polyol sugars and reduced capsular sizes in mannitol-enriched media, but the strain remains virulent *in vivo* and *in vitro* [135,136] |
| *Δptp1, Δptp2* | Reduced growth in *Δptp2* and *Δptp1Δptp2* at >37oC, similar to *Δhog1; Δptp2* showed osmotic and oxidative stress sensitivity, poor vegetative growth, a complete absence of unilateral and bilateral mating even when crossed with *MATα Δhog1,* complete defective pheromone production and cell fusion contrary to *MATα Δhog1* but highly improved filamentation, pheromone production, and cell fusion when co-deleted with *Hog1* in *MATα Δhog1Δptp2 x MAT****a***unilateral crossing. Like *Δcac1*, *Δptp2* and *Δptp1Δptp2* mutants displayed a drastic reduction in capsule formation and failed to produce melanin at 30oC with 0.1% glucose or at 37oC with 0.5% glucose, contrary to *Δhog1* and *Δhog1Δptp2* mutants. Deletion of *ptp2* promotes azole resistance in *Δhog1* background mutant in an *Erg*-independent manner. The *Δptp1Δptp2* are completely hypersensitive to 5-FC than individual mutants and *Δhog1.* Similar urease activity levels in *Δptp1, Δhog1,* and *Δhog1Δptp2* but still less compared with the *wt*. Further reduction of urease activity in *Δptp2* and *Δptp1Δptp2*. A *wt* virulence in *Δptp1* but severe attenuation of virulence in *Δptp2* and a more severe attenuation in *Δptp1Δptp2* with a great reduction of fungal burden in the lung (indication of a severe defect in colonisation, proliferation, and dissemination) and no trace of fungal recovery in the brain [137] |
| *Ptp1ovex, Ptp2ovex, Δptp1, Δptp2, Δhog1* | The *Ptp2ovex* and *Ptp2ovexΔhog1* mutants are hyper-resistant to cadmium toxicity and, together with the *Δhog1* mutant, displayed similar hyper-resistant to FDX, FCZ, and diamide contrary to the hypersensitivity of *Δptp2* mutant. The *Ptp2ovexΔhog1* and *Δhog1* mutants produced a similar level of hypercapsulation but no capsule in the *Ptp1ovexΔptp2* mutant. At 0.1% glucose, a *wt* level of melanisation was observed in *Δhog1, Δptp1, Ptp1ovex*, *Ptp2ovex*, and *Ptp2ovexΔptp1;* drastically reduced melanisation in *Ptp1ovexΔptp2* but no melanin in *Δptp2* mutant. At 0.5% glucose, melanisation disappeared in all the mutants except in *Δhog1.* Highly improved mating efficiency in *Ptp2ovex* and *Ptp2ovexΔhog1* similar to *Δhog1* mutants; no mating in *Δptp2* and *Ptp1ovex* mutants but aslightly reduced mating in *Ptp1ovexΔhog1* compared with the *wt*. Overexpression of *Ptp1* may inhibit filament growth, agar adhesion and invasion [137] |
| *Δpuf4* | Defective *Hxl1* *m*RNA slicing when the mutant is transferred from 30 to 37oC or by adding TCM at 30oC, and this is characterised by stabled *Hxl1* transcript, low accumulation of sliced *Hxl1* *m*RNA, and slow accumulation of downstream Kar2 protein (a temperature stress sensor); defective *Hxl1* *m*RNA decay; defective growth at >37oC with considerable tissue dissemination without any compromise in the virulence when assessed in the MIMC [43,138]. The mutants displayed hyper-filamentation traits but a reduced level of Mfα1and a defective basidiospore formation; attenuated virulence in *G. mellonella* larvae but moderately attenuated in murine intranasal instillation while *Δcrz1Δpuf4* mutants in the same condition remained avirulent [43] |
| *Δqsp1* | Attenuated infection in normal and C5 complement-deficient mice; lower pulmonary burden; induced Th2-dominant response during infection, which is a weak immune response against the cryptococcal cell clearance; induced Type 2 cytokines (IL-4, IL-5, and IL-13) in the lung similar to the *wt*; reduced macrophage intracellular accumulation; larger capsule at 25oC, which reduced to *wt* size at 37oC with normal chitin/chitosan components; hypomelanisation at 37oC; thinner cell wall with a weak distinctive layers |
| *Δrac1, Δcdc42* | Unaffected thermotolerance but a severe defect in haploid filamentation and mating in *Δrac1* mutants; normal actin organisation (i.e., the formation of actin patches and cables) but a poor and ineffective endocytic process in *Δrac1* mutants characterised by the late-appearing unfused small vacuole, which the expression of autoactivated Wsp1p can suppress. Normal actin patches but an absence of cables in *Δcdc42* mutants; defective actin organisation in *Δcdc42Δrac1* mutants [26] |
| *Δrac1, Δrac2, Δcdc42, Δcdc420* | Normal growth at 30/37oC contrary to hyper-defective growth of *Δcdc42Δcdc420Δrac2, Δcdc42Δcdc420,* and *Δras1* mutants at >30oC; normal haploid/diploid cell cycle growth in each mutant at 30oC; a very slight growth improvement could be restored with the overexpression of *Rac1* or *Rac2* in *Δras1* mutant; both mutants are virulence in murine infection model; each mutant is relatively bigger in cell size compared to the *wt* due to heat stress but without any defect in cell morphology, budding, and cytokinesis contrary to the biggest *Δras1* cell size with defective cell morphology; order of sensitivity to Latrunculin B, LatB (actin filament disruptor) increased from *Δrac2, Δcdc42Δcdc420, Δcdc42Δcdc420Δrac2* to *Δras1* but *Δrac1* showed a *wt* LatB sensitivity. Morphologically defective and delayed hyphal formation are observed respectively in bilateral and unilateral mating of *Δrac1* mutant, yet with mating having normal fused clamp cells, binucleate **a**/*α* hyphal segment, and co-segregation of **a** and *α* mating types. The *Δrac2* showed a delayed hyphal formation in unilateral and bilateral mating of mutant; normal hyphal morphology and mating but broader and richer in haustoria (that may be involved in nutrients absorption) than the *wt*. The delayed hyphal formation is caused by a defect in initial fusion mating types though *Mfα1* expression is greatly reduced in *MATα Δrac1 x MAT****a*** *Δrac1* than in *MATα Δrac2 x MAT****a*** *Δrac2* bilateral mating. Also, there is a more significant alteration in ROS localisation during vegetative hyphal growth in *MATα Δrac1 x MAT****a*** *Δrac1* than *MATα Δrac2 x MAT****a*** *Δrac2* [139] |
| *Δram1* | Defective cell growth due to poor and incomplete cytokinesis; actin delocalisation and uneven distribution leading to wrinkled morphology; defective haploid fruiting/differentiation in *C. neoformans,* but *S. cerevisiae* appeared to have an alternative route of circumventing these phenotypic traits associated with this gene disruption [140] |
| *Δras1, Δcdc24* | Both mutants showed increased actin depolarisation from 30 to 37oC; *Δras1Δcdc24, Δras1,* and *Δcdc24* mutants showed growth defect at ≥37oC with enlarged unbudded cell size (poor cytokinesis) similar to *Δcdc42* and *Δcdc420* mutants; however, this could be restored by the overexpression of *Cdc42* or *Cdc420* [23,24]; *Δras1* mutants displayed attenuated virulent irrespective of the temperature in *C. elegans* (25oC) [3] or murine (37oC) |
| *Δras1, Δras2* | The *Δras1Δras2* and *Δras1* mutants are viable at non-permissive temperatures but are sterile at >37oC with a drastic reduction in CSF recovery from infected animal model, which could be restored by the overexpression of Pak kinase *Ste20α* or *Rac1* in either of these background mutants [141];overexpression of *Cdc24* and *Cdc42* but not *Dch2* could also restore the growth defect phenotype of *Δras1* mutants [23]; *Δras2* showed no significant defect relative to the *wt* and overexpression of *Ras2* in the *Δras1* backgroundpartially suppressed thermosensitive vegetative growth, defective actin polarisation (enlarged cell size due to mislocalisation of Rac1p and Rac2p), poor budding, defective mating, poor cell differentiation, and poor filamentation, which are phenotypic attributes of *Δras1* mutants; *Δras1* and *Δras2* mutants showed similar virulence expression as compared to the *wt* [142,143] but are attenuated/avirulent in animal models of cryptococcosis [23] |
| *Δrho1, Δrho10, Δrho11* | The *rho1* mutants under the *Pctr4* repression are thermosensitive at 30oC; *Δrho10* mutants are sensitive >30oC and become hypersensitive at >37oC, which could be partially restored with 1 M sorbitol at 37oC but not at 39oC; *Δrho11* mutants showed a *wt* growth response irrespective of the cultivating temperature or osmotic stabiliser; the *Δrho1Δrho10* double-mutants displayed *Δrho10* features, but sorbitol restored *Δrho10Δrho11* growth defect at 39oC better than the *Δrho10* nevertheless the growth efficiency is still less compared to the *wt* [144] |
| *Δrim101* | Impaired regulation of Cfo1, Cft1, Sit1, Cig1, Ctr4, Ugd1, Cmt1, and Pmm proteins; produce capsule but with poor cell wall integrity; more virulent and survive macrophage better [71,145]. Reduced growth at higher pH and temperature and phosphate availability does not affect the growth. Normal growth in YPD irrespective of the carbon sources and in the presence of heavy metals, zinc, sodium, manganese, CsA, and CsA+calcium [40] |
| *Δrim101, Δrim20, Δvps*25 | Defective melanin and capsule production, sensitive to copper limitation, and increased phagocytosis [54] |
| *Δrom2* | Attenuated virulence and defective cell morphology; *ts* mutants with hyper-elongation phenotype among sub-population at 37oC accompanied with poor actin and microtubules organisation [146] |
| *Δsav1* | Blockage of GXM exocytic secretion, which is accumulated as undischarged vesicles and polarised within the septate and buds; the polarised located vesicles can be perturbed by LatB, which disrupted F-actin formation, but the frequency of the vesicles within the deformed cytokinesis remained significantly higher than the *wt;* more vesicles in the buddings than the parent cells but the presence of LatB reversed this distribution due to defective F-actin; significant reduction of secreted acid phosphatase just as found in *Δsec4* yeast mutant; significant growth reduction from 25 to 30oC and the mutant failed to grow at 37oC (*Δsav1* serotype D mutant is more thermosensitive than serotype A) [147] |
| *Δsac6* | Poor growth under 1% O2 accompanied by the vacuole accumulation of Cap1 protein; normal budding, cell polarity, and cytokinesis; impaired endocytic process but can be rescued with 1 M sorbitol at 1% O2; virulence in mice was unaffected [1] |
| *Δsec14-1, Δsec14-2, Δsfh5, Δplb1* | *Δsec14-1* and *Δsec14-1Δsfh5* mutants are hypovirulent with a delay CNS dissemination (similar to *Δplb1* but more delayed dissemination) due to impaired secretion of acid phosphatase encoded by *Aph1* and phospholipase B1 encoded by *Plb1,* which also caused a reduction in the lung burdens [7,148]; *Δsec14-1, Δsec14-2, Δsfh5, Δsec14-1Δsfh5,* and *Δsec14-2Δsfh5* mutantsshowed a *wt* melanisation; however, *Δsec14-1, Δsec14-1Δsfh5,* and *Δplb1* were hypercapsulated compared to the *wt* while other mutants displayed a *wt* capsule size; reduced macrophage vomocytosis in *Δsec14-1* and *Δplb1; Δsec14-2, Δsfh5,* and *Δsec14-2Δsfh5* mutants showed unperturbed phospholipase secretion and displayed a *wt* virulence; *Sec14-1* and *Sec14-2* are redundant genes capable of restoring phenotypic deficiency in *Δsec14-1* mutant (better restoration with *Sec14-1* than *Sec14-2*), and together with *Sfh5,* are compensatory; *Δsec14-1, Δsec14-1Δsfh5* and *Δplb1* mutants are sensitive to SDS and CFW due to the absence of GPI-anchored Plb1(indication of cell wall defect) but displayed a comparable growth to the *wt* [148] |
| *Δsgl1* | Non-pathogenic mutant in MIMC but capable of inducing CD4+ T-cell-independent immunity against cryptococcal infections; no dissemination nor invasion but a rapid systemic clearance as early as day 3 post-infection [149] |
| *Δsit1* | Impaired iron acquisition through non-reductive siderophore uptake from ferrioxamine B, impaired cAMP-cascade event, increased melanin production (probably by the rescuing Cfo1/Cft1 reductive iron-uptake in this mutant), cell wall integrity with full virulence [150] |
| *Δsnf1, Δsnf5, Δmbf1* | The tendency of melanisation decreased with temperature (delayed melanisation), and the mutant failed to melanise at 37oC; severely attenuated virulence and poor tissue dissemination due to poor growth at 37oC in the presence of alternative carbon sources, mainly sucrose, acetate, and ethanol; poor anti-nitrosative response; defective mating; *Δsnf1* mutants appeared to have *wt* capsulation [2,151] |
| *Δsod1-Cu-Zn* | Conspicuously sensitive to ROS (such as *t*-BOOH) and redox cycling agent (MND) due to significant reduction in Sod activity as temperature increased from 30 to 37oC; fragmentation of large vacuole within the cytoplasm; attenuated virulence and defective growth in the macrophage; susceptible to *in vitro* PMN (neutrophil) killing; impaired activity of laccase, urease, and phospholipase but no defects in the growth, capsule formation, sporulation, mating, stationary phase nutrient limiting survival and auxotrophy for S-containing amino acids unlike *S. cerevisiae Δsod1* mutant [152,153] |
| *Δsod2-Mn* | The avirulent mutants become *ts* with increased susceptibility to superoxide anion [154] |
| *Δspe3-Δlys9* | Impaired spermidine and lysine biosynthesis, growth defect at 30oC, avirulent, and failed to survive *in vivo* in an MIMC*.* The *in vivo* survival *Δspe3-Lys9* mutant displayed a reduced growth at 30oC, avirulent with a drastic capsule reduction but delayed melanin production (just as found in *Δspe3-Δlys9* mutants). The thermosensitive and avirulent *Spe3-Δlys9* mutants failed to grow in the absence of Lys. All the mutants showed poor growth at 37oC [155] |
| *Δsre1, Δstp1, Δscp1* | Poor growth under low O2 and CoCl2 enrich media; loss of virulence (though without a major defect in the melanin and capsule formation); disseminate but with poor proliferation; impaired sterol biogenesis; hypersensitive to azole and failure to grow in iron-limiting media. The *Δsre1* mutant showed defective *Erg5* (C-22 sterol desaturase) under normoxic conditions but remained induced under hypoxic conditions, showed defective *Erg1* (squalene epoxidase) and *Erg3* (C-5 sterol desaturase) in hypoxic condition [156-159] |
| *Δssn8* | Functional mating filamentation and monokaryotic fruiting due to natural overexpression of *Cwc1* in the absence of *Ssn8;* no significant*;* no major defect in the mutant growth compared to the *wt* or overexpressed mutant (*pGpd1::Ssn8*) in the major media or with alternative carbon sources except a poor growth in 2% galactose similar to *S. cerevisiae;* overexpression of Ssn8 prevents heterothallic *α****/a*** mating, and *α*/*α* or **a/a** monokaryotic fruiting but deletion promotes mating and fruiting; compromised cell wall structure and integrity; enhanced agar invasion, melanin, and capsule formation but significantly attenuated for virulence; significant reduction in CFU recovery from the brain, CSF, and liver except in the lung similar to *S. cerevisiae;* overexpression of Ssn8 prevents *α****/a***  effective mating and *α*/*α* or **a/a** monokaryotic fruiting, but deletion promotes mating and fruiting; compromised cell wall structure and integrity [160] |
| *Δste12* | Serotype A *MATα*mutant displayed typical hyphae formation, but the mating frequency was moderately affected; haploid fruiting (sporulation) and filamentation were utterly defective in response to nitrogen starvation; the mutant strain virulence remained unaffected [48,161]. Serotype D *MATα* mutant*,* however, showed a drastic reduction in virulence as assessed in an animal model due to capsular size-reduction [161]. |
| *Δste20* | Serotype A mutant (*Δste20**α*) are sterile for bilateral mating due to the lack of clamp connections in the filaments, which is responsible for mating and haploid fruiting. Defective mating, cytokinesis (formed pseudohyphae-like morphology due to unsegregated septates), grown at 39oC, and virulent in a similar way to serotype D (*Δste20α* and *Δste20***a**); however, serotype D mutants showed a normal growth at 37oC only with full virulence. *Δste20α* practically displayed attenuated virulence, unlike *Δpak1* mutant, due to a reduced capsular size formation in animal models infected with *Δste20α*.  Complete phenotypic restoration is possible in *Δste20α::Ste20α* reconstituted mutant except fora full capsule restoration, which re-iterates the reason for the moderate virulence observed in this reconstituted mutant compared to the *wt* [112] |
| *Δste20Δpak1* | Irrespective of the mating-type strain, the double mutation is synthetically lethal, and the strains are inviable [112] |
| *Δsxi1α* | Either serotype Α/D mutant remained virulence (survived 37oC with prototrophic growth; melanin and capsule production and urease activity remained unaffected), but the mating was defective with limited filamentation, no basidia, no basidiospores [105] |
| *Δtco1, Δtco2* | *Δtco1* mutants showed reduced virulence without any significant defect in hypoxia-responsive genes observed; *Δtco2* is as virulent as the *wt* [156,162] |
| *Δtor1, Δfrr1, Δvad1* | Mutant and heterozygous mutants (*Δtor1* and *Tor1*/*Δtor1*) with *Δfrr1* are susceptible to RPM [163], and with *Δvad1* are susceptible to autophagy [164], temperature (*Mpk1* activity is repressed), DNA damage (*Rad53* activity is repressed) with poor actin/cytoskeletal organisation [163] |
| *Δtps1, Δtps2, Δnth1* | The glycolytic pathway was impaired in each mutant; mutants displayed a *wt* urease activity, melanin, and capsule formation.  *Δtps1* and *ΔTps2* mutants showed growth defect at 37oC, especially in the presence of 2% glucose but not galactose, and this could be restored with 1 M Sorbitol. The *Δtps2* mutant failed to survive at 37oC in the CFS media; the generation time of *Δtps2* and *Δnth1* mutants is the same as the *wt* but slightly lower in *Δtps1* either in the presence of glucose/galactose; occurrence of temperature-dependent accumulation of toxic trehalose-6-phosphate in *Δtps2* mutants leading to cell death at 37oC; *Δtps1* mutants are avirulent in immunocompromised rabbits and mice with adequate and complete clearance from the host body*;* only *Δtps1* mutant displayed attenuated virulence in *C. elegans;* these mutations do not affect the intracellular survival of the mutants in macrophage as all survived anaerobic conditions equally for five days.  All major antifungal susceptibilities of the mutants are mostly the same as the *wt* except *Δtps1* with less susceptibility to FCZ; expression of *Tps1, Tps2,* and *Nth1* is not affected in *Δcna1, Δmga1, Δcpa1,* and *Δcpa2* mutants[165,166] |
| *Δtrp3, Δtrp5* | The *Δtrp* mutants (created by RNA*i*) showed a slightly reduced effect of NCR in the Pro medium; however, *wt* growth could not be achieved with an increased concentration of alternative nitrogen sources, including Trp and dipeptides, which still means a significant effect of NCR on amino acid permeases in this mutant contrary to *Δhom3* and *Δthr1* repressible mutants [167] |
| *Δtsa1, Δtsa3* | *Δtsa1 or Δtsa1Δtsa3* mutants are sensitive to oxidative and nitrosative stress with retarded growth at 25 and 38.5oC and reduced fungal tissue burden in the brain and lungs; however, *Δtsa3* mutant showed a reduced tissue burden in the lung only; there is a total loss of virulence in *Δtsa1* and *Δtsa1Δtsa3* but not in *Δtsa3* mutant [168] |
| *Δtuf1* | Mutants created by RNA*i* showed a significant growth reduction in nonfermentable carbon sources such as glycerol (attribute similar to *Δvad1*), but the growth was slightly affected in the presence of glucose; laccase activity remained unaffected, but virulence is severely attenuated like *Δvad1* mutant; evidence of similar phenotype with *Δaox1* mutant is highly predictable [115] |
| *Δura5* | The mutant and the reconstituted variants (stable transformants with non-specific ectopic integration and unstable transformants with autonomously replicating plasmid) significantly displayed less virulence compared to the *wt* [169] |
| *Δure1* | No growth defect, no impaired virulence factors; drastic reduction in mortality associated with infection; no inflammatory response induced due to poor tissue invasion/dissemination [170,171] |
| *Δuxs1* | Mutant capsule lacked xylose units [92] |
| *Δvad1* | Upregulation of universal repressor of transcription (*Not1*); increased activation of the global transcriptional regulator (*Sre1*) in the presence of FCZ; downregulation of other virulence determinants like *Pck1* (in gluconeogenesis), *Tuf1* (mitochondria elongation factor for protein synthesis), and *Mpf3* (in cell wall integrity) and the mutant failed to utilise glycerol. Completely avirulent in a mouse model of infection accompanied with significantly reduced *Lac1* expression due to accumulation of *Not1* global negative regulator of transcription; however, capsule formation and urease activity remained unchanged; mating was moderately reduced, and finally, *in situ* hybridisation had proved the occurrence of *Vad1* transcripts in the *Cryptococcus* infected HIV patient [115]  Overexpression of *Tuf1* in this mutant restored respiratory growth (mitochondria function) and FCZ sensitivity (both in the *Δvad1* mutant and tetracycline-induced FCZ-resistant strain) but failed to cleave *Sre1* in response to FCZ for the virulence to be restored [115,172]. |
| *Δvtc4, Δepp1,* and *Δxpp1* | Mutants showed no visible growth defect in phosphate-limiting conditions. The *Δvtc4* is drastically susceptible to zinc toxicity showing the scavenging effect of cytoplasmic polyphosphate because of zinc uptake by Pho proteins. The *Δxpp1* and *Δxpp1Δepp1* are more susceptible to zinc than *Δepp1* alone at 30oC but not 37oC. The *Δvtc4, Δxpp1,* and *Δxpp1Δepp1* mutants are resistant to CsA or CsA+calcium; however, *Δepp1* is resistant only at 30oC but becomes susceptible at 37oC. Generally, each single/double mutant showed no growth defect in YPD [40] |
| *Δvph* | Mutants generated by insertional mutation showed defective vacuolar acidification with cytoplasmic vacuoles aggregated into a single enlargement; impaired copper assimilation that rendered laccase inactive but can be restored by exogenous copper [173]; failed to produce capsule, melanin (despite the evidence of *Lac1* transcripts), and urease activity; *ts* mutants with increased doubling time from 30 – 37oC (indication of an increased growth defect); completely avirulent in MIMC [173,174] |
| *Δvps34* | Defective formation of Atg8 autophagy vesicles during phagocytosis, and the mutant was unable to survive starvation. Drastic reduction of melanin production but capsule formation, urease activity, and survival at 37oC remained unaffected, yet the mutant is avirulent in MIMC with zero CFU recovery from the lung or brain, and this is unrelated to poor melanin formation because *Δlac1* mutant was pathogenic albeit with a smaller reduction in virulence when compared to the *wt*.  Compared to the hypovirulent *Δcap60* and *Δlac1* mutants that are systemically half-cleared within 24 *hpi, Δvps34* is rapidly and completely cleared from the lung in about 15 *hpi,* accompanied by a high level of TNF*α* in the mice within 5 *hpi*. Similarly, *Δvps34* mutants are more rapidly killed within 24 hours after macrophage phagocytosis but induced similar *wt* macrophage autophagy following the conversion of lipidated Atg8 homologue, LC3-I to LC3-II in the infected macrophage. The *wt* expression of *Vps34* complemented *S. cerevisiae Δvps34* mutant to restore the thermosensitivity and autophagy body formation in nutrient-limiting conditions [175] |
| *Δvps45* | Poor iron acquisition; defective growth from 30 – 37oC; disruption of vacuolar accumulation of Cfo1 protein; susceptible to hyperosmotic stress, CFW, caffeine, >0.01% SDS, >3 mM chloroquine (CQ), and 0.4 mM quinacrine. The cell wall integrity and major virulence factors remain unaffected, but macrophage clearance increases due to the reduced intracellular survival of this mutant [176] |
| *Δwos2* | Slight growth reduction in the presence of Zn from 30 to 37oC; no major significant effect on the growth and all phenotypic associated virulence traits [177] |
| *Δwsp1* | Severe defect in endocytosis; defective cell morphology/vacuolar biogenesis due to poor actin organisation, which could be partially restored by the expression of the active domain of Cin1p and could be fully restored with the constitutive expression of Cdc42p fused to the active domain of Cin1p. Furthermore, the mutants showed mislocalisation of Rac1p (a vacuolar membrane-associated Rho GTPase) due to poor co-localisation of Wsp1-GBP (active)/Wsp1-B-GBP (more active) and Rac1p. Other defects include poor chitin formation and distribution; Sav1p mislocalisation leading to a poor secretory process of polysaccharide components of cryptococcal GXM; defective mating due to poor pheromone response; acapsular with deformed cell morphology; decreased melanin and urease activity; susceptible to phagocytosis and attenuated virulence with moderate tissue invasion. Most of these defects can partially to fully be restored by the expression of Wsp1-GBP (active)/Wsp1-B-GBP (more active) [26,178] |
| *Δyfh1* | Excessive accumulation of iron within the mitochondria, which led to ionic imbalance and mitochondrial rupture [179] |
| *Δznf1* | Normal cell fusion and hyphal formation in *MAT****a***-*α* mating or normal filamentation in *α-α* mating [49] |
| *Δznf2* (located on chromosome 8) | Severely impaired filamentation in bilateral mating but slightly reduced formation of hyphae and basidiospores in the unilateral mating; effective cell fusion (either homozygous *α-α* fusion/heterozygous ***a****-α* fusion diploid) mutants that failed to filament during bilateral mating but rather bud continuously; the effective and elevated pheromone expression and production that could induce conjugation tube/monokaryotic hyphae in the proximal *wt* *MATα* mating-type contradicted MAT**a** *ΔMat2* and *ΔSte7* mutants; no conjugation tube induced in MAT*α ΔZnf2* mutant in proximity to *wt* *MAT****a*** mating type; apparent virulence improvement in MIMC without any significant effect on melanisation, capsulation, and thermotolerance[49] |
| *Δznf2Δcpk1* | The double mutant showed severely impaired filamentation during unilateral mating with *wt* *MAT****a*** in a similar way to *ΔCpk1* mutant [49] |

Phenotypic expression and virulence exist for the *GATA* transcription factors (*Gat1, Gat201, Gat204, Bwc2,* and *Cir1*), *Tup1,* and *Rim101*. Fungal GATA transcriptional factors are usually characterised with Cys-X-X-Cys-X17-20-Cys-X-X-Cys-5-12 basic residues DNA binding domain recurring zinc finger motif [69]. **AdF =** anidulafungin; **BAPTA =** 1,2-bis(2-aminophenoxy)ethane-N,N,N,N,-tetraacetic acid; **BBB =** blood-brain-barrier; **Caffeine** inhibits signal transduction pathways necessary for cell wall biogenesis; **Congo red** is a stain that binds *β*-1,4-glucans to disrupt cell wall biogenesis. **5-FC =** 5-fluorocytosine/flucytosine (FCT); **AmpB =** amphotericin B (polyene drug); **CFU =** colony forming unit; **CNS =** central nervous system; **CpF =** caspofungin; **CQ =** chloroquine; **CsA =** cyclosporine A; **CSF =** cerebrospinal fluid; **CFW =** Calcofluor White; **DAG =** diacylglycerol; **DPI =** diphenyleneiodonium; **ECC =** echinocandin; **FCZ =** fluconazole (azole drug); **FDX =** fludioxonil; **GXM =** glucuronoxylomannan; **hCFS =** human cerebrospinal fluid; **hpi =** hours post-infection; **ICZ =** itraconazole; **PP-IP =** diphosphoinositol phosphate/inositol pyrophosphate; **IP4/IP5/IP6/IP7/IP8 =** *myo-*inositol tetra/penta/hexa/hepta/octa – kisphosphates; **LatB =** Latrunculin B; ***L*-DOPA =** 3,4-dihydroxylphenylalanin; **LIM =** low-iron medium; **LPL =** lysophospholipase; **LPTA =** lysophospholipase/transacylase; **Lys =** lysine; **McF =** micafungin; **MCZ** **=** miconazole; **MIMC =** mouse infection model of cryptococcosis; **MND =** menandione; **MNS =** monensin; **NCR =** nitrogen catabolite repression; **NMC =** neomycin; **NTC =** nourseothricin; **ovex =** overexpression; **PCZ =** posaconazole; **PI-PLC =** phosphatidylinositol‐specific phospholipase C; **PMN =** polymorphonuclear leukocytes; **Pro =** proline **ROS =** reactive oxygen species; **RPM =** rapamycin; **SD =** synthetic dextrose; **SDS =** sodium dodecyl sulphate (a detergent and protein destabiliser); **SHAM =** salicylhydroxamic acid; **TCA =** tricarboxylic acid cycle; **TCM =** tunicamycin; **Trp =** tryptophan; ***ts* =** temperature sensitive; **VCZ =** voriconazole; ***wt* =** wild-type; **YNB =** yeast nitrogen base; **YPD =** yeast peptone dextrose media.

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