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PEARLS

Environmental pH modulation by pathogenic fungi as a strategy to conquer the host

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The ability of microorganisms to sense and adapt to changes in the environment is essential to their survival. This is particularly important for species with an intimate association with host organisms, such as pathogens, symbionts, and commensals. Host environments vary greatly in pH, ranging from highly acidic in the stomach (pH < 2) to mildly acidic on the skin and plant surfaces ($6.5 <$ pH < 4.5), neutral in the blood (pH 7.4), and basic in parts of the intestine (pH < 8.5) [1–4], and fungi have developed multiple mechanisms to adapt to pH variations. This Pearl will focus on the ability of pathogenic fungi to respond to and actively modulate the host's pH.

Fungal adaptation to changes in ambient pH

In fungi, the adaptive responses induced by changes in ambient pH have been extensively studied in model organisms. For example, the response to weak acid stress has been characterized in *Saccharomyces cerevisiae* [5], while the role of the Pal/Rim alkaline response pathway, one of the most specialized and conserved signaling cascades in fungi, has been delineated in *S. cerevisiae*, *Aspergillus nidulans*, *Yarrowia lipolytica*, and several fungal pathogens [6–8]. The mechanisms of pH sensing and adaptation in fungi have been reviewed elsewhere [9–11].

Fungal pathogens can modulate the pH of their host

Another aspect of pH regulation is the ability of microorganisms to actively modify the pH of their environment. Fungi can achieve this by secreting acids or alkali. The ability of fungi to secrete natural organic acids (such as butyrate, oxalate, malate, citrate, gluconate, and succinate) is well utilized in the industry, particularly with nonpathogenic *Aspergillus* sp. and *Rhizopus* sp. The magnitude of pH change depends on the nutrient availability, the organic acids being produced, and on the ability of the fungus to remove ammonium ions from ammonium sulfate salt or to excrete H^+-ions as a byproduct of NH_4^+ assimilation [12, 13]. Acidifying fungi can also raise extremely low pH levels to a favorable level.

Certain pathogenic fungi acidify the environment as a strategy to damage host tissues. Many plant-necrotizing fungi secrete significant amounts of acid: *Sclerotinia sclerotiorum* and *Butrytis* sp. produce oxalic acid [14], while *Pennicilium* sp. and *Aspergillus* sp. secrete mainly gluconic and citric acids [15, 16]. The produced acids not only acidify the tissues but can also lower the activity of reactive oxygen species produced by the host [17]. Fusaric acid produced by *Fusarium oxysporum* acidifies plant surfaces and activates the membrane H^+-ATPase, a pH-regulated process that leads to the expression of proteases and subsequent tissue invasion [18]. Similarly, the human pathogen *Candida albicans* acidifies the environment in a carbohydrate-dependent fashion, allowing production of aspartyl proteases, which are potent virulence factors [19].

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Ammonia as a key player in environmental alkalinization

Environmental alkalinization in fungi is common yet not a well-understood phenomenon. Often, this process is mediated by ammonia, a multifunctional biological molecule with diverse roles in eukaryotes. In fungi, it supports communication between colonies in *S. cerevisiae* [20], aging of surface-ripened cheeses by *Y. lipolytica* and *Debaryomyces hansenii* [21], and expression of pectin lyase, a key virulence factor in *Colletotrichum* sp. [22], among other functions.

Ammonia is generated either extracellularly or within the fungal cell as a byproduct of protein and amino acid catabolism, common nutrients in many host niches [23, 24]. Excess intracellular ammonia is secreted or exported from the cell or exported as urea and subsequently converted to NH_4^+ by secreted ureases. Accumulation of this highly basic compound in the immediate environment raises the pH. An excellent example of this process is found in the phytopathogen *Colletotrichum gloeosporioides*, a cause of anthracnose fruit rot. The fungus utilizes L-glutamate or glutamine to produce ammonia, which elevates the environmental pH of healthy fruit from 5.6 to 8.5. This results in the activation of fungal pathogenicity factors, such as production of pectate lyase, induction of appressorium formation during host penetration, and stimulation of host cell death mechanisms [22] (Fig 1). In other fungal species, the

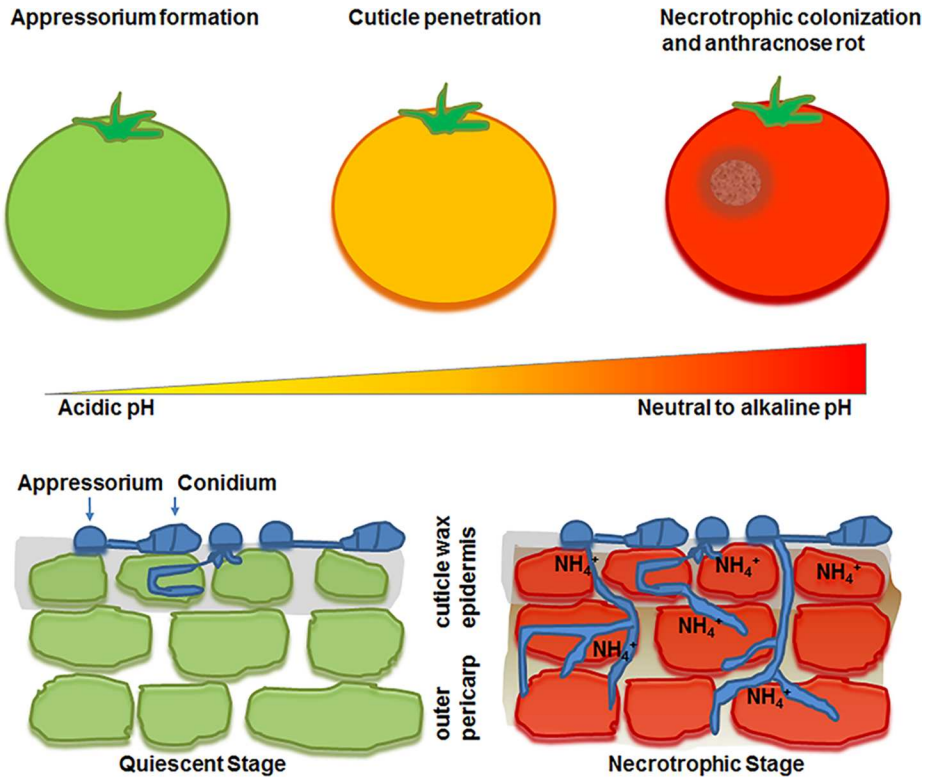

Fig 1. Modulation of host pH by the phytopathogen *Colletotrichum gloeosporioides* increases fungal virulence. *C. gloeosporioides* infects the tomato fruit in a process initiated upon attachment of the fungal conidia to the plant surface. During the quiescent stage of infection, fruit physiological factors such as nutrient availability, acidic pH, and surface waxes determine the rate of fungal growth and germination. As the fruit ripens, conidia germinate into a specialized structure, named appressorium, which eventually becomes melanized. Melanin alters the permeability of the plant cell wall, creating a hypertonic environment that allows the fungus to penetrate the host epidermis using turgor pressure. This process is accompanied by active metabolism of amino acids, such as glutamate and glutamine, and gradual environmental alkalization. The fungus transitions into the necrotrophic stage, characterized by a dramatic shift in fungal metabolism and activation of pathogenicity factors, such as proteases and lyases, resulting in anthracnose fruit rot.

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alkalinized environment also activates the expression of virulence traits: production of asexual spores and secretion of lytic enzymes in *Magnaporthe oryzae*, melanin formation and capsule production in *Cryptococcus neoformans*, and hyphal morphogenesis, adhesion, and invasion in *C. albicans*, among others [25–29].

Typically, fungi increase the environmental pH at a steady but slow pace. A clear exception is *C. albicans*, which is capable of ammonia-driven alkalinization at a remarkable rate. Upon growth on amino acids as the sole carbon source, this human pathogen can modulate the environmental pH from 4 to ~7.5 within few hours, a process also driven (albeit more slowly) by other *Candida* sp. [23, 30] The alkalinization mechanism has been studied extensively and includes sensing of amino acids from the extracellular milieu via the SPS (Ssy1, Ptr3, and Ssy5) sensor system, followed by activation of the transcription factor Stp2p in an SPS-dependent manner and induction of amino acid influx [31]. As a result, ammonia is generated and exported via ammonium transporters to raise the environmental pH and allow fungal transition to the more virulent hyphal form [32, 33]. Deletion of genes involved in any step of this mechanism leads to impaired generation of ammonia and neutralization of the medium in response to these nutrients. Thus, metabolism of amino acids is critical for pH modulation by this fungus.

C. albicans is closely associated with the host and has evolved to utilize a variety of nonpreferred carbon sources available in different anatomical sites. Metabolism of organic acids and N-acetylglucosamine also results in environmental alkalinization [23, 34, 35]. However, genes required for alkalinization on amino acids do not affect growth or pH changes on these nutrients, suggesting different mechanisms for pH modulation [35]. Most importantly, *C. albicans* cells grown on organic acids do not generate ammonia. How *C. albicans* generates a basic extracellular environment under these conditions is currently unknown. It is also not clear if other fungal species share the ability to neutralize the environment upon utilization of these nutrients.

Regulation of ammonia production

Fungi regulate the production of ammonia depending on environmental cues. Ammonia production by *M. anisopliae* is tightly regulated by amino acids, a signal for the presence of proteinaceous nutrients in the environment. *M. anisopliae* grown in media containing low levels of single amino acids yields higher levels of ammonia than when amino acids are abundant, implying either induction of catabolite repressible enzyme(s) or regulation of enzyme activity via substrate inhibition [28]. Generation of ammonia by *N. crassa* and *A. fumigatus* is a loosely regulated process triggered by nutrient deprivation [36]. Presence of glucose in the environment represses the process, presumably due to the metabolic switch from gluconeogenesis to glycolysis or repression of deaminases and ammonia transporters. It is also possible that the higher growth rate in the presence of glucose allows for complete utilization of ammonia released from amino acid catabolism.

Environmental alkalinization as a virulence factor

Many fungal pathogens modulate environmental pH as a means to escape host immune responses, facilitate destruction of the host tissues, and/or stimulate reproduction. Most fungi inhabit mildly acidic environments, such as soil, plant, and animal surfaces. On the other hand, for some fungi, such as the phytopathogens *C. gloeosporioides* and *M. oryzae*, acidic pH favors fungal colonization and invasion [12, 28]. The mildly acidic pH of the plant surface favors both germination of attached conidia and rapid differentiation of the germ tube into a specialized cell named appressorium. Once the appressorium penetrates the plant tissues, the fungus switches to necrotrophic development, associated with rapid ammonia release and

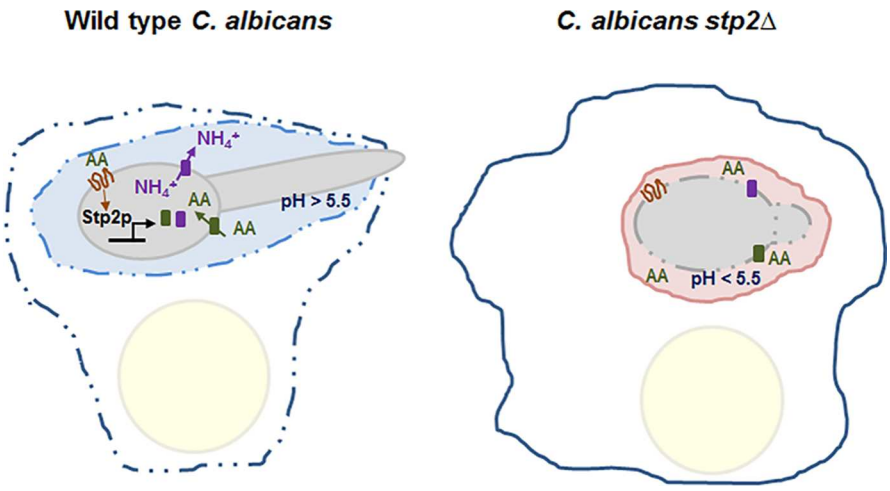

Fig 2. Neutralization of the macrophage phagosome by the fungal pathogen *C. albicans* is essential for host damage. Upon phagocytosis by the macrophages, *C. albicans* responds to the presence of amino acids and other alternative carbon sources abundant in the phagosomal milieu. Amino acids (AA) in particular are sensed via the SPS sensor system (orange), leading to activation of the transcription factor Stp2p, which induces the expression of genes encoding for amino acid permeases (green rectangles) and ammonium transporters (purple rectangles). This results in uptake of amino acids into and release of NH_4^+ from the fungal cell and increase of phagosomal pH. Hyphal growth causes physical damage to the macrophage membranes, leading to leakage of cellular content and death. *C. albicans* cells defective in utilization of amino acids and/or extrusion of NH_4^+, such as the *stp2Δ* mutant, fail to modulate the pH of the phagosome and are readily cleared by the immune cells.

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increase in environmental pH, which triggers the expression of virulence factors [13, 22, 28, 37]. Thus, the acidic environment serves as a signal in this fungus to switch from saprotrophic to necrotrophic growth and damage the host (Fig 1).

Neutralization of acidic niches is a very common microbial strategy to evade host immune responses. For example, *C. albicans* neutralizes the macrophage phagosome, a process essential for germination and escape from the immune cell [31–33]. *C. albicans* genes essential for pH modulation in vitro fail to induce macrophage damage, highlighting the importance of this process in immune evasion (Fig 2) [31–33]. Similarly, *Candida glabrata* mutants with alkalinization defect in vitro, such as cells lacking functional mannosyltransferases, fail to effectively modify phagosomal pH and damage the macrophage [38]. The spherules of *Coccidioides* spp., causative agents of coccidioidomycosis, release enzymatically active urease and ammonia to raise the environmental pH and destroy the host tissue [39, 40]. Disruption of the urea degradation pathway in this organism significantly attenuates fungal virulence and survival in mice [39]. Thus, fungal pathogens can alkalinize the host environment to modulate their virulence, underlining the importance of this process on microbial and human physiology.

In summary, adaptation of fungi to pH variations in the host is critical for their survival, and fungal pathogens are capable of actively modulating the environmental pH. Acidification of the host tissues promotes expression and activity of fungal proteases. Many fungi utilize nitrogen or carbon metabolism pathways to generate ammonia, which is released from the cell to raise the extracellular pH. Generation of alkaline pH favors morphogenetic and reproductive processes in fungi, such as germination, hyphal growth, and formation of fruiting bodies, all critical for disease progression. The alkaline pH increases fungal virulence by facilitating penetration into host surfaces and hindering or evasion of immune responses. Thus, the ability

to control extracellular pH is an important aspect of fungal physiology that contributes to fitness within the host.

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References

1. Arbenz H. [Studies on pH values of the surface of the normal skin]. Dermatologica. 1952; 105(6):333–53. PMID: 13043261
2. Krahulec IL, Poncova-Kozouskova V. [Determination of pH in the oral cavity]. Cesk Stomatol. 1951; 51 (6–7):210–22. PMID: 14887035
3. Dubois D. A GLASS ELECTRODE FOR TESTING THE pH OF BLOOD. Science. 1932; 76(1976):441–3. doi: 10.1126/science.76.1976.441 PMID: 17744347
4. Lang WR. Vaginal acidity and pH; a review. Obstet Gynecol Surv. 1955; 10(4):546–60. PMID: 13244967
5. Piper P, Calderon CO, Hatzixanthis K, Mollapour M. Weak acid adaptation: the stress response that confers yeasts with resistance to organic acid food preservatives. Microbiology. 2001; 147(Pt 10):2635–42. doi: 10.1099/00221287-147-10-2635 PMID: 11577142
6. Blanchin-Roland S, Da Costa G, Gaillardin C. ESCRT-I components of the endocytic machinery are required for Rim101-dependent ambient pH regulation in the yeast Yarrowia lipolytica. Microbiology. 2005; 151(Pt 11):3627–37. doi: 10.1099/mic.0.28196-0 PMID: 16272384
7. Penalva MA, Arst HN Jr. Regulation of gene expression by ambient pH in filamentous fungi and yeasts. Microbiol Mol Biol Rev. 2002; 66(3):426–46, table of contents. PubMed Central PMCID: PMC120796. doi: 10.1128/MMBR.66.3.426-446.2002 PMID: 12208998
8. Xu W, Smith FJ Jr., Subaran R, Mitchell AP. Multivesicular body-ESCRT components function in pH response regulation in Saccharomyces cerevisiae and Candida albicans. Mol Biol Cell. 2004; 15 (12):5528–37. PubMed Central PMCID: PMC532031. doi: 10.1091/mbc.E04-08-0666 PMID: 15371534
9. Maeda T. The signaling mechanism of ambient pH sensing and adaptation in yeast and fungi. FEBS J. 2012; 279(8):1407–13. doi: 10.1111/j.1742-4658.2012.08548.x PMID: 22360598
10. Davis D. Adaptation to environmental pH in Candida albicans and its relation to pathogenesis. Curr Genet. 2003; 44(1):1–7. doi: 10.1007/s00294-003-0415-2 PMID: 12819929
11. Bignell E. The Molecular Basis of pH Sensing, Signaling, and Homeostasis in Fungi. Adv Appl Microbiol. 2012; 79:1–18. doi: 10.1016/B978-0-12-394318-7.00001-2 PMID: 22569515
12. Prusky D, McEvoy JL, Leverentz B, Conway WS. Local modulation of host pH by Colletotrichum species as a mechanism to increase virulence. Mol Plant Microbe Interact. 2001; 14(9):1105–13. doi: 10.1094/MPMI.2001.14.9.1105 PMID: 11551075
13. Bi F, Barad S, Ment D, Luria N, Dubey A, Casado V, et al. Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity in phytopathogenic fungi. Mol Plant Pathol. 2016; 17(8):1178–95. doi: 10.1111/mpp.12355 PMID: 26666972
14. Manteau S, Abouna S, Lambert B, Legendre L. Differential regulation by ambient pH of putative virulence factor secretion by the phytopathogenic fungus Botrytis cinerea. FEMS Microbiol Ecol. 2003; 43 (3):359–66. doi: 10.1111/j.1574-6941.2003.tb01076.x PMID: 19719667
15. Ruijter GJ, Visser J. Characterization of Aspergillus niger phosphoglucose isomerase. Use for quantitative determination of erythrose 4-phosphate. Biochimie. 1999; 81(3):267–72. PMID: 10385009
16. Prusky D, Yakoby N. Pathogenic fungi: leading or led by ambient pH? Mol Plant Pathol. 2003; 4 (6):509–16. doi: 10.1046/j.1364-3703.2003.00196.x PMID: 20569410
17. Chen C, Dickman MB. Proline suppresses apoptosis in the fungal pathogen Colletotrichum trifolii. Proc Natl Acad Sci U S A. 2005; 102(9):3459–64. PubMed Central PMCID: PMC552905. doi: 10.1073/pnas.0407960102 PMID: 15699356
18. Brandao RL, Castro IM, Passos JB, Nicoli JR, Thevelein JM. Glucose-induced activation of the plasma membrane H(+)-ATPase in Fusarium oxysporum. J Gen Microbiol. 1992;138 Pt 8:1579–86.
19. Naglik JR, Challacombe SJ, Hube B. Candida albicans secreted aspartyl proteinases in virulence and pathogenesis. Microbiol Mol Biol Rev. 2003; 67(3):400–28, table of contents. PubMed Central PMCID: PMC193873. doi: 10.1128/MMBR.67.3.400-428.2003 PMID: 12966142
20. Palkova Z, Janderova B, Gabriel J, Zikanova B, Pospisek M, Forstova J. Ammonia mediates communication between yeast colonies. Nature. 1997; 390(6659):532–6. doi: 10.1038/37398 PMID: 9394006

21. Gori K, Mortensen HD, Arneborg N, Jespersen L. Ammonia production and its possible role as a mediator of communication for Debaryomyces hansenii and other cheese-relevant yeast species. J Dairy Sci. 2007; 90(11):5032–41. doi: 10.3168/jds.2006-750 PMID: 17954742

22. Shnaiderman C, Miyara I, Kobiler I, Sherman A, Prusky D. Differential activation of ammonium transporters during the accumulation of ammonia by Colletotrichum gloeosporioides and its effect on appressoria formation and pathogenicity. Mol Plant Microbe Interact. 2013; 26(3):345–55. doi: 10.1094/MPMI-07-12-0170-R PMID: 23387470

23. Vylkova S, Carman AJ, Danhof HA, Collette JR, Zhou H, Lorenz MC. The fungal pathogen Candida albicans autoinduces hyphal morphogenesis by raising extracellular pH. MBio. 2011; 2(3):e00055–11. PubMed Central PMCID: PMC3101780. doi: 10.1128/mBio.00055-11 PMID: 21586647

24. Schindler J, Sussman M. Ammonia determines the choice of morphogenetic pathways in Dictyostelium discoideum. J Mol Biol. 1977; 116(1):161–9. PMID: 563469

25. Sun Y, Cao C, Jia W, Tao L, Guan G, Huang G. pH Regulates White-Opaque Switching and Sexual Mating in Candida albicans. Eukaryot Cell. 2015; 14(11):1127–34. PubMed Central PMCID: PMC4621312. doi: 10.1128/EC.00123-15 PMID: 26342021

26. Nobile CJ, Solis N, Myers CL, Fay AJ, Deneault JS, Nantel A, et al. Candida albicans transcription factor Rim101 mediates pathogenic interactions through cell wall functions. Cell Microbiol. 2008; 10 (11):2180–96. PubMed Central PMCID: PMC2701370. doi: 10.1111/j.1462-5822.2008.01198.x PMID: 18627379

27. Davis D, Edwards JE Jr., Mitchell AP, Ibrahim AS. Candida albicans RIM101 pH response pathway is required for host-pathogen interactions. Infect Immun. 2000; 68(10):5953–9. PubMed Central PMCID: PMC101559. PMID: 10992507

28. Landraud P, Chuzeville S, Billon-Grande G, Poussereau N, Bruel C. Adaptation to pH and role of PacC in the rice blast fungus Magnaporthe oryzae. PLoS ONE. 2013; 8(7):e69236. PubMed Central PMCID: PMC3712939. doi: 10.1371/journal.pone.0069236 PMID: 23874922

29. Vecchiarelli A, Pericolini E, Gabrielli E, Kenno S, Perito S, Cenci E, et al. Elucidating the immunological function of the Cryptococcus neoformans capsule. Future Microbiol. 2013; 8(9):1107–16. doi: 10.2217/fmb.13.84 PMID: 24020739

30. Priest SJ, Lorenz MC. Characterization of Virulence-Related Phenotypes in Candida Species of the CUG Clade. Eukaryot Cell. 2015; 14(9):931–40. PubMed Central PMCID: PMC4551586. doi: 10.1128/EC.00062-15 PMID: 26150417

31. Miramon P, M CL. The SPS amino acid sensor mediates nutrient acquisition and immune evasion in Candida albicans. Cell Microbiol. 2016.

32. Danhof HA, Lorenz MC. The Candida albicans ATO Gene Family Promotes Neutralization of the Macrophage Phagolysosome. Infect Immun. 2015; 83(11):4416–26. PubMed Central PMCID: PMC4598414. doi: 10.1128/IAI.00984-15 PMID: 26351284

33. Vylkova S, Lorenz MC. Modulation of phagosomal pH by Candida albicans promotes hyphal morphogenesis and requires Stp2p, a regulator of amino acid transport. PLoS Pathog. 2014; 10(3):e1003995. PubMed Central PMCID: PMC3953444. doi: 10.1371/journal.ppat.1003995 PMID: 24626429

34. Naseem S, Araya E, Konopka JB. Hyphal growth in Candida albicans does not require induction of hyphal-specific gene expression. Mol Biol Cell. 2015; 26(6):1174–87. PubMed Central PMCID: PMC4357515. doi: 10.1091/mbc.E14-08-1312 PMID: 25609092

35. Danhof HA, Vylkova S, Vesely EM, Ford AE, Gonzalez-Garay M, Lorenz MC. Robust Extracellular pH Modulation by Candida albicans during Growth in Carboxylic Acids. MBio. 2016; 7(6).

36. Wiame JM, Grenson M, Arst HN Jr. Nitrogen catabolite repression in yeasts and filamentous fungi. Adv Microb Physiol. 1985; 26:1–88. PMID: 2869649

37. Alkan N, Davydov O, Sagi M, Fluhr R, Prusky D. Ammonium secretion by Colletotrichum coccodes activates host NADPH oxidase activity enhancing host cell death and fungal virulence in tomato fruits. Mol Plant Microbe Interact. 2009; 22(12):1484–91. doi: 10.1094/MPMI-22-12-1484 PMID: 19888814

38. Kasper L, Seider K, Gerwien F, Allert S, Brunke S, Schwarzmuller T, et al. Identification of Candida glabrata genes involved in pH modulation and modification of the phagosomal environment in macrophages. PLoS ONE. 2014; 9(5):e96015. PubMed Central PMCID: PMC4006850. doi: 10.1371/journal.pone.0096015 PMID: 24789333

39. Mirbod-Donovan F, Schaller R, Hung CY, Xue J, Reichard U, Cole GT. Urease produced by Coccidioides posadasii contributes to the virulence of this respiratory pathogen. Infect Immun. 2006; 74(1):504–15. PubMed Central PMCID: PMC1346605. doi: 10.1128/IAI.74.1.504-515.2006 PMID: 16369007

40. Wise HZ, Hung CY, Whiston E, Taylor JW, Cole GT. Extracellular ammonia at sites of pulmonary infection with Coccidioides posadasii contributes to severity of the respiratory disease. Microb Pathog. 2013;59–60:19–28. PubMed Central PMCID: PMC3656146.