Kurdistan Regional Government- Iraq

Ministry of Higher Education & Scientific Research

Salahaddin University – Erbil

Directorate of Higher Education & Postgraduate Studies

Education College - Biology Department

Anti-Fungal Resistance

A Review artic in Medical Mycology

In Partial Fulfillment of the Requirements for the Degree of Ph.D.

Prepared by:

Sherko Muhammed Abdul-Rahman

Supervised by:

Assist. Pro. Dr. Karzan Abdulmuhsin Mohammad

(2023-2024)

Anti-Fungal Resistance

[a] Sherko Muhammed Abdul-Rahman, [b] Karzan Abdulmuhsin Mohammad

[a] Department Biology, Education College, Salahaddin University -Erbil,
"Email: sherko.abdulrahman@su.edu.krd"
[b] Department Biology, Education College, Salahaddin University -Erbil,
"Email: karzan.mohammad@su.edu.krd"

Abstract

Despite improvement of antifungal therapies over the last 30 years, the phenomenon of antifungal resistance is still of major concern in clinical practice. In the last 10 years the molecular mechanisms underlying this phenomenon were extensively unraveled. In this article, after a brief overview of currently available antifungals, molecular mechanisms of antifungal resistance will be detailed. It appears that major mechanisms of resistance are essential due to the deregulation of antifungal resistance effector genes. This deregulation is a consequence of point mutations occurring in transcriptional regulators of these effector genes. Resistance can also follow the emergence of point mutations directly in the genes coding antifungal targets. In addition, we further describe new strategies currently undertaken to discover alternative therapy targets and antifungals. Identification of new antifungals is essentially achieved by the screening of natural or synthetic chemical compound collections. Discovery of new putative antifungal targets is performed through genome-wide approaches for a better understanding of the human pathogenic fungi biology.

Keywords: Antifungal resistance, Fungal Infections, *Candida albicans*, antifungal targets

1. Introduction

Fungus (pl: fungi) are a diverse group of organisms with the common characteristic of lacking chloroplast and the presence of a chitin cell wall (Naranjo-Ortiz and Gabaldón, 2019). Fungi belong to the eukaryotic domain of life (Woese et al., 1990). As predicted, around 2.2 to 6 million species of fungi may exist, although only around 120 000 have been described (Hawksworth and Lücking, 2017). Fungi are natural decomposers essential in recycling environmental nutrients (Frąc et al., 2018). Apart from their vital role in the environment, fungi are also helpful to humans in several ways. For example, several species of fungi (known as a mushroom) are a source of food and nutrition (Ho et al., 2020).

Fungi are essential in baking, beverage industries (beer, wine, alcohol), soya sauce, and cheese preparation. Fungi also found their importance in pharmaceutical companies. For example, drugs like ergometrine, cortisone, and cyclosporine are all derived from fungi. They also remain the source of essential antibiotics (for example, penicillin); also used for producing beneficial chemicals (like citric acid), a source of valuable enzymes, a host for the production of heterologous proteins, a biological model, and controlling pests in agriculture (Hyde et al., 2019).

Like viruses and bacteria, many fungi are also cause several infectious diseases (parasites) in plants, animals, humans. A disease or infection caused by fungi is known as mycosis (Richardson and Warnock, 2012). Based on site, fungal infection can be superficial, cutaneous, subcutaneous, systemic (affecting the entire body) and opportunistic (Dixon and Walsh, 1996). Because of the reasons mentioned in the coming sections, treating, or managing fungal infections is becoming more challenging and a matter of great concern. Plant pathogenic fungi are able to cause extensive damage and losses to agriculture and forestry including the rice blast fungus (Vandeputte *et al.*, 2012a), Dutch elm disease, and chestnut blight. Some other fungi can cause serious diseases in humans, several of which may be fatal if left untreated. These include aspergillosis, candidosis, coccidioidomycosis, cryptococcosis, histoplasmosis, mycetomas, mucormycosis, and para-coccidioidomycosis. The so-called dermatophytic and keratinophilic fungi can attack eyes, nails, hair, and especially skin and cause local infections such as ringworm and athlete's foot. Fungal spores are also a cause of allergies, and fungi from different taxonomic groups can provoke allergic reactions (Diamond, 1991).

A healthy and immunocompetent individual maintaining optimum hygiene rarely gets a fungal infection. However, due to the rapid emergence and spread of anti-fungal resistance against commonly used anti-fungal drugs, there is a dire need to find alternative ways to treat better and manage the global fungal burden (Fisher et al., 2022). Discussion of the reasons and molecular mechanism involved in development of anti-fungal resistance is skipped in this review as this topic have been discussed by others (Cowen et al., 2015).

Antifungal drugs treat fungal infections by killing or stopping the growth of dangerous fungi in the body. Fungi can develop resistance to antifungal drugs the same way bacteria can develop resistance to antibiotics (Ostrowsky et al., 2020). Resistance happens when germs

develop the ability to defeat the drugs designed to kill them. That means the germs are not killed and continue to grow (Tornimbene et al., 2018).

Currently, only a small number of antifungal drug types exist, so resistance can severely limit treatment options. Some types of fungi, like *Candida auris*, can become resistant to all the antifungal drugs normally used to treat these infections(Ostrowsky et al., 2020). Resistance is especially concerning for patients with invasive fungal infections severe infections that affect the blood, heart, brain, eyes, or other parts of the body(Shah et al., 2012).

The aim of this review article briefly discusses ways to better manage the increasing global fungal burden and rising anti-fungal resistance, after a brief presentation of the medical impact of fungal infections at the global level and a summary of clinical treatments available today for clinicians, we will review the mechanisms underlying in vitro resistance to antifungals in fungal species of major importance in human medicine. Discovery of new putative antifungal targets is performed through genome-wide approaches for a better understanding of the human pathogenic fungi biology. Lastly, an overview of ongoing research undertaken to develop new therapeutic strategies to fight against fungal infections will be exposed.

2. Basics of fungal biology

It is crucial to understand fungal biology to develop more effective treatment and prevention methods for fungal infections. Fungal pathogens can be divided into two major groups: filamentous fungi and yeasts (Vandeputte *et al.,* 2012b). Most of the primary pathogens are filamentous fungi, while most of the opportunistic pathogens (which cause infections when a patient's immune defenses are compromised) are yeasts (Vandeputte *et al.*, 2012b). The basic biology of filamentous fungi and their lifecycle is shown in Fig.1 (a) (Worrall, 1999). Filamentous fungi are generally composed of elongated, branched hyphae with cell walls, and typically reproduce sexually or asexually by producing a multitude of spores (as shown in Fig.1 (a) (Worrall, 1999). Their hyphae are multicellular, filamentous, branching structures, comprising apically growing series of colorless cellular compartments separated by cell walls, as shown in Fig.1 (b) (Webster and Weber, 2007). The structures of the hyphae are unique and are not found in other kingdoms of multicellular life such as plants and animals (Di Mambro et al., 2019) Each fungus grows a vast number of hyphae that are intertwined into a tangled web called mycelium (Webster and Weber, 2007). In contrast, yeasts

grow as discrete cells. Some of them proliferate via fission, but proliferation by budding is more common as depicted in Fig.1 (c) (Martin and Arkowitz, 2014).

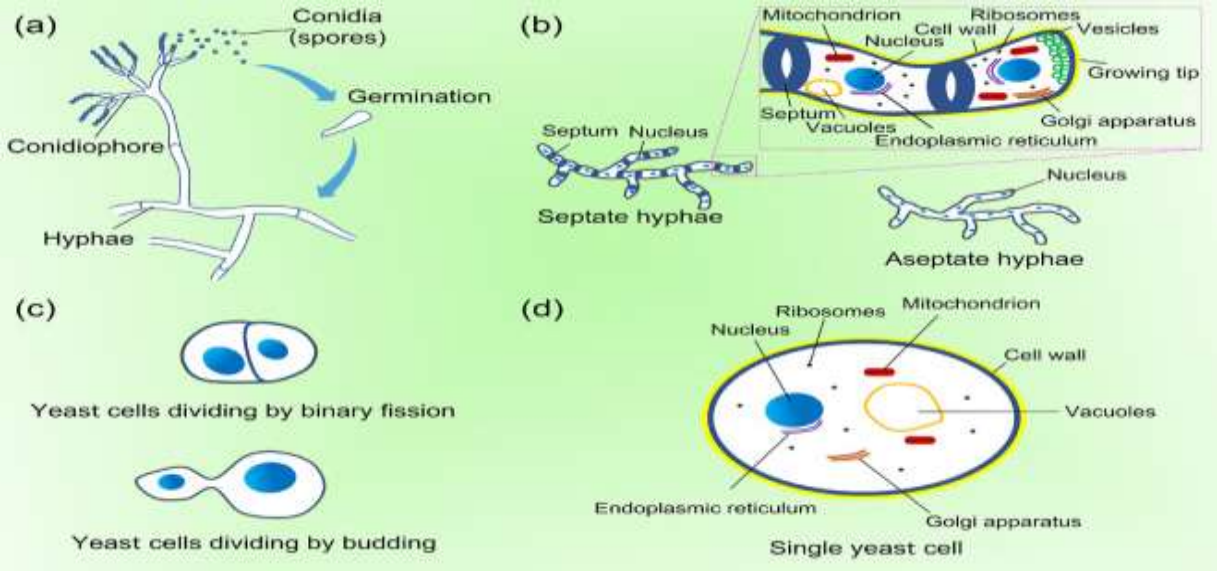

Fig. 1. (a) Representative structures and life cycle of fungi; (b) septate branched hyphae and aseptate branched hyphae; (c) schematic of yeast cells dividing by binary fission or by budding; (d) schematic of a single yeast cell.

3. Current Antifungal Drugs and Challenges

3.1. Current Antifungal Drugs

The overlap in cellular machinery between fungi and mammalian cells hinders the development of antifungal drugs that only target fungi, without causing additional undesirable side effects for the mammalian cells (Eucker *et al.*, 2001). Compared with antibiotics, the number of antifungal agents is small, and many antifungal drugs are toxic to eukaryotic host cells (Giles *et al.*, 2018). Ideas and techniques that have been effective for the development of antibacterial drugs have not shown similar efficacy upon translation to the development of antifungal drugs, as bacteria and fungi have critically different structures and components (Ablordeppey *et al.*, 1999).

At present, approximately 80 types of antifungal drugs are clinically available. They can be categorised into five classes, including polyenes (such as amphotericin B), allylamines, azoles (such as fluconazole, itraconazole, posaconazole), pyrimidine analogues, and echinocandins (such as caspofungin, micafungin and anidulafungin) (Denning and Hope, 2010). The antifungal mechanisms of these drugs can be further divided into four common targeting techniques, which are outlined in Table.1. The first common target for antifungal

drugs is ergosterol (Mathew and Nath, 2009). Ergosterol is required for the function and biogenesis of the plasma membrane, and inhibiting its synthesis, binding to it, or damaging it can be lethal to fungi (Bard *et al.*, 1993, Alcazar-Fuoli and Mellado, 2013). Other common targets for antifungal drugs include the cell envelope (both membrane-active and cell wall-active drugs are effective here) (Hatano *et al.*, 2002); inhibiting the biosynthesis of nucleic acids, proteins, chitin, and mannan (Hatano *et al.*, 2002); and other mechanisms, such as producing reactive oxygen species (ROS) or depleting adenosine triphosphate (ATP) (Chen *et al.*, 2013). Sulphur metabolism has been recently reported as a potential new antifungal target as sulphur-related processes are a fundamental aspect of the fungal physiology of some species, such as *Aspergillus fumigatus* (Amich, 2022). However, no antifungal drug that targets sulphur metabolism has been reported so far.

Table 1. Antifungal mechanisms of current antifungal drugs.

Category of antifungal mechanisms	Antifungal mechanism	Representative drugs	Refs.
Targeting ergosterol	Perturbing membrane function through binding to ergosterol	Amphotericin B	(Vandeputte *et al.*, 2012b)
	Inhibiting ergosterol biosynthesis	Imidazoles, triazoles, allylamines and morpholines	(Odds *et al.*, 2003)
Targeting the cell envelope	Disrupting membrane via pore formation	Antimicrobial peptides	(Lee *et al.*, 2002)
	Inhibiting synthesis of fungal cell wall polysaccharides	Triazoles and echinocandins	(Odds *et al.*, 2003)
Targeting biosynthesis	Inhibiting fungal protein synthesis	Sordarins	(Odds *et al.*, 2003)
	Interfere with microtubule assembly	Griseofulvin	(Odds *et al.*, 2003)
	Inhibits DNA synthesis	Flucytosine	(Odds *et al.*, 2003)
	Inhibiting expression of mRNAs	Hinokitiol	(Komaki *et al.*, 2008)
	Inhibiting glycolysis	Benoate	(Krebs *et al.*, 1983)
Other mechanisms	Promoting ROS production	Amphotericin B, Dill seeds essential oil	(Mesa-Arango *et al.*, 2014)
	Depleting ATP	Benoate	(Krebs *et al.*, 1983)
	Inhibiting the respiratory electron transport system	Pyrrolnitrin	(Tripathi and Gottlieb, 1969)

Because yeasts and filamentous fungi differ in structure and composition, some antifungal mechanisms may be effective against only one or the other. For example, flucytosine exerts its antifungal activity by incorporating into RNA and causing premature chain termination or by inhibiting DNA synthesis. However, for this to occur, it must be internalized into the cell and converted to its active form through the activity of two enzymes. Most filamentous fungi lack these enzymes, so the antifungal activity of flucytosine is limited to yeasts (Odds *et al.*, 2003). As another example, the allylamines inhibit squalene epoxidase, which is involved in an early step in the ergosterol biosynthesis pathway in susceptible species, which include many filamentous fungi but few pathogenic yeasts (Odds *et al.*, 2003).

3.2. Current Antifungal Challenges

Current antifungal drugs aid in the management of fungal infections; however, many challenges remain. Specifically, many antifungal drugs exhibit toxicity, and fungi are increasingly developing resistance (Odds *et al.*, 2003). The rise of antifungal resistance is particularly concerning, as this will limit the efficacy of future treatments, as is occurring for antibacterial drugs. The number of reports of drug-resistant human pathogenic fungi has increased rapidly over the past decades (Fisher *et al.*, 2018). Drug resistance to common antifungal agents has greatly increased the difficulty of treating fungal infections clinically (Elad *et al.*, 1992). The global spread of azole-resistant *Aspergillus* spp. and rise of multidrug-resistant *Candida* spp. are particularly concerning due to the high mortality associated with invasive infections caused by these species (Van der Linden *et al.*, 2015, Sanguinetti *et al.*, 2015). Azoles are not only used for the health protection of human, animal and crop, but also used for antifouling coatings and wood preservatives. The widespread use of azole has accelerated the evolution of azole-resistant fungi (Fisher *et al.*, 2018). In one surveillance study, the rates of resistant *Candida* spp. increased from 4.2% in 2008 to 7.8% in 2014 [40], and some institutional studies have reported higher rates of 10% or more (Alexander *et al.*, 2013, Xu *et al.*, 2021). For instance, the prevalence of fluconazole-resistant Candida albicans infections in The Ninth People's Hospital of Chongqing in China increased dramatically from 36% to 64% in only two years (Xu *et al.*, 2021).

Fungi have developed several resistance mechanisms including alterations in drug targets, alteration in cellular pathways (such as alteration in sterol biosynthesis), reduction in the intercellular concentration of target enzyme, overexpression of the antifungal drug target,

activation of stress response signalling, and overexpression of efflux pump proteins (Fig. 2) (Revie *et al.*, 2018, Hokken *et al.*, 2019). Besides, fungi have their intrinsic resistance mechanisms to antifungal drugs, including biofilm formation, differences in cellular permeability, and some mechanisms overlapping with those implicated in acquired resistance including target incompatibility, stress response signalling, and efflux pump proteins overexpression (Fig. 2).

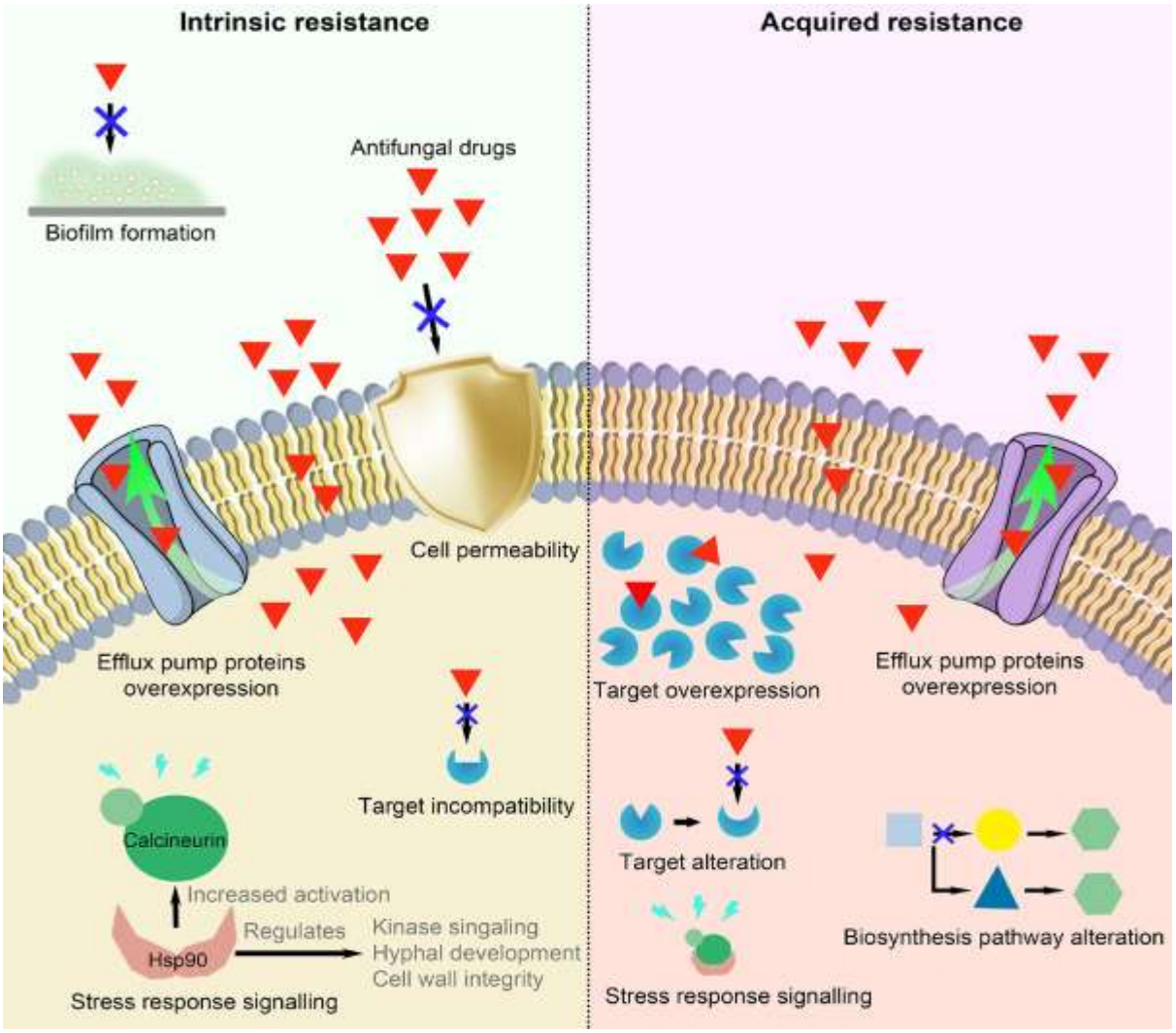

Fig. 2. The mechanisms of fungal acquired and intrinsic resistance which can be developed through several mechanisms. Examples include overexpression of drug targets, target alteration by amino acid replacements, signalling through stress response pathways, overexpression of efflux pump proteins, or alterations in cellular pathways. Intrinsic resistance includes several mechanisms overlapping with those described in acquired resistance, such as target incompatibility, stress response signalling, and efflux pump overexpression. Besides, intrinsic resistance can be caused by the formation of fungal biofilms and differences in cellular permeability.

3.3. Biofilms

"United we stand, divided we fall". This statement is certainly true concerning the fight between fungi and antifungals. It is well characterized that microbial communities engulfed in a polysaccharides-rich extracellular matrix, also known as biofilm, are by far more resistant to antifungal drugs than isolated cells. Fortunately, few pathogenic species within the fungal kingdom are able to form biofilms (Pettit *et al.*, 2010). The mostly known and widely studied of those species able to form biofilms are the species of the *Candida* genus (Mukherjee and Chandra, 2004). Another yeast frequently responsible for biofilm-associated infections is *Cryptococcus neoformans* (Pettit *et al.*, 2010). Some clinical cases have also reported the involvement of other yeast species, such as *Pichia fabianii* or *Trichosporon asahii* (Hamal *et al.*, 2008, Di Bonaventura *et al.*, 2006). Additionally, it is now accepted that filamentous fungi, and particularly those of the *Aspergillus* genus, can grow as biofilms in humans (Ueno *et al.*, 2009) Fungal biofilms are frequently polymicrobial biofilms, meaning that bacterial species frequently associate with one or several fungi (Harriott and Noverr, 2009).

In medical mycology, biofilms constitute a real concern in the fields of invasive and dental medicine. They constitute a nonnegligible source of nosocomial fungal infections, essentially through the use medical devices. Moreover, fungal biofilms are resistant to almost all the currently used antifungals, with the exceptions of echinocandins and lipid formulations of AmB(Chandra *et al.*, 2005). The molecular mechanisms underlying the persistence of the fungal biofilms despite antifungal treatment remain unclear. It is likely that biofilm resistance is the result of a combination multiple factors, among them an increased expression of efflux pumps, a modification of plasma membrane composition, and the biofilm-produced extracellular matrix itself (Mukherjee and Chandra, 2004).

4. Development of New Antifungal Strategies

Current antifungal treatments are limited in their capacity to treat infections, especially systemic infections and no considerable advancements in antifungal therapies were developed recently. New therapies are therefore needed against pathogenic fungi. Several approaches were developed during the last several years in order to find new solutions. Researchers aim to discover new antifungal drugs either by testing already

existing medical compounds, compounds from natural sources such as plants, sea, microorganisms or by systematic screens of chemical compound libraries. Researchers also strive to elucidate the underlying biology of fungal microorganism both in vitro and in vivo. Host-fungal interactions play a critical role for all fungal pathogens. Targeting this interaction provides novel therapies, which could be used alone or in combination with existing antifungal drugs. Such a combination may also determine the development of antifungal drug resistance.

4.1. Development of New Antifungal Active Compounds

Much effort has gone towards analyzing the antifungal properties of what is called natural compounds (NP) or natural bioactive compounds isolated from plants, other microorganisms, or marine organisms (Mayer *et al.*, 2011). Some such compounds are investigated because their known triggering mechanisms important for fungi, while other compounds are tested blindly for their antifungal properties. Currently, none of these studies have produced a compound suitable for the clinical trial stage although interesting results were obtained (Mayer *et al.*, 2011).

Other studies focused on *in vitro* screens of several drugs currently used in clinical practice for their potentiation of the antifungal effect of the fungistatic agent fluconazole (FLC) on *Candida albicans*. This facilitated the discovery of several compounds, such as inhibitors of the calcineurin (Marchetti *et al.*, 2000) or Tor pathways (Cruz *et al.*, 2001), efflux pump inhibitors (derived compounds of milbemycin)(Sharma *et al.*, 2009), and more recently, antibodies against heat-shock 90 protein (HSP90)(Pachl *et al.*, 2006). In particular, inhibitors of the calcineurin pathway were shown to be fully active *in vivo* in the potentiation of fluconazole, and they also led to a dramatic decrease in fungi virulence (Bader *et al.*, 2006).

Systematic screening of chemical compounds libraries was also undertaken, essentially by industrial laboratories as an attempt to discover new antifungal compounds (Ting *et al.*, 2011). High throughput screening of the legacy Schering-Plough compound collection has recently led to the discovery of a new glucan synthase inhibitor effective again *C. albicans* and *C. glabrat*a (Walker et al., 2011).

Some analysis used reverse genetic assay in which, *C. albicans* heterozygous deletion or transposon disruption mutants' collection was screened for growth under treatment with collections of chemical compounds (Oh *et al.*, 2010).

4.2. Genome-Wide Studies to Detect Potential New Antifungal Targets

The improvement of already existing antifungal drugs and the limitation of drugs resistance apparition has helped to elucidate the basic biology of the fungal pathogen. For this purpose, several groups made efforts to develop collection of systematic mutants essentially for *C. albicans*. An important difficulty for antifungal therapy is to develop drugs that exploit factors unique to fungi, which can be challenging considering that organism is eukaryotic and share many conserved biological pathways. Genes that are essential to fungal survival are possible targets for drug development.

Using the GRACE (gene replacement and conditional expression) or CPR (conditional promoter replacement) technologies, some research groups have assessed the essentiality of *C. albicans* and *Aspergillus fumigatus* genes (Roemer *et al.*, 2003b). One study identified 567 essential genes in *C. albicans* (Roemer *et al.*, 2003b). And another study screened 54 genes of *A. fumigatus* based on ortholog functions and essentiality in *C. albicans* and *S. cerevisiae* (Hu *et al.*, 2007), of which 35 were defined as essential in *A. fumigatus*. Authors were able to show that while the ERG11 gene family (CYP51A and ERG11B) is essential in *A. fumigatus*, the individual genes themselves are not. These analyses provide interesting and fully informative data for antifungal drug design and improve upon previous *in silico* analyses that when using *S. cerevisiae* data were only able to identify 61% of homologous genes reported in the genes found in the Roemer et al. analysis (Roemer *et al.*, 2003a).

The diploid state of the genome presents a major problem to the development of a mutant collection. Therefore, some collections consist of heterozygous deletion (Xu *et al.*, 2007) or transposon disruption mutants (Oh *et al.*, 2010). Other collections contain homozygous transposon disruption mutants based on the random insertion thanks to the Tn7 transposon to a UAU cassette (Bruno and Mitchell, 2004). These collections were first restricted to the transcription factors of *C. albicans* (Nobile and Mitchell, 2005), but continue to be enlarged for the entire genome (Epp *et al.*, 2010, Nobile and Mitchell, 2009). Other collections consist of deletion mutants constructed with PCR generated deletion cassettes, with two different markers for each allele in the case of *C. albicans*. Such collections are now being constructed for *C.* glabrata (Homann et al., 2009).

Three kinds of analyses detailed below were performed with these collections. They aimed a better understanding of the modifications occurring in the fungi submitted to antifungal treatments or of the relationship developed between the fungus and its host all along the infectious process. Such knowledge might improve the actual therapy to avoid resistance development or might allow playing on the host-fungus equilibrium to improve recovery of patients (Bruno *et al.*, 2006).

First of all, treating strains with already known antifungal drugs and analyzing for example, growth modification and later transcriptional rewiring, some authors try to better understand drugs mechanisms of action and/or to find synergistic effect between them (Homann *et al.*, 2009). Gene encoding the transcription factor Cas5 was found to be involved in the response to caspofungin (Bruno *et al.*, 2006). Other studies showed that *AGE3,* which encodes an ADP-ribosylation factor GTPase activating effector protein, if deleted, abrogates fluconazole tolerance in *C. albicans*. Interestingly, Brefeldin A, an inhibitor of ADP-ribosylation factor, resulted in a synergistic effect with other drugs for *C. albicans* as well as for *Aspergillus* (Epp et al., 2010). Finally, Homann et al. screened a collection of 143 transcription factor mutants under 55 distinct conditions among which exposition to fluconazole and 5FC, and they conclude in their analysis that nearly a quarter of the knockout strains affected sensitivity to commonly used antifungal drugs (Homann *et al.*, 2009).

Other studies were geared better understand the biology of fungal species. For this purpose, mutant collections were subjected to a wide range of environmental conditions, modifying elements such as pH, salt concentration, carbon sources, oxidative conditions, temperature, and availability of essential elements such as metals (iron, copper, zinc, etc.) (Homann *et al.*, 2009, Epp *et al.*, 2010).

5. Conclusion

These last years were very rich in better knowledge of molecular basis of antifungal resistance and more generally of the metabolism of pathogenic fungi. Antifungal drug resistance appears to essentially be due to point mutations in either drug targets or transcription factors regulating actors of the resistance. In the near future, high throughput diagnostic tools could be used in the course of treatment of fungal infections in order to detect resistance and adjust therapeutic strategies accordingly before any clinical evidence and therefore allow a rapid adjustment of the antifungal treatment. One of the challenges of finding new antifungal targets in C.

albicans was the lack of sophisticated screening technologies often employed with other fungal species such as Saccharomyces cerevisiae. The recent application of genome-wide studies to pathogenic fungi for both host-pathogen interactions and the biological study will hopefully encourage and facilitate the development of new effective therapeutic strategies. Such improvements in antifungal treatment may lead to a better clinical outcome.

References

ABLORDEPPEY, S. Y., FAN, P., ABLORDEPPEY, J. H. & MARDENBOROUGH, L. J. 1999. Systemic antifungal agents against AIDS-related opportunistic infections: current status and emerging drugs in development. *Current medicinal chemistry,* 6**,** 1151-1196.

ALCAZAR-FUOLI, L. & MELLADO, E. J. 2013. Ergosterol biosynthesis in Aspergillus fumigatus: its relevance as an antifungal target and role in antifungal drug resistance. *Frontiers in microbiology,* 3**,** 439.

ALEXANDER, B. D., JOHNSON, M. D., PFEIFFER, C. D., JIMÉNEZ-ORTIGOSA, C., CATANIA, J., BOOKER, R., CASTANHEIRA, M., MESSER, S. A., PERLIN, D. S. & PFALLER, M. A. J. 2013. Increasing echinocandin resistance in Candida glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clinical infectious diseases,* 56**,** 1724-1732.

AMICH, J. J. 2022. Sulfur metabolism as a promising source of new antifungal targets. *Journal of Fungi,* 8**,** 295.

BADER, T., SCHRÖPPEL, K., BENTINK, S., AGABIAN, N., KÖHLER, G. & MORSCHHÄUSER, J. J. 2006. Role of calcineurin in stress resistance, morphogenesis, and virulence of a Candida albicans wild-type strain. *Infection immunity,* 74**,** 4366-4369.

BARD, M., LEES, N., TURI, T., CRAFT, D., COFRIN, L., BARBUCH, R., KOEGEL, C. & LOPER, J. J. 1993. Sterol synthesis and viability oferg11 (cytochrome P450 lanosterol demethylase) mutations inSaccharomyces cerevisiae andCandida albicans. *Lipids,* 28**,** 963-967.

BRUNO, V. M., KALACHIKOV, S., SUBARAN, R., NOBILE, C. J., KYRATSOUS, C. & MITCHELL, A. P. J. 2006. Control of the C. albicans cell wall damage response by transcriptional regulator Cas5. *PLoS pathogens,* 2**,** e21.

BRUNO, V. M. & MITCHELL, A. P. J. 2004. Large-scale gene function analysis in Candida albicans. *Trends in microbiology,* 12**,** 157-161.

CHANDRA, J., ZHOU, G. & GHANNOUM, M. A. J. 2005. Fungal biofilms and antimycotics. *Current Drug Targets,* 6**,** 887-894.

CHEN, Y., ZENG, H., TIAN, J., BAN, X., MA, B. & WANG, Y. J. 2013. Antifungal mechanism of essential oil from Anethum graveolens seeds against Candida albicans. *Journal of medical microbiology,* 62**,** 1175-1183.

COWEN, L. E., SANGLARD, D., HOWARD, S. J., ROGERS, P. D. & PERLIN, D. S. J. 2015. Mechanisms of antifungal drug resistance. *Cold Spring Harbor perspectives in medicine*.

CRUZ, M. C., GOLDSTEIN, A. L., BLANKENSHIP, J., DEL POETA, M., PERFECT, J. R., MCCUSKER, J. H., BENNANI, Y. L., CARDENAS, M. E. & HEITMAN, J. J. 2001. Rapamycin and less immunosuppressive analogs are toxic to Candida albicans and Cryptococcus neoformans via FKBP12-dependent inhibition of TOR. *Antimicrobial agents chemotherapy,* 45**,** 3162-3170.

DENNING, D. W. & HOPE, W. W. J. 2010. Therapy for fungal diseases: opportunities and priorities. *Trends in microbiology,* 18**,** 195-204.

DI BONAVENTURA, G., POMPILIO, A., PICCIANI, C., IEZZI, M., D'ANTONIO, D. & PICCOLOMINI, R. J. 2006. Biofilm formation by the emerging fungal pathogen Trichosporon asahii: development, architecture, and antifungal resistance. *Antimicrobial agents chemotherapy,* 50**,** 3269-3276.

DI MAMBRO, T., GUERRIERO, I., AURISICCHIO, L., MAGNANI, M. & MARRA, E. J. 2019. The yin and yang of current antifungal therapeutic strategies: how can we harness our natural defenses? *Frontiers in pharmacology,* 10**,** 80.

DIAMOND, R. D. J. R. O. I. D. 1991. The growing problem of mycoses in patients infected with the human immunodeficiency virus. 13**,** 480-486.

DIXON, D. M. & WALSH, T. J. J. 1996. Medical Microbiology. *S. Baron*.

ELAD, Y., YUNIS, H. & KATAN, T. J. 1992. Multiple fungicide resistance to benzimidazoles, dicarboximides and diethofencarb in field isolates of Botrytis cinerea in Israel. *Plant Pathology* 41**,** 41-46.

EPP, E., VANIER, G., HARCUS, D., LEE, A. Y., JANSEN, G., HALLETT, M., SHEPPARD, D. C., THOMAS, D. Y., MUNRO, C. A. & MULLICK, A. J. 2010. Reverse genetics in Candida albicans predicts ARF cycling is essential for drug resistance and virulence. *PLoS pathogens,* 6**,** e1000753.

EUCKER, J., SEZER, O., GRAF, B. & POSSINGER, K. J. 2001. Mucormycoses. *Mycoses* 44**,** 253-260.

FISHER, M. C., ALASTRUEY-IZQUIERDO, A., BERMAN, J., BICANIC, T., BIGNELL, E. M., BOWYER, P., BROMLEY, M., BRÜGGEMANN, R., GARBER, G. & CORNELY, O. A. J. 2022. Tackling the emerging threat of antifungal resistance to human health. *Nature reviews microbiology,* 20**,** 557-571.

FISHER, M. C., HAWKINS, N. J., SANGLARD, D. & GURR, S. J. J. 2018. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science,* 360**,** 739-742.

FRĄC, M., HANNULA, S. E., BEŁKA, M. & JĘDRYCZKA, M. J. F. I. M. 2018. Fungal biodiversity and their role in soil health. 9**,** 707.

GILES, C., LAMONT-FRIEDRICH, S. J., MICHL, T. D., GRIESSER, H. J. & COAD, B. R. J. 2018. The importance of fungal pathogens and antifungal coatings in medical device infections. *Biotechnology advances,* 36**,** 264-280.

HAMAL, P., OSTRANSKY, J., DENDIS, M., HORVÁTH, R., RUZICKA, F., BUCHTA, V., VEJSOVA, M., SAUER, P., HEJNAR, P. & RACLAVSKY, V. J. 2008. A case of endocarditis caused by the yeast Pichia fabianii with biofilm production and developed in vitro resistance to azoles in the course of antifungal treatment. *Sabouraudia,* 46**,** 601-605.

HARRIOTT, M. M. & NOVERR, M. C. J. 2009. Candida albicans and Staphylococcus aureus form polymicrobial biofilms: effects on antimicrobial resistance. *Antimicrobial agents chemotherapy,* 53**,** 3914-3922.

HATANO, K., MORISHITA, Y., NAKAI, T. & IKEDA, F. J. 2002. Antifungal mechanism of FK463 against Candida albicans and Aspergillus fumigatus. *The Journal of Antibiotics,* 55**,** 219-222.

HAWKSWORTH, D. L. & LÜCKING, R. J. 2017. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology spectrum,* 5**,** 10.1128/microbiolspec. funk-0052-2016.

HO, L.-H., ZULKIFLI, N. A. & TAN, T.-C. J. 2020. Edible mushroom: nutritional properties, potential nutraceutical values, and its utilisation in food product development. *An introduction to mushroom,* 10.

HOKKEN, M. W., ZWAAN, B., MELCHERS, W. & VERWEIJ, P. J. 2019. Facilitators of adaptation and antifungal resistance mechanisms in clinically relevant fungi. *Fungal genetics Biology,* 132**,** 103254.

HOMANN, O. R., DEA, J., NOBLE, S. M. & JOHNSON, A. D. J. P. G. 2009. A phenotypic profile of the Candida albicans regulatory network. 5**,** e1000783.

HU, W., SILLAOTS, S., LEMIEUX, S., DAVISON, J., KAUFFMAN, S., BRETON, A., LINTEAU, A., XIN, C., BOWMAN, J. & BECKER, J. J. 2007. Essential gene identification and drug target prioritization in Aspergillus fumigatus. *PLoS pathogens,* 3**,** e24.

HYDE, K. D., XU, J., RAPIOR, S., JEEWON, R., LUMYONG, S., NIEGO, A. G. T., ABEYWICKRAMA, P. D., ALUTHMUHANDIRAM, J. V., BRAHAMANAGE, R. S. & BROOKS, S. J. 2019. The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Diversity,* 97**,** 1-136.

KOMAKI, N., WATANABE, T., OGASAWARA, A., SATO, N., MIKAMI, T. & MATSUMOTO, T. J. 2008. Antifungal mechanism of hinokitiol against Candida albicans. *Biological Pharmaceutical Bulletin,* 31**,** 735-737.

KREBS, H. A., WIGGINS, D., STUBBS, M., SOLS, A. & BEDOYA, F. J. 1983. Studies on the mechanism of the antifungal action of benzoate. *Biochemical Journal,* 214**,** 657-663.

LEE, D. G., PARK, Y., KIM, H. N., KIM, H. K., KIM, P. I., CHOI, B. H. & HAHM, K.-S. J. B. 2002. Antifungal mechanism of an antimicrobial peptide, HP (2–20), derived from N-terminus of Helicobacter pylori ribosomal protein L1 against Candida albicans. *Biochemical biophysical research communications,* 291**,** 1006-1013.

MARCHETTI, O., ENTENZA, J., SANGLARD, D., BILLE, J., GLAUSER, M. & MOREILLON, P. J. 2000. Fluconazole plus cyclosporine: a fungicidal combination effective against experimental endocarditis due to Candida albicans. *Antimicrobial agents chemotherapy,* 44**,** 2932-2938.

MARTIN, S. G. & ARKOWITZ, R. A. J. F. 2014. Cell polarization in budding and fission yeasts. *microbiology reviews,* 38**,** 228-253.

MATHEW, B. P. & NATH, M. J. 2009. Recent approaches to antifungal therapy for invasive mycoses. *ChemMedChem: Chemistry Enabling Drug Discovery,* 4**,** 310-323.

MAYER, A. M., RODRÍGUEZ, A. D., BERLINCK, R. G. & FUSETANI, N. J. 2011. Marine pharmacology in 2007–8: Marine compounds with antibacterial, anticoagulant,

antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. *Comparative Biochemistry Physiology Part C: ,* 153**,** 191-222.

MESA-ARANGO, A. C., TREVIJANO-CONTADOR, N., ROMÁN, E., SÁNCHEZ-FRESNEDA, R., CASAS, C., HERRERO, E., ARGÜELLES, J. C., PLA, J., CUENCA-ESTRELLA, M. & ZARAGOZA, O. J. 2014. The production of reactive oxygen species is a universal action mechanism of amphotericin B against pathogenic yeasts and contributes to the fungicidal effect of this drug. *Antimicrobial agents chemotherapy,* 58**,** 6627-6638.

MUKHERJEE, P. K. & CHANDRA, J. J. 2004. Candida biofilm resistance. *Drug resistance updates,* 7**,** 301-309.

NARANJO-ORTIZ, M. A. & GABALDÓN, T. J. 2019. Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. *Biological Reviews,* 94**,** 2101-2137.

NOBILE, C. J. & MITCHELL, A. P. J. 2005. Regulation of cell-surface genes and biofilm formation by the C. albicans transcription factor Bcr1p. *Current Biology,* 15**,** 1150-1155.

NOBILE, C. J. & MITCHELL, A. P. J. 2009. Large-scale gene disruption using the UAU1 cassette. *Candida albicans: Methods Protocols***,** 175-194.

ODDS, F. C., BROWN, A. J. & GOW, N. A. J. F. 2003. Antifungal agents: mechanisms of action. *Trends in microbiology,* 11**,** 272-279.

OH, J., FUNG, E., SCHLECHT, U., DAVIS, R. W., GIAEVER, G., ST. ONGE, R. P., DEUTSCHBAUER, A. & NISLOW, C. J. 2010. Gene annotation and drug target discovery in Candida albicans with a tagged transposon mutant collection. *PLoS pathogens,* 6**,** e1001140.

OSTROWSKY, B., GREENKO, J., ADAMS, E., QUINN, M., O'BRIEN, B., CHATURVEDI, V., BERKOW, E., VALLABHANENI, S., FORSBERG, K. & CHATURVEDI, S. J. 2020. Candida auris isolates resistant to three classes of antifungal medications—New York, 2019. *Morbidity Mortality Weekly Report,* 69**,** 6.

PACHL, J., SVOBODA, P., JACOBS, F., VANDEWOUDE, K., VAN DER HOVEN, B., SPRONK, P., MASTERSON, G., MALBRAIN, M., AOUN, M. & GARBINO, J. J. 2006. A randomized, blinded, multicenter trial of lipid-associated amphotericin B alone versus in combination with an antibody-based inhibitor of heat shock protein 90 in patients with invasive candidiasis. *Clinical infectious diseases,* 42**,** 1404-1413.

PETTIT, R. K., REPP, K. K. & HAZEN, K. C. J. 2010. Temperature affects the susceptibility of Cryptococcus neoformans biofilms to antifungal agents. *Medical Mycology,* 48**,** 421-426.

REVIE, N. M., IYER, K. R., ROBBINS, N. & COWEN, L. E. J. 2018. Antifungal drug resistance: evolution, mechanisms and impact. *Current opinion in microbiology,* 45**,** 70-76.

RICHARDSON, M. D. & WARNOCK, D. W. 2012. *Fungal infection: diagnosis and management,* John Wiley & Sons.

ROEMER, T., JIANG, B., DAVISON, J., KETELA, T., VEILLETTE, K., BRETON, A., TANDIA, F., LINTEAU, A., SILLAOTS, S. & MARTA, C. J. 2003a. Large-scale

essential gene identification in Candida albicans and applications to antifungal drug discovery. *Molecular microbiology,* 50**,** 167-181.

ROEMER, T., JIANG, B., DAVISON, J., KETELA, T., VEILLETTE, K., BRETON, A., TANDIA, F., LINTEAU, A., SILLAOTS, S. & MARTA, C. J. M. M. 2003b. Large-scale essential gene identification in Candida albicans and applications to antifungal drug discovery. *Molecular microbiology,* 50**,** 167-181.

SANGUINETTI, M., POSTERARO, B. & LASS-FLÖRL, C. J. 2015. Antifungal drug resistance among Candida species: mechanisms and clinical impact. *Mycoses,* 58**,** 2-13.

SHAH, D. N., YAU, R., LASCO, T. M., WESTON, J., SALAZAR, M., PALMER, H. R. & GAREY, K. W. J. 2012. Impact of prior inappropriate fluconazole dosing on isolation of fluconazole-nonsusceptible Candida species in hospitalized patients with candidemia. *Antimicrobial agents chemotherapy,* 56**,** 3239-3243.

SHARMA, M., MANOHARLAL, R., SHUKLA, S., PURI, N., PRASAD, T., AMBUDKAR, S. V. & PRASAD, R. J. 2009. Curcumin modulates efflux mediated by yeast ABC multidrug transporters and is synergistic with antifungals. *Antimicrobial Agents Chemotherapy,* 53**,** 3256-3265.

TING, P. C., KUANG, R., WU, H., ASLANIAN, R. G., CAO, J., KIM, D. W., LEE, J. F., SCHWERDT, J., ZHOU, G. & WAINHAUS, S. J. 2011. The synthesis and structure–activity relationship of pyridazinones as glucan synthase inhibitors. *Bioorganic medicinal chemistry letters,* 21**,** 1819-1822.

TORNIMBENE, B., EREMIN, S., ESCHER, M., GRISKEVICIENE, J., MANGLANI, S. & PESSOA-SILVA, C. L. J. 2018. WHO global antimicrobial resistance surveillance system early implementation 2016–17. *The Lancet Infectious Diseases,* 18**,** 241-242.

TRIPATHI, R. K. & GOTTLIEB, D. J. 1969. Mechanism of action of the antifungal antibiotic pyrrolnitrin. *Journal of bacteriology,* 100**,** 310-318.

UENO, A., HAMANO, T., FUJII, A., MATSUNAGA, A., NAGANUMA, S., YONEDA, M., ITOH, H. & KURIYAMA, M. J. 2009. Effects of voriconazole and vascular lesions in invasion of aspergillosis into the central nerve system. *Rinsho Shinkeigaku= Clinical Neurology,* 49**,** 468-473.

VAN DER LINDEN, J., ARENDRUP, M., WARRIS, A., LAGROU, K., PELLOUX, H., HAUSER, P., CHRYSSANTHOU, E., MELLADO, E., KIDD, S. & TORTORANO, A. J. 2015. Prospective multicenter international surveillance of azole resistance in Aspergillus fumigatus. *Emerging infectious diseases,* 21**,** 1041.

VANDEPUTTE, P., FERRARI, S. & COSTE, A. T. J. 2012a. Antifungal resistance and new strategies to control fungal infections. *nternational journal of microbiology,* 2012.

VANDEPUTTE, P., FERRARI, S. & COSTE, A. T. J. I. J. O. M. 2012b. Antifungal resistance and new strategies to control fungal infections. 2012.

WALKER, S. S., XU, Y., TRIANTAFYLLOU, I., WALDMAN, M. F., MENDRICK, C., BROWN, N., MANN, P., CHAU, A., PATEL, R. & BAUMAN, N. J. 2011. Discovery of a novel class of orally active antifungal β-1, 3-d-glucan synthase inhibitors. *Antimicrobial agents chemotherapy,* 55**,** 5099-5106.

WEBSTER, J. & WEBER, R. 2007. *Introduction to fungi*, Cambridge university press.

WOESE, C. R., KANDLER, O. & WHEELIS, M. L. J. P. O. T. N. A. O. S. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. 87**,** 4576-4579.

WORRALL, J. J. 1999. *Structure and dynamics of fungal populations*, Springer Science & Business Media.

XU, D., JIANG, B., KETELA, T., LEMIEUX, S., VEILLETTE, K., MARTEL, N., DAVISON, J., SILLAOTS, S., TROSOK, S. & BACHEWICH, C. J. 2007. Genome-wide fitness test and mechanism-of-action studies of inhibitory compounds in Candida albicans. *PLoS pathogens,* 3**,** e92.

XU, J.-L., LUO, Y.-X., YUAN, S.-H., LI, L.-W. & LIU, N.-N. J. 2021. Antifungal nanomaterials: current progress and future directions. *Innovations in Digital Health, Diagnostics, Biomarkers,* 1**,** 3-7.