NBU Journal of Plant Sciences

Vol. 3, No. 1 March 2015 p. 14-17
ISSN No. 0974-9027

Antifungal Agents for Treatment of Mycoses

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Being eukaryotes the similarity of fungi with animals in great extent, it is very difficult to develop suitable antifungal compounds which target only to the fungi and spare the host compare to anti-bacterials. Concerted and systematic programmes to discover and develop new antibiotics and anti-fungals have been driven to a considerable extent by the development of resistance by these organisms to the drugs commonly used against them as well as the side effects they exerted on host body. Following are the unique cellular or biochemical targets available in true fungi which could be very effective in designing antifungal drugs:

- Apical tip hyphal growth or by budding.
- Presence of chitin and β-glucans in the cell wall
- Presence of nuclear membrane during cell division.
- Presence of ergosterol in the cell membrane.
- Microtubules are insensitive to colchicines but sensitive to griseofulvin and benzimidazole.
- Lysin biosynthesis by amino adipic acid pathway.
- Stop codon UGA codes tryptophan in fungi

Before discussion about antifungal agents a brief knowledge about different fungal diseases of human beings would help us in understanding the subject. Fungal diseases are usually divided into five groups according to the level of infected tissue and mode of entry into the host which are: superficial, cutaneous, subcutaneous, systemic, and opportunistic infections.

The superficial mycoses occur mainly in the tropics and include black piedra, white piedra, and tinea versicolor. The cutaneous mycoses are which infect the outer layer of skin such as ringworms, tinea, or dermatomycosis. These diseases occur worldwide and represent the most common fungal diseases of humans. Most common such types of mycoses are:

Tinea capitis: Disease of Scalp hair (*Tricophyton* spp. and *Microsporum* spp.)
Tinea corporis: Due to social exchanges and contacts (*Trichophyton* spp.)
Tinea cruris: Disease of joints and groins, itching (*Epidermophyton* sp.)
Tinea pedis: Athletes foot, in bengali 'haza' (*T. rubrum*)
Tinea manuum: similar disease on hands (*T. rubrum*)
Tinea unguium: Attacking nails (*T. rubrum*)

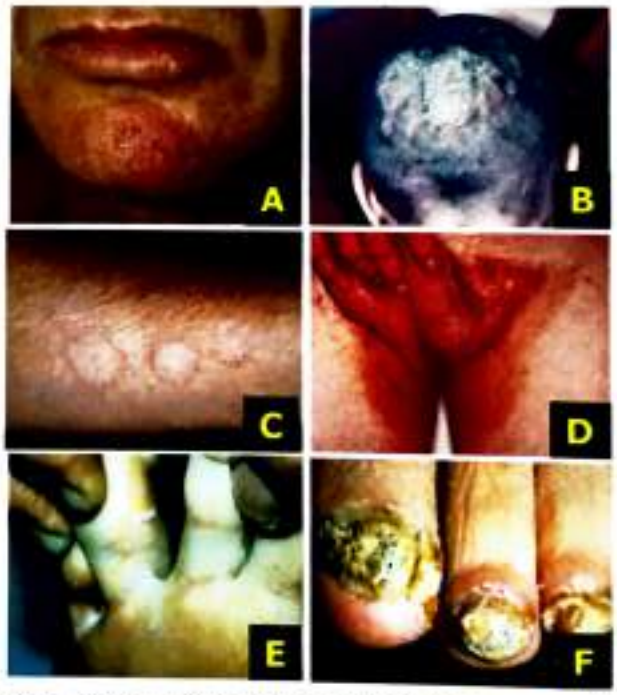

Fig.1. Different types of cutaneous mycoses caused by human pathogenic fungi; A. *Tinea barbae*; B. *Tinea capitis*; C. *Tinea corporis*; D. *Tinea cruris*; E. *Tinea pedis*; F. *Tinea unguium*

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Fungi that infect below the upper layer of skin are subcutaneous pathogens. Normally they come from soil and must be introduced into the body beneath the cutaneous layer. Examples of such mycoses include chromomycosis, maduromycosis, sporotrichosis. The systemic mycoses are the most serious of the all fungal infections in the normal host because the responsible fungi can disseminate throughout the body. Examples include blastomycosis, coccidiomycosis, cryptococcosis, and histoplasmosis. And lastly, the opportunistic mycoses can create life threatening situations in the compromised hosts. Examples of such diseases include aspergillosis, candidiasis, and *Pneumocystis carinii* pneumonia. Systemic and opportunistic mycoses are prevalent in immunocompromised persons such as AIDS patient or in persons suffering from prolong malnutrion. The systemic pathogens may even invade lung tissues, pericardial membrane to reach heart valves, meninges and spinal cord by a basidiomycetous yeast *Cryptococcus neoformans*, blood stream by *Histoplasma capsulatum* and threaten the lives of patients.

A number of antifungal agents are available today for treatment of mycoses targeting at different sites of cellular and biochemical machineries of fungal cells essential to fungal living. One main difference between the fungal cell and the mammalian cell is the presence of a fungal cell wall, and thus cell wall inhibitors could represent a promising class of antifungal agents. However, only recently have efficient cell wall biosynthesis inhibitors called echinocandins such as caspofungin, micafungin been studied as antifungal agents. These compounds mainly inhibit fungal glucan biosynthesis which makes their cell wall. Beauty of these compounds is the total absence of their cross resistance and minimum toxicity, and they are fungicidal in their mode of action. An antibiotic nikkomycin Z also does the same function by inhibiting chitin biosynthesis.

Membrane lipids including sterols have great role for its stabilization and functioning. Sterols are important constituents of both mammalian and fungal cell membranes, but there is a significant difference that allows fungal cells to be selectively targeted. Mammalian cell membranes contain cholesterol as the predominant sterol component, while fungal cell membranes contain ergosterol as the primary steroid component. These two sterols are quite similar in structure, but this structural difference has become the basis for the activity of many available antifungal agents.

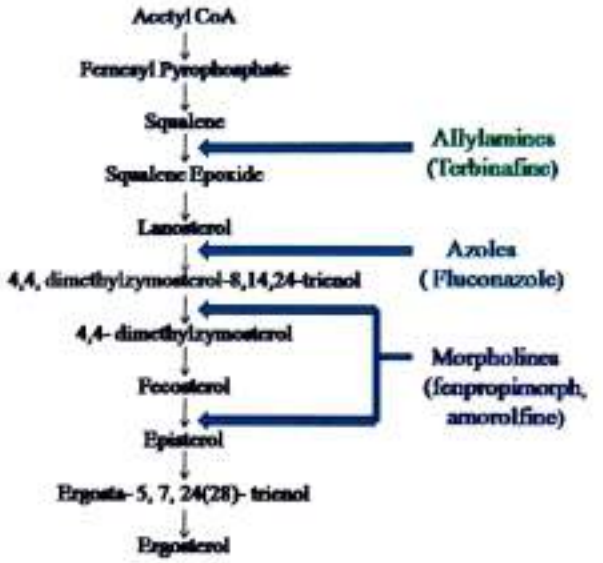

Fig. 2: Inhibition of ergosterol bio-synthesis by different antifungal drugs.

The discovery of polyene antifungal agents can be considered the first significant breakthrough in antifungal therapy. Polyene antibiotics such as nystatin and amphotericin B have an affinity for cell membranes that contain ergosterol rather than cholesterol, and as such are reasonably well targeted to fungal cell membranes. These antibiotics integrate themselves into the cell membrane of fungi, causing the membranes to become leaky, and ultimately to lyse, killing the organism. However, both drugs are quite toxic to the mammalian host, and thus must be used with caution. Nystatin is too toxic to be used systemically. However, it has very poor bioavailability when given orally, and thus it can be used to treat fungal infections of the mouth and GI tract. Amphotericin B has a low enough toxicity to be used systemically by IV administration, but can produce significant nephrotoxicity that limits its use as a systemic antibiotic. Some newer formulations of amphotericin B such as different lipid formulations have been developed with a somewhat attenuated toxicity profile.

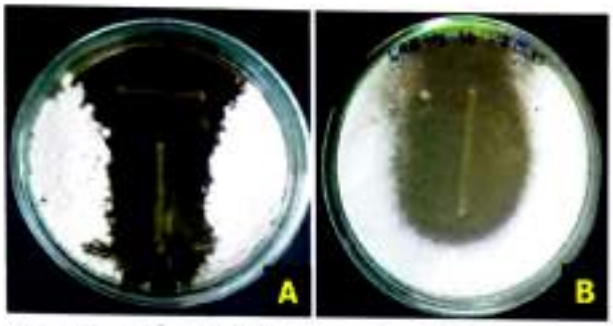

Fig. 3: Zones of inhibition produced by lactic acid bacteria against fungal pathogens: A- *Mucor* sp. B- *Curvularia* sp.

A number of agents have been developed that target the biosynthesis of ergosterol, which is unique to the organism. A key step in the fungal biosynthesis of ergosterol is the cytochrome P450 enzyme 14-·- demethylase (known as CYP51), and many of the available agents target this enzyme as their primary mechanism of action. Treatment with an azole results in the accumulation of sterols still bearing a 14- methyl substituent, and these results in permeability changes, leaky membranes and malfunction of membrane-bound proteins. The first azole based drug marketed was ketokonazole for therapeutic purpose in 1958 although its efficacy was determined a decade ago. Later the second generation azoles like fluconazole and recently with very high therapeutic indexed azoles like voriconazole and posaconazoles are marketed by renouned companies like Pfizer and Schering.

A group of allyl amines and some related derivatives have been developed that inhibit the fungal enzyme squalene synthase, a step in the synthesis of cholesterol and ergosterol. Selectivity for the fungus is based on the fact that fungal squalene synthase is much more sensitive to drug treatment than the mammalian form of the enzyme. The most important drugs in this regard are terbinafine and tolnaftate.

Of the other antifungal agents that can be used to treat fungal infections, sodarins are important. This is a group of compounds inhibit many fungus-specific proteins with moderate precision thus inhibit their growth effectively in fungi, the drug flucytosine is converted to 5-fluorouracil by fungal cytosine deaminase, and then through a series of steps to 5-fluorodeoxyuridine monophosphate. 5- Fluoro-deoxyuridine monophosphate acts as a thymidylate synthase inhibitor in fungi, which interferes with the ability to synthesize RNA and some proteins, resulting in the death of the organism. These transformations do not occur in mammalian cells. However, some bacteria in the human intestinal flora can convert flucytosine to 5-fluorouracil, which is used as a cytotoxic agent in cancer chemotherapy, so human toxicity can result. Resistance to flucytosine is a significant problem, and as such the drug is generally used in combination with amphotericin B.

Fungal microtubules of the spindle are seriously damaged by Griseofulvin, an antibiotic produced by *Penicillium patulum* can and thus stop fungal growth. It can be used orally for the treatment of fungal infections of the fingernails and toenails. Topical griseofulvin does not

Table 1. A list of fungi susceptible to inhibitory compounds produced by lactic acid bacteria

LAB Isolate	Activity Spectrum	Compound(s)	References
Lactobacillus plantarum MiLAB14	Broad antifungal Spectrum	Hydroxy fatty acids, Phenyl lactic acid, Cyclo (Phe-Pro), Cyclo(Phe-OH-Pro)	Sjogren et al., 2003
Lactobacillus rhamnosus	*Penicillium* spp. *Aspergillus* spp. *Fusarium* spp. *Alternaria* spp.	Sodium acetate1	Stiles et al., 2002
Lactobacillus casei	*Penicillium* spp.	Possibly proteinaceous	Gourama, 1997
Pediococcus pentosaceus MiLAB24	Broad Spectrum	Cyclo (Phe-OH-Pro)	Magnusson et al., 2003
Lactobacillus pentosus	*Candida albicans*	Pentocin TV35b	Okkers et al., 1999
Pediococcus acidilactici LAB5	Broad antifungal spectrum	Phenyl lactic acid, An unknown molecule of 83kDa	Mandal et al., 2013

penetrate skin or nails, but when given orally, it is incorporated into keratin precursor cells, and ultimately into the keratin that makes up skin and nail tissue. This form of keratin cannot support fungal growth. The mechanism of action for griseofulvin involves binding to tubulin, which inhibits cell division, and it may also interfere with DNA replication.

Undecylenic acid is widely employed in OTC preparations, and is fungistatic when applied topically, presumably because it interacts with constituents of the fungal cell membrane.

Application of probiotic microorganisms also reduced the food and fungi mediated allergies in human beings. These also maintain the healthy micrbiota of our intestinal system and help us to combat many pathogenic infections particularly *Candida albicans* thus offering a great prospect for future use against fungal diseases. Many species of *Lactobacillus* and some species of *Pediococcus* and *Lactococcus* including *P. acidilactici* LAB5, *L. lactis* sub species *lactis* LABW1 and *L. lactis* sub species *lactis* LABW 3 of our isolates could kill a significant number of human- as well as plant- pathogenic fungi. The *Pediococcus* isolate produces phenyl lactic acid and an unknown compound of 83 KD as the key molecules responsible for antifungal activity.

A number of different mechanisms contribute the development of resistance. They include molecular changes of the drug itself; over expression of drug target-thus swamping the antifungal agent; the reverse over expression, namely reduction in concentration of drug target-thus eliminating it as a site of action; changes in molecular biosynthesis; and pumps that actively eliminate the antifungals. Dissection of each of these mechanisms reveals new weaknesses in the pathogens and is used as a strategy to combat the problem of resistance. Genomic decoding of many of the pathogenic fungi will help accelerate the validation of multiple targets against which new generations of antifungals are beginning to develop.

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