21st Century Guidebook to Fungi, Second Edition of the online version, by David Moore, Geoffrey D. Robson and Anthony P. J. Trinci

[URL: http://www.davidmoore.org.uk/21st_Century_Guidebook_to_Fungi_PLATINUM/]

Chapter 18: Molecular biotechnology

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We start this Chapter by examining the molecular aspects of antifungal agents; those that target the fungal membrane, and those that target the fungal wall. Clinical control of systemic mycoses in the 21st century is still centred on use of azoles and polyenes, but ***combinatorial therapy*** is a promising molecular approach to managing development of resistance. In the agricultural sector control of fungal disease in the 21st century is dominated by the strobilurins, fungicides produced by fungi, and ***integrated pest management programmes*** use tactics to avoid fungicide resistance very similar to those employed in combinatorial therapy.

In the rest of the Chapter we venture into territory that is more generally recognised as representing 'molecular biology'. We discuss fungal genetic structure and the sequencing, annotation and comparison of fungal genomes. We then describe methods of manipulating genomes to enable commercial production of recombinant protein and other metabolites by filamentous fungi.

But we start this account of 21st century fungal molecular biology by looking back to the end of the 19th century, to note that **Paul Ehrlich**, winner of the **Nobel Prize in Physiology or Medicine in 1908**, developed, so long ago, the concept of **selective toxicity** (visit the Nobel Prize website) by insisting that the chemistry of drugs used must be studied in relation to their mode of action and their affinity for the cells of the organisms against which they were directed. His aim was, as he expressed it, to find chemical substances to serve as '**magic bullets**' which would go straight to the disease organisms at which they were aimed and do minimum damage to the diseased host.

Ehrlich's team used the now-common approaches of screening many newly synthesised compounds for anti-microbial activity followed by optimisation of the biological activity of a lead compound through systematic chemical modifications. In 1909 the team discovered the organic arsenical compound '**Salvarsan**' to treat syphilis; it was the 606th compound the team had synthesised for testing and was the **first organic antibiotic**.

Of course, thirty years after this, **penicillin** became the ultimate magic bullet antibacterial as the result of a chance discovery (see Section 17.15), but Ehrlich established the systematic approach that has become known as **drug discovery**.

18.1 Antifungal agents that target the membrane

Because fungi are themselves eukaryotes, drug discovery programmes aimed at finding antifungal agents with the required specificity must concentrate on targeting features that are unique to fungi. Attention must also be given to the type of material being researched. By convention ***fungicides*** are used to treat ***plants***, whilst **antifungal drugs** are used to treat **animals**. Either of these may be (a) ***fungistatic***, meaning that the agent stops fungal growth without killing the fungus and thereby 'buys time' for the diseased organism's intrinsic defences to operate; or ***fungicidal***, meaning that the agent is fatally toxic to the fungus, without, hopefully, being in the least damaging to the diseased organism.

A **protectant** fungicide or antifungal drug is a chemical that acts outside the plant or animal and protects it from fungal infection. Protectants usually have multiple sites of action:

- Milardet's '**Bordeaux mixture**' (a paint-like mixture of calcium hydroxide and copper sulfate) was originally developed as a fungicide in vineyards to control downy mildew on grape vines, is also used to protect potato plants from *Phytophthora infestans* infection and is an effective protectant of all sorts of plants against all sorts of stem and foliar fungal and fungus-like diseases.
- **Whitfield's ointment** (a greasy mixture of benzoic and salicylic acids) is used to treat athlete's foot and is effective against similar superficial disease fungi.

A compound like Bordeaux mixture affects a range of sensitive sites in the fungus but its mode of action at some of these sites may be the same (it is the copper ion which is fungitoxic). The contrast here is between an antifungal that affects a single fungal site, which hopefully is unique, and an antifungal that affects several sites in the fungus. The former will exhibit more selective toxicity than the latter. On the other hand, resistance of the fungus to the latter type of antifungal will develop much more slowly than to the former. So, it's a trade-off between reduction in crop yield, because of lower selective toxicity (for which aspect single site antifungals perform better than multiple site ones), and the rate at which resistant strains arise in the fungal population (for which aspect multiple site antifungals perform better than single site ones). Of course, selective toxicity is more important for ***antifungal drugs*** than for ***agricultural fungicides***.

Systemic fungicides and antifungal drugs penetrate the plant or animal tissues from the site of application and spread through the tissues of the host, eliminating established infections. Systemic antifungal agents usually have a highly specific (generally single) site of activity, for example:

- benzimidazole, targets microtubules of fungal pathogens of plants,
- ketoconazole, targets sterol biosynthesis of fungal pathogens of animals,
- and see Table 18.1.

Table 18.1. Overview of the sites of action of some systemic antifungal agents

Cellular target	Host (i.e. diseased) organism	Agent	Mode of action
Plasma membranes	animals	Polyenes (Fig. c2)	bind to ergosterol
	animals & plants	Azoles (Figs 18.5 & 18.6)	inhibit ergosterol synthesis
	plants	Edifenphos (an organophosphorus fungicide) (Fig. 18.11)	inhibits phosphatidyl choline synthesis
Nucleic acid biosynthesis	plants	Acylalanines (e.g. metalaxyl) (Fig. 18.11)	inhibit RNA polymerase
	animals	5-fluorocytosine	inhibits RNA synthesis
Mitochondrial respiration	plants	Strobilurins (Figs 18.12 & 18.13)	bind to cytochrome b, disrupting electron flow and ATP synthesis
Cell wall	plants	Polyoxins, Nikkomycins (Fig. 18.8)	inhibit chitin synthesis
	plants	Tricyclazole (Fig. 18.11)	inhibits melanin synthesis
	man	Echinocandins (Fig. 18.9)	inhibit β-1,3 glucan synthase
Cytoskeleton	plants	Benzimidazoles (Fig. 18.10)	bind to β-tubulin
	animals	Griseofulvin (Fig. 18.10)	binds to microtubule associated proteins

Other potential targets include: mannoproteins involved in adhesion, translation elongation factor, and proteases.

The basis of the selective toxicity of antifungal agents that target plasma membranes is the relative sterol composition of those membranes (see the *Plasma membrane and signalling pathways* section in Chapter 5 and *The fungal wall as a clinical target* in Chapter 6; CLICK on the section titles to view the pages now).

Resources Box

Information about toxicity

Rapid access to internationally peer reviewed information on chemicals commonly used throughout the world, including those that occur as contaminants in the environment and in food, is provided by the **INCHEM** website of the International Programme on Chemical Safety. It consolidates chemical safety information from a number of intergovernmental organisations whose goal it is to assist in the sound management of chemicals.

View this URL: http://www.inchem.org/