Exploiting the Stress Response:
Anti-Fungal Drug Susceptibility Testing in Near Real-Time

Zavizion B., Rieder R.J., and Zhao, Z.

BioSense Technologies, Inc.
Woburn, MA

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Abstract

Background. Until recently, the need for fungal testing in clinical microbiology laboratories has been minimal because of the low frequency of infections encountered. As a result, most laboratories out-source their testing needs, delaying the availability of results, or perform in-house testing requiring labor intensive methods due to the lack of available instrumentation. The increase in the rate of fungal infections has resulted in the need for rapid and easy-to-use diagnostic methods. A novel method that monitors the stress response developed by susceptible fungal cells in the presence of a drug is presented as the basis of a new platform for determining the drug susceptibility testing (DST) of fungi in near real-time.

Methods. The dielectric permittivity of drug-treated and untreated fungal suspensions was measured using the Z-Sense™ Differential Impedance Platform to monitor metabolic changes associated with the corresponding drug induced stress responses. Measurements were performed using two identical 100 µL test volume chambers embedded within a disposable credit-card sized cassette. The respective impedances were continuously recorded by a PC-based data acquisition system. Fungal strains used in this study were routinely cultured using corresponding agar plates or liquid broth.

Results. Data were obtained for strains of *C. albicans* known to be both resistant and sensitive to fluconazole, an anti-fungal drug commonly prescribed for fungal infections. The responses from the resistant and sensitive cells were unique and could be distinguished from each other in less than 30 minutes. The former profile was consistent with the absence of stress in the presence of the drug while the latter reflected a strong stress response. All results were in good agreement with data obtained with conventional methods.

Conclusions. Continuously monitoring the drug induced stress response of fungi is an effective way to identify drug resistance/susceptibility in near real-time.

Introduction

Assessing the growth of fungi in the presence of antifungal drugs is the conventionally accepted method for determining drug susceptibility. We present here an alternative method for characterizing the effect of an antifungal drug on fungi by obtaining more rapid information about the metabolic state of those cells (see Fig.1).

The approach is based on recognizing that some chemical compounds (including antifungal drugs) trigger a measurable and immediate stress response in susceptible fungi. The physiological stress response is a set of metabolic pathways distinct from conventional growth (i.e. the process responsible for increasing the numbers of cells) which ensures organism survival and adaptation to a new environment.

Since the development of stress response does not depend on the rate of cell division, anti-fungal susceptibility results can be obtained in near real-time. This is compared to days currently needed to obtain results with existing culture-based anti-fungal susceptibility testing methods.

We have found that a practical method for sensing the development of the stress response is to monitor changes in the dielectric permittivity, an easily measured electrical property, of a drug-treated fungal suspension to quantify subtle changes in its charge distribution.

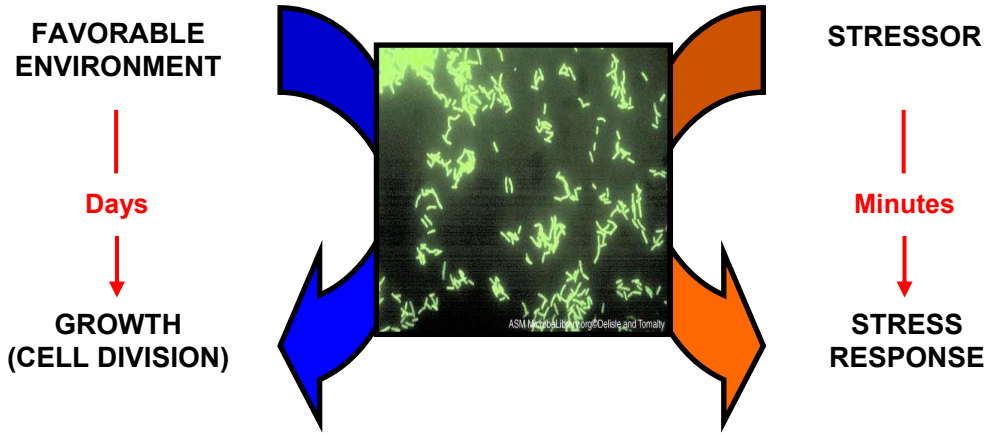

Fig. 1. Two Possible Metabolic Pathways for Measuring the Susceptibility of Fungi to Antifungal Drugs.

Rapid Antibiotic Susceptibility Testing: Stress versus Growth

A fungal strain is judged to be susceptible or resistant to a drug based on the value of the minimum inhibitory concentration (MIC), the amount of antifungal needed to inhibit its proliferation *in vitro*.

Our approach provides information regarding organism susceptibility by quantifying the physiological stress response developed by the drug-treated cells.

For example, an untreated susceptible strain maintained under optimal growth conditions will develop no stress, while the same strain exposed to a lethal concentration of an antifungal compound will be highly stressed in the cells' quest to survive.

Similarly, a fully resistant strain exposed to the same drug concentration will develop no stress response at all.

Methods

The dielectric properties of drug-treated and -untreated *Candida albicans* (*C. albicans*) were measured using the BioSense Z-Sense™ differential impedance sensing platform (Fig. 2). The resulting Normalized Impedance Response (NIR) profiles continuously register the difference between stressed and unstressed fungi.

Clinical isolate strains of *C. albicans* known to be resistant to fluconazole (FLU) (FLUR; MIC>128 mg/mL) and susceptible to amphotericin B (AMP) (AMPS; MIC=0.125 mg/mL) and a second strain known to be susceptible to both FLU (FLUS; MIC=0.25 mg/mL) and AMP (AMPS; MIC=0.125 mg/mL) were generously provided by Dr. Theodore White from the Seattle Biomedical Research Institute.

One chamber of the Z-Sense™ test cassette was manually filled with *C. albicans* cells suspended in growth medium RPMI 1640 containing the corresponding antifungal compound. The adjacent reference chamber was filled with the same cells and medium without the antifungal compound. The filled cassette was then inserted into the analyzer set at 37ºC and the impedance signals from each chamber were continuously recorded and analyzed. The cell concentration was determined at the start and end of each experiment and all experiments were repeated at least three times (not consecutively).

Measurements with sterile medium, medium with drugs (no cells added), and with untreated cells (no drug added) in both chambers were also obtained and served as a negative control.

Fig. 2. Z-Sense™ Differential Impedance Sensing Platform.

Results

The measured Normalized Impedance Response (NIR) profiles for the susceptible and resistant strains of *C. albicans* treated with fluconazole are plotted in Fig. 3A and 3B.

The NIR profiles for the antifungal-treated susceptible strains are qualitatively similar irrespective of the mechanism of action of the drug used. These profiles are characterized by an immediate and continuous decrease in the NIR values with an intensity that is proportional to the drug concentration used.

In contrast, the NIR profiles obtained for the strain having established resistance to FLU, show no difference between the antifungal-treated and -untreated cells. However, when this same strain was treated with AMP for which it was susceptible, the NIR profile decreased in value similar to the profiles obtained for the other susceptible strain. Differences in the NIR profiles for susceptible and resistance strains are easily distinguish by visual inspection in under 30 minutes.

Additional control experiments were conducted to ensure that the presence of the drug alone (with no cells added) did not influence the NIR profiles. No detectable differences in the shape of the NIR profiles obtained with medium or with medium supplemented with antifungals were found (data not shown).

To ensure that the NIR profiles were not due to cell death, aliquots of the drug-treated and -untreated yeast cells were enumerated in parallel with the generation of the NIR profiles (Fig. 3C; 3D). Despite the development of a sizeable NIR profile, no differences in cell numbers were seen.

Summary and Conclusions

Two important conclusions can be drawn from this set of results: 1.) Monitoring the development of the stress response using differential impedance sensing technology enables infections caused by susceptible fungal strains to be easily distinguished from those caused by resistant strains in near real-time; 2.) The decrease in NIR values is drug concentration dependent indicating a correlation between the amount of the drug and the degree of the stress-related changes in cellular metabolic activity.

Overall, the data presented support our suggestion that sensing the fungal stress response is a powerful, fast, and sensitive tool for improving the routine diagnostic screening of fungi infections and promoting the prescription of targeted antimicrobial therapies.

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Contact Information. For more information on our product development or collaboration please contact Dr. Ronald Rieder or Dr. Boris Zavizion at BioSense Technologies, Inc. 4 Arrow Drive, Woburn, MA 01801 Tel: (781) 933-3635 or email them at info@biosensetech.com).

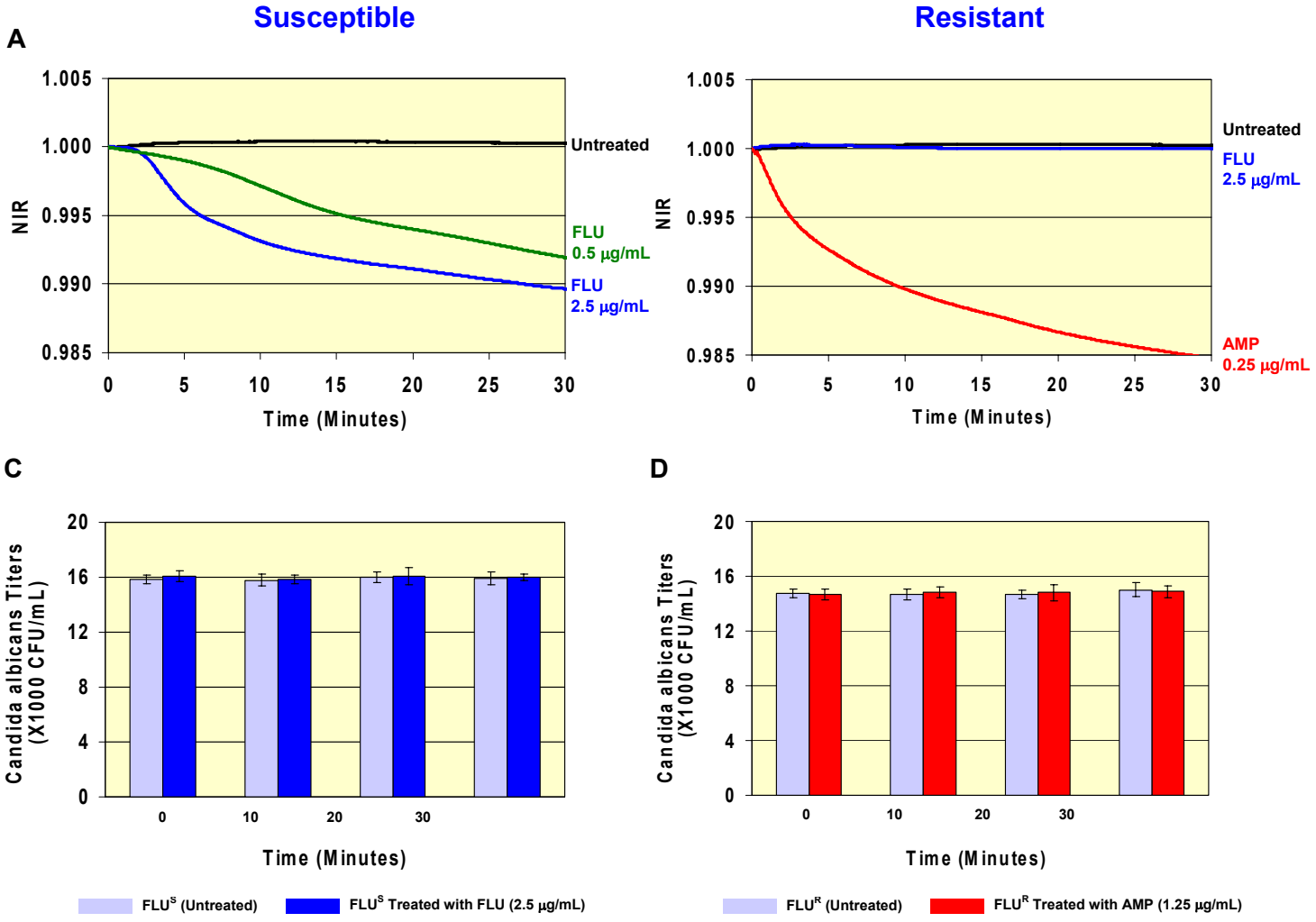

Fig. 3. Z-Sense™ Measurements for *C. albicans* Treated with Antifungals:. A. Susceptible strain of *C. albicans* suspended in RPMI 1640 (10^4 CFU/mL) exposed to no drug (black line; untreated) and fluconazole [0.5 µg/mL (2xMIC) - green line, 2.5 µg/mL (10xMIC) - blue line]; **B.** Resistant strain of *C. albicans* suspended in RPMI 1640 (10^4 CFU/mL) exposed to no drug (black line; untreated); fluconazole [2.5 µg/mL (10xMIC) - blue line]; and amphotericin B [0.25 µg/mL (2xMIC) - red line]; **C.** and **D.** Corresponding cell numbers associated with **A.** and **B.**