Impedance-based de-replication of antifungal drug classes

J. Wuyts⁽¹⁾, B. Pauwels⁽¹⁾, M. Holtappels⁽¹⁾, S. Verguts⁽²⁾, W. Luyten⁽³⁾, B. Landuyt^(2,3), P. Van Dijck⁽¹⁾

(1) VIB-KU Leuven Center for Microbiology, Kasteelpark Arenberg 31, 3001 Leuven, Belgium

(2) CellSine, ICAB, Witte Paterstraat 4, 1040 Brussels, Belgium

(3) KU Leuven, Department of Biology, Division of animal physiology and neurobiology, Naamsestraat 59, 3000 Leuven, Belgium

Introduction

Although three major classes of antifungal drugs are currently in use to treat systemic fungal infections, patient mortality rates are still extremely high, indicating there is still a high need for novel antifungal drugs. This is further exemplified by the recent initiatives to stimulate antifungal drug development (e.g. GAIN act). However, antifungal drug development is hard. First, fungi, like humans, are eukaryotes often resulting in high toxicity of potential antifungal drugs. Second, target-based drug discovery has largely failed because the vast majority of proposed drug targets are false positives¹. Finally, although natural products remain the most important source to develop novel antifungals, discovering novel antifungals from natural products is hard because these efforts are plagued by rediscovery of known compounds. **In this work, we have used *in situ* cultivation to isolate and screen novel microorganisms for the production of antifungals. Next, we have developed an impedance based de-replication platform to characterize unfractionated microbial extracts and to determine the mode-of-action of the responsible compounds.**

Principle

Figure 1: Impedance spectroscopy can be used to de-replicate microbial fermentation broths with antifungal activity. A blank measurement is performed on the medium inside a well in a modified 96-well plate by subjecting the wells to a small alternating current (AC). In this way, we measured impedance, the AC analogue of resistance. Next, we add *Candida albicans* SC5314 strain (wild type) to the wells and allow the cells to adhere to the wells. Adherence of the cells is reflected by an increase in impedance over time, expressed as antifungal index (AI). After adhesion, the non-adhered cells are washed away and fresh medium containing an antifungal compound is added. The compound exerts its effect on the adhered *C. albicans* cells and this results in an altered impedance profile. This profile is specific for the antifungal drug class and can be used to determine the mode-of-action of microbial extracts with antifungal activity.

Results

Figure 2: Impedance response profiles can be used to de-replicate fermentation broths with antifungal activity. The top row of graphs show the impedance profile of pure, characterized antifungal compounds. The two bottom rows show the impedance response profile of unidentified microbial extracts with antifungal activity. The similarity of the profiles suggest that the unidentified compound in the microbial extracts responsible for the antifungal activity has a similar mode-of-action as the characterizes compounds.

Conclusions

Although we still need to identify the compound responsible for the antifungal activity in the microbial extracts, impedance spectroscopy can become a valuable tool in high-throughput screening campaigns to discover novel antifungals with a novel mode-of-action from natural products, especially because it is label-free, an easy read-out and fast.

References

¹ Perfect, J. (2017). The antifungal pipeline: a reality check. *Nature Reviews Drug Discovery*, 16(9), pp.603-616.

Contact

Jurgen.wuyts@vib.kuleuven.be