Assessing Microbial Metabolism Using a Simple Oxygen Consumption Assay

Introduction

MitoXpress®-Xtra is a water-soluble oxygen-sensitive phosphorescent probe produced by Luxcel Biosciences that facilitates microtitre-plate based analysis of microbial oxygen consumption. The 'mix & measure' procedure allows rapid and specific detection of microbial oxygen consumption providing a simple yet sensitive means of assessing the impact of a given manipulation on cellular function. Areas of application include, mode of drug action elucidation, screening for antimicrobial compounds, the assessment of bacterial load and the optimisation of culture conditions.

The **MitoXpress®-Xtra** assay provides information on the rate of microbial oxygen consumption. Such measurements can provide insight into the metabolic effect of a specific manipulation or can be used as a measure of survival and replication.

The assay is based on the ability of dissolved O_2 to quench the phosphorescence of the soluble, oxygen sensitive probe (**MitoXpress®-Xtra**). Probe emission is quenched by molecular oxygen via a physical (collisional) mechanism; whereby depletion of dissolved oxygen causes an increase in probe emission. Changes in probe signal therefore reflect changes in oxygen concentration within the sample.

Method

Materials:

- **MitoXpress®-Xtra**, Luxcel Biosciences, (Cat. No. MX-100)
- Clear 96 or 384 well plates from Costar
- FLUOstar Omega, BMG LABTECH

Assay Format:

The assay is a simple 'mix and measure' test:

1. *Microbes are dispensed into the wells of a 96 well plate in 100 µl volumes in the appropriate growth medium.*
2. *10 µl of **MitoXpress®-Xtra** probe is added to each well.*
3. *100 µl of mineral oil is added to exclude ambient O_2*
4. *The plate is measured kinetically at the required temperature.*
5. *Oxygen profiles are then related to metabolic activity.*

Multiplexing of both growth and microbial metabolism is achieved using the BMG LABTECH scripting function. Bacterial growth is measured by absorbance at 600–nm and oxygen consumption is measured using the **MitoXpress®-Xtra** probe which is measured using dual delay, time-resolved measurements. Optimal measurement wavelengths are 380nm for excitation and 650nm for emission. Delay times of 30 and 70 µs are used, both with a measurement window of 30 µs respectively. These dual intensity measurements are used to calculate emission lifetime using the following function $\tau = t_2\text{-}t_1 / \text{Ln}(D_1/D_2)$ [t=delay time, D=measured intensity value]. Scripts and protocols can be obtained through your local BMG LABTECH representative.

Results and Discussion

Analysis of bacterial Growth:

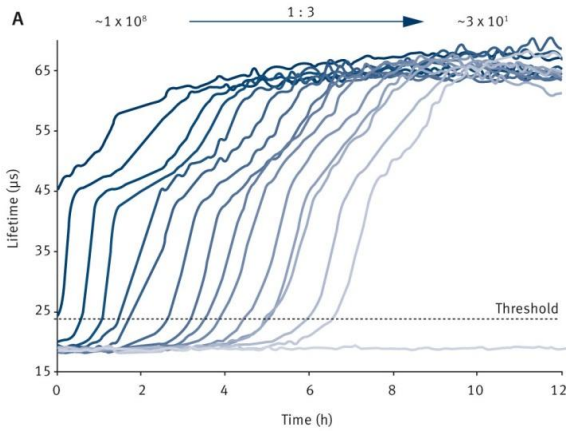

Fig. 1: Oxygen-based growth curves from serial dilution of *E.coli*. As bacteria replicate, oxygen consumption rate increases. At a critical point, oxygen consumption exceeds back diffusion. This is seen as an increase in probe signal.

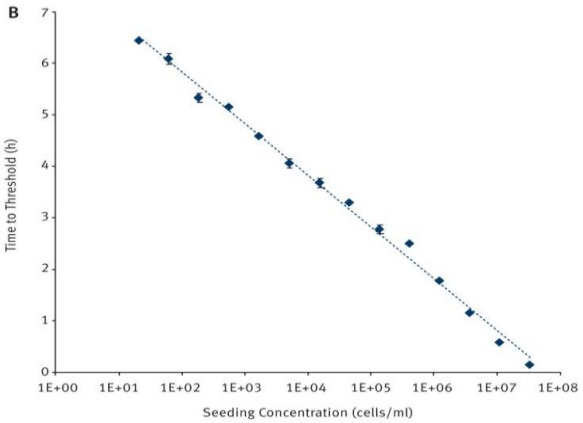

Fig. 2: Correlation between *Time-To-Threshold* and Seeding Concentration. The time required to reach the threshold signal (24 µs) reflects the seeding concentration and is dependant on the replication rate and cellular oxygen consumption rate.

Comparison with Optical Density Measurements:

Oxygen and OD_{600} data can be obtained from the same well thereby allowing multiparametric analysis of cell growth. OD600 values reflect microbial replication rate while oxygen-based analysis reflects both growth and alterations in cell metabolism. Oxygen gradients are detectable considerably earlier than increases in OD600 (Fig. 2) and give a more robust read-out. This multiparametric approach can be useful when probing cell metabolism and elucidating modes of action and can detect any shift from aerobic to anaerobic metabolism in facultative anaerobes.

MitoXpress®-Xtra

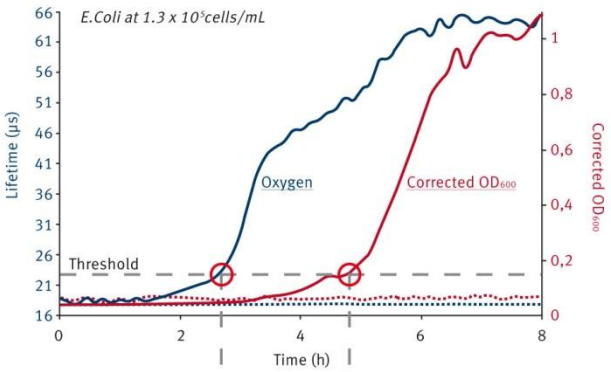

Fig. 3: Comparison between O_2 and OD600 profiles (Multiplexed measurement - *E.coli* seeded at 1.3x10^5 cells/ml)

Antibiotic Treatment:

Microtitre plate-based analysis of microbial oxygen consumption allows the high throughput generation of IC_{50} and MIC values and can be used to screen for compounds that perturb cell metabolism. A dose response analysis demonstrating this is presented in Figure 4.

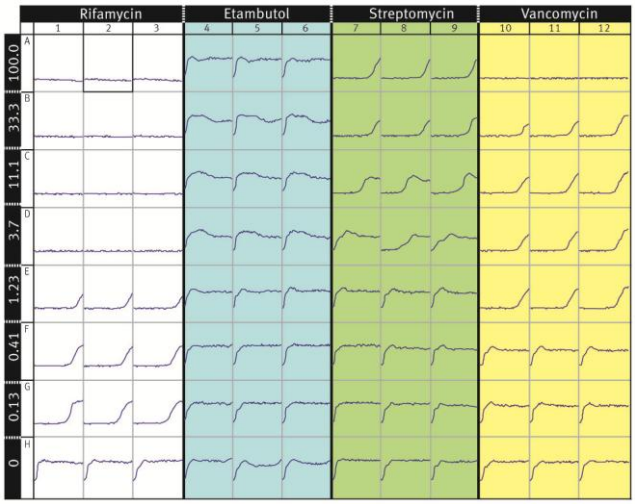

Fig. 4: *S.aureus* seeded at ~1x10^7 cells/ml in EB, exposed to increasing concentrations of the indicated antibiotic and measured kinetically at 37°C

Analysis of Fungal Growth – *C.albicans*

Data indicates that analysis of *C.albicans* can be assessed using the described assay (Fig. 5A) with the dependence on seeding concentration presented in Figure 5B. Short term analysis (High cell numbers measured for ~20min) allows the assessment of immediate effects on cell metabolism while longer term analysis (lower cell numbers & extended measurement times) facilitates analysis of effect on cell growth and metabolism.

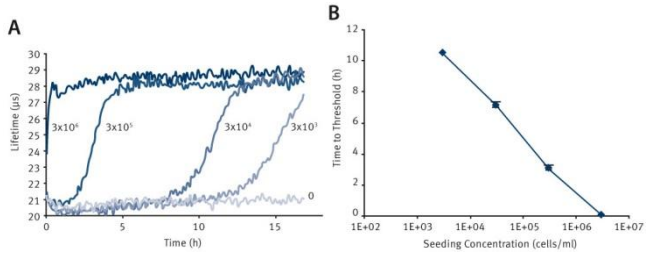

Fig.5A: *C.albicans* oxygen profile measured in EC at increasing seeding concentrations

Fig. 5B: Relationship between *Time-To-Threshold* and *C.albicans* seeding concentration

Drug Treatment:

The electron transport chain inhibitor Antimycin (Fig. 6A) and the polyene antifungal Amphotericin B (Fig. 6B) cause an immediate and dose dependant decreases in oxygen consumption while the triazole antifungal Fluconazole (Fig. 6C) caused no appreciable decrease in oxygen consumption. These observations correlate with mode of drug action and demonstrate how such measurements can be used to assess the specific metabolic affects of compound treatment.

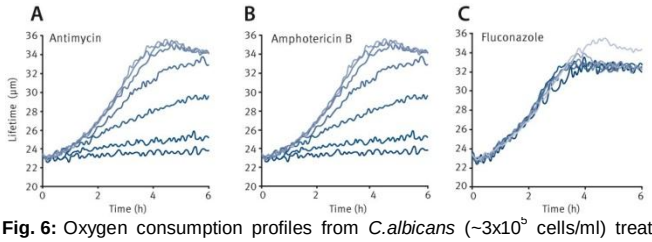

Fig. 6: Oxygen consumption profiles from *C.albicans* (~3x10^5 cells/ml) treated with increasing concentrations Antimycin (from 30 µM), Amphotericin B (from 16 µg/ml) and Fluconazone (from 65 µg/ml) in RPMI.

Conclusion

MitoXpress®-Xtra facilitates simple and convenient probing of microbial metabolism and can be applied to the analysis of both bacteria and yeast. The metabolism implications of treatments such as drug exposure, genetic manipulation or altered culture conditions can be easily accessed and elucidation of mode of action is facilitated. The assay provides the throughput and resolution necessary for screening and is capable of detecting antimicrobial activity and generating IC_{50} or MIC data.

BMG LABTECH GmbH, Hanns-Martin-Schleyer-Str. 10, D-77656 Offenburg/Germany.
Phone: +49 781 96968-0, Fax +49 781 96968-67. www.bmglabtech.com