Live imaging to monitor plant defensin activity on yeast cells *in vitro*

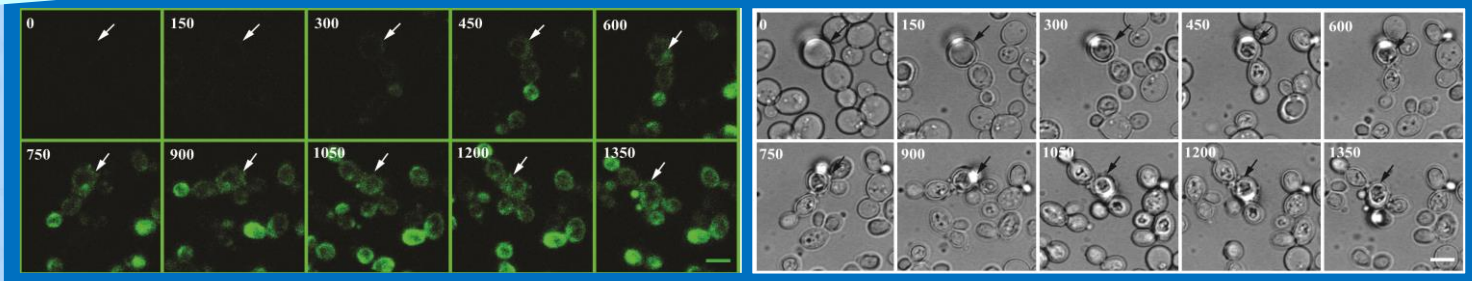

Fig. 1. (a)Time series of live yeast cells treated with BODIBY-labeled NAD1 peptide solution (in green) over 1350 seconds showing the interaction of the labelled peptide on the surface of the yeast cells and their eventual death (white arrows) and (b) the same images in (a) under transillumination mode showing yeast cell death (black arrows) using a Zeiss LSM 510 laser scanning Axiovert 200M inverted confocal microscope (La Trobe Bioimaging Platform, La Trobe University)

Plant defensins are small cationic peptides with antimicrobial activity and are integral part of the plant innate immune system. They have been found in leaves, tubers, flowers, pods and seeds predominantly within stomatal and peripheral plant cells. They have antifungal activity but they are also effective against plant bacterial pathogens. Through genetic engineering, plant defensins isolated from different plant species have been overexpressed in transgenic crop plants for enhanced resistance against phytopathogens. To select potential candidate peptides, one must be able to screen the activity of such compounds and live imaging is one way to monitor and to study the activity of such peptides *in vitro*.

Method: This is a summarized version of the method by Bleackley et al (2014) where the class-II anti-fungal plant defensin previously isolated from the flowers of the ornamental tobacco, *Nicotiana alata* Defensin 1 (NaD1) was labeled with the fluorophore BODIPY-FL EDA (Molecular Probes). Yeast cells (*S. cerevisiae* strain BY4741) grown overnight in YPD liquid medium, were treated with 0 or 25 µl of 2.5 mg/ml BODIPY-labeled NaD1 peptide stock solution. The yeast cells were then visualized by confocal microscopy using a Zeiss LSM 510 laser scanning Axiovert 200M inverted confocal microscope with a Plan Apochromat 100×/1.4 oil differential interference contrast (DIC) objective. For live-cell imaging, the cells were excited at 488 nm with an argon laser using band-pass filters BP505-530 or BP505-550 to detect BODIPY fluorescence (green). Time series images at 30-s and 60-s intervals over 36 min were acquired using the Zen 2009 image acquisition software (Carl Zeiss MicroImaging GmbH). The images were analyzed using Fiji software (ImageJ 1.47h version) (see http://imagej.nih.gov/ij/) and Zeiss LSM Image Browser version 4.2.0.121 (Carl Zeiss MicroImaging GmbH).

Results and possible applications: Live imaging was useful in observing the antagonistic effects of NAD1 on yeast cells i.e. Interaction of the molecule to the yeast cell membrane/wall surface and the subsequent killing of the cells (shown above). Its possible application could be for the rapid screening of plant defensins' antifungal activity on yeast cells as a model system *in vitro.*

Advantages: Yeast cells are easy to grow and do not require PC2 testing facilities**.** Various dyes such as Propidium iodide and FM-64 can be used as counterstains. This could be used to test modified synthetic versions of plant defensins *in vitro* prior to testing in transgenic plants in glasshouses or in field trials.

Disadvantages: Yeast cells as a model for antifungal activity may be limiting as many plant including human fungal pathogens have differences in cell wall structure which may affect interaction and the activity of plant defensins on such pathogens. Complementary testing of plant defensins should be done on the fungal pathogen of interest to confirm such activities.

Further Reading: Bleackley et al. (2014) Antimicrob Agents Chemother. 58(5):2688-98; Francisco and Georgina, J Plant Physiol Pathol 2017, 5:1 DOI: 10.4172/2329-955X.1000159; Latgé, J.-P. (2007), Mol Microbiol, 66: 279-290; Rocio et al. (2020) Frontiers in Microbiol 10: 2993. DOI:10.3389/fmicb.2019.02993; Sher Khan et al. (2019) 3 Biotech 9, 192. https://doi.org/10.1007/s13205-019-1725-5; Xie X, and Lipke PN. (2010) Yeast. 27(8):479–488. doi:10.1002/yea.1787

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