

The development of a panel of bacterial reporter strains for the detection of antimicrobial compounds at sub-inhibitory levels

T.K.S Janssens¹, M. Naderman¹, B. van der Burg¹ and B. Brouwer^{1,2}

¹BioDetection Systems BV, Science Park 406, 1098XH Amsterdam, the Netherlands
²VU University Amsterdam, Department of Animal Ecology, de Boelelaan 1085, 1081HV Amsterdam, the Netherlands



Introduction

Mining (meta)genomic libraries or isolates for useful small antimicrobial molecules requires screening of biological activities

Problem: false negatives in inhibition assays due to sub-inhibitory concentrations in inhibitory assays

Detection of antimicrobial compounds based on structural and mechanistic properties by transcriptional regulatory mechanisms of

- antibiotic resistance systems
- intra- and inter-specific signalling
- general stress pathways

Aim

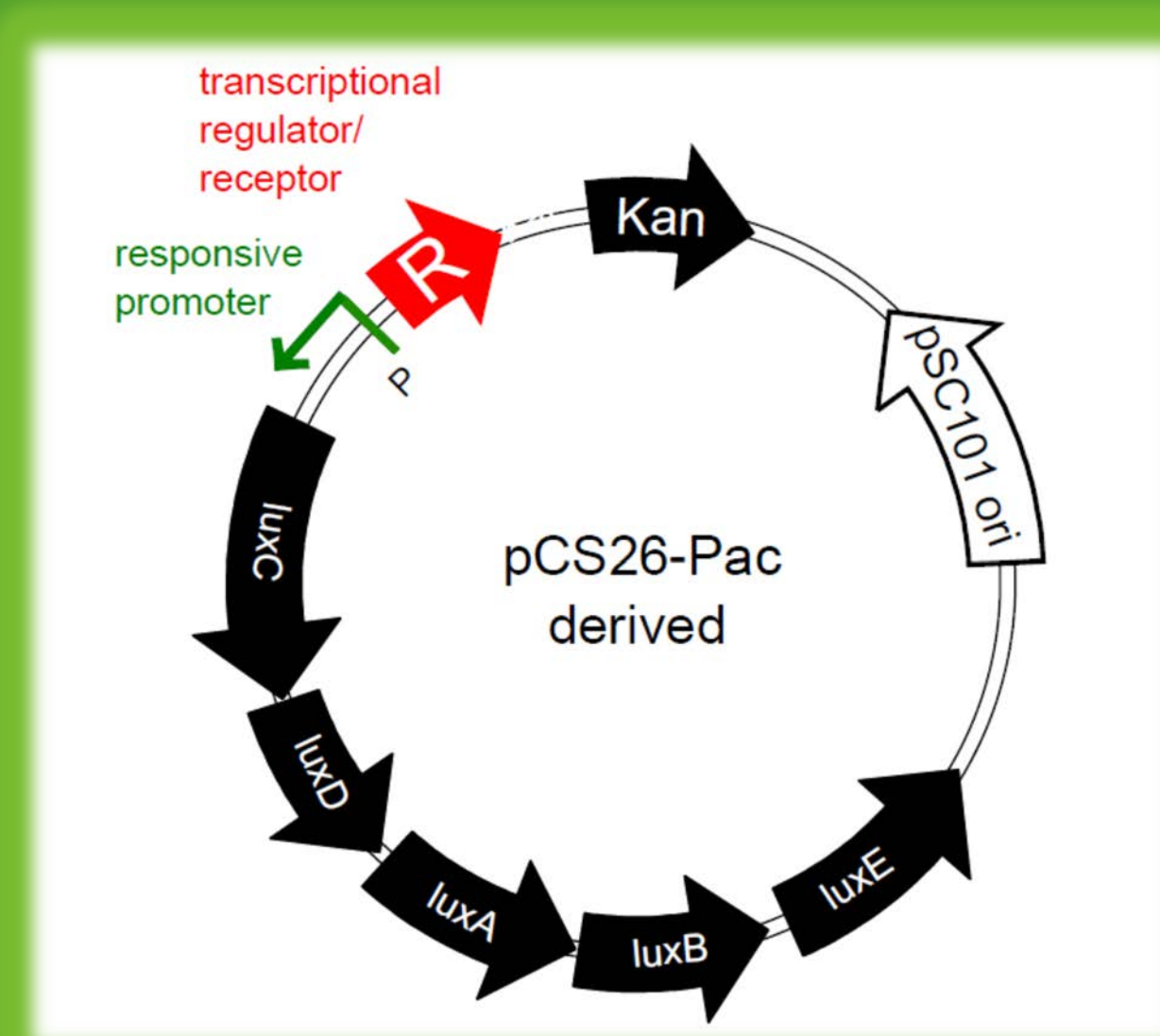
Development of a panel of microbial reporters for biomining purposes based on biological activities based on mechanisms reported in the literature.

To be applied in:

- De-replication of downstream screens
- Promising clone selection
- Mode of action assessment of new compounds

Proof of principle in host *E. coli* DH5a

Low copy number *luxCDABE* plasmid (pCS26-Pac) constructs



Future panel

Class specific

Receptor mediated

- macrolides
- tetracyclines
- phloroglucinoles
- streptogramins
- β-lactams

Sensor kinase signaling

- cationic polypeptides
- glycopeptides

Mode of action

Inhibition of

- Quorum Sensing
- Replication
- Transcription
- Translation
- Cell wall synthesis
- Fatty acid synthesis

Stress responses

- Cell cycle arrest
- Cell envelope
- Genotoxicity
- Oxidative

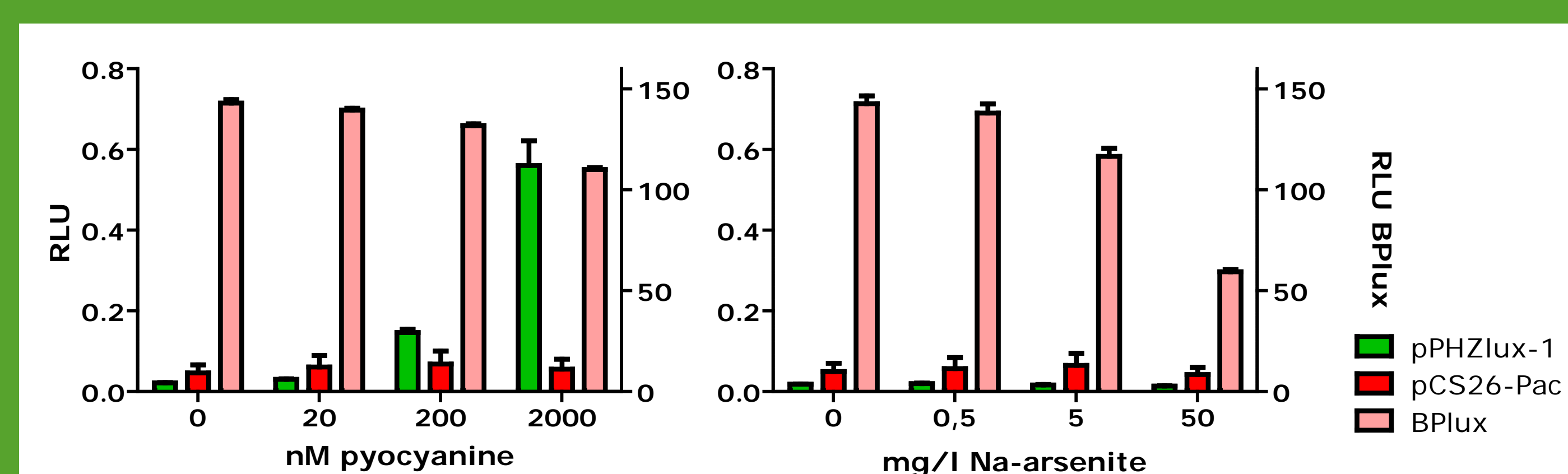
Whole cell biosensor

Results

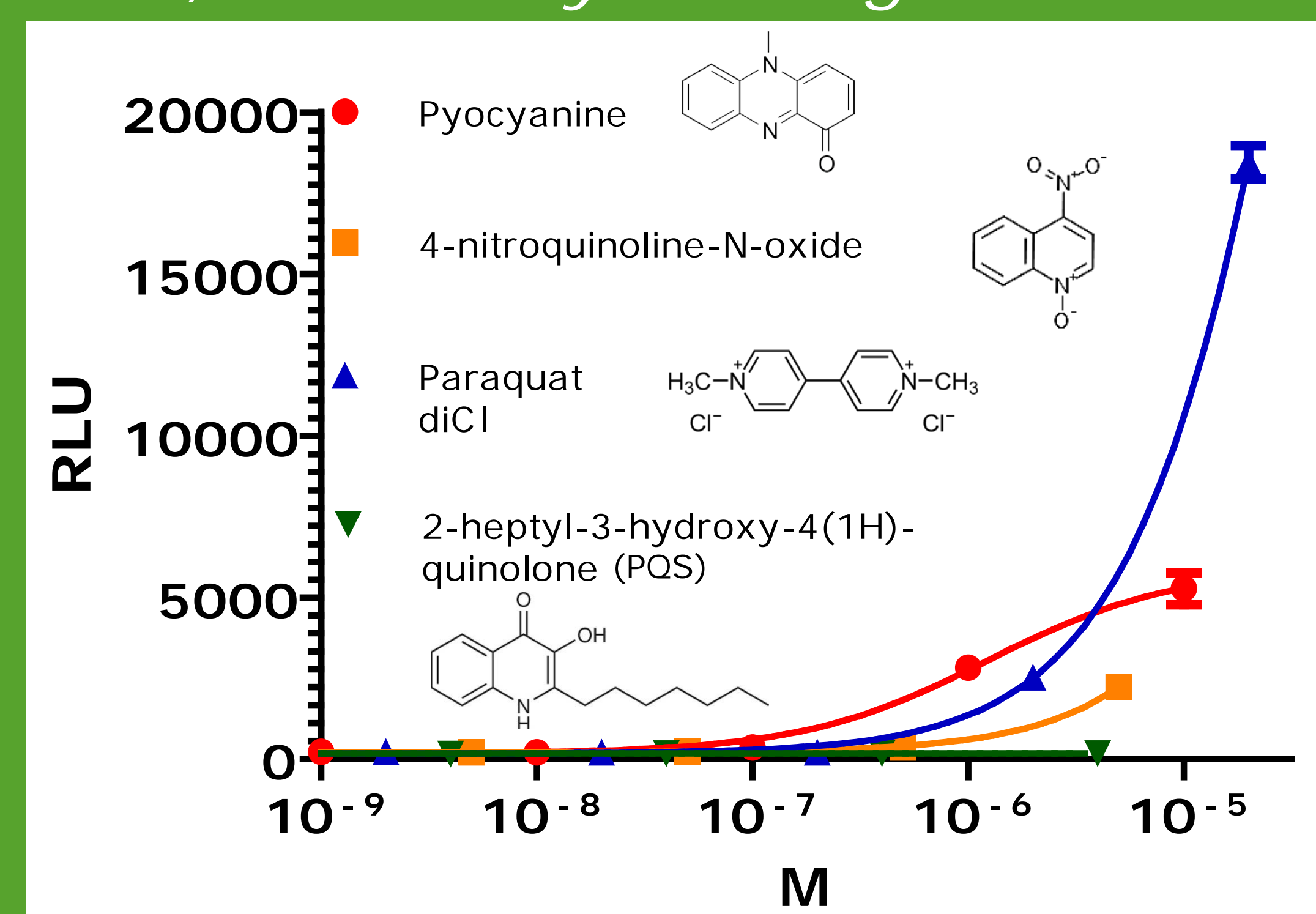
pPHZlux-1: reporter for redox-active compounds (*P. aeruginosa* SoxR and mono-oxygenase promoter)

BPlux: whole cell bioreporter for cytotoxicity (strong synthetic basal promoter)

pPHZlux-1 responds to redox-cycling small molecules (e.g. the phenazine pyocyanine) directly but not to ROS

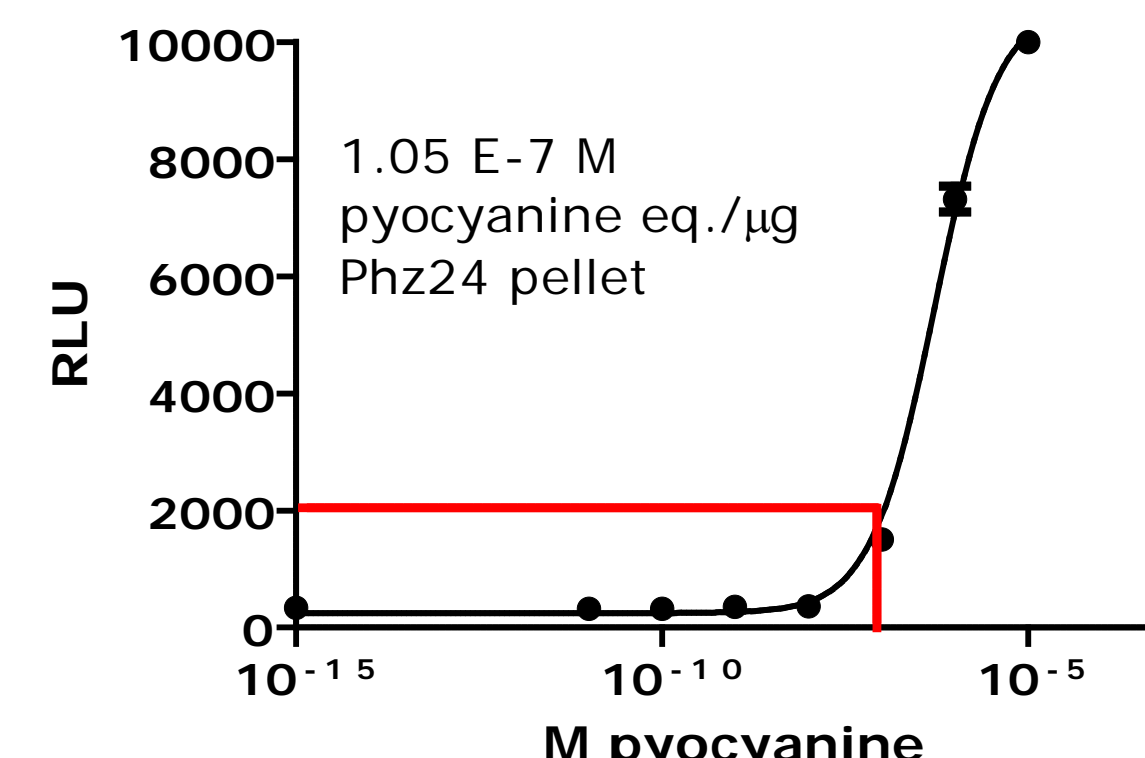
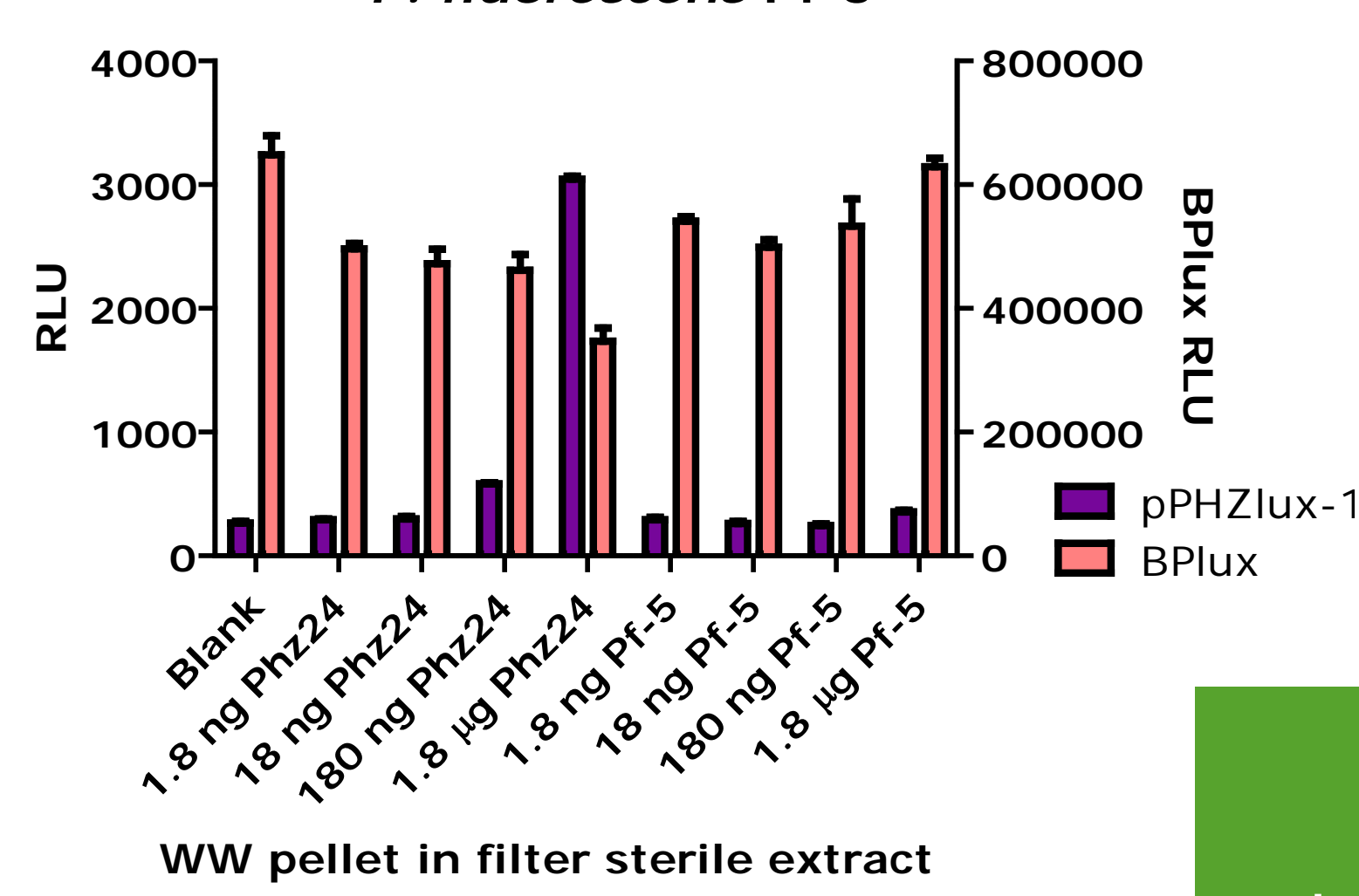


pPHZlux-1 is induced by pure redox-active model compounds, but not by the negative control PQS



pPHZlux-1 detects redox activity in microbial cultures at sub-inhibitory concentrations

Activities in pellets of biocontrol strains *Pseudomonas chlororaphis* Phz24 and *P. fluorescens* Pf-5



P. fluorescens Pf-5: control
P. chlororaphis Phz24: phenazine-1-carboxylate producer

Conclusion/Outlook

The combined application of a specific and a whole cell bioreporter on redox active producing biocontrol strains, confirms the feasibility of the approach.

The application of a panel of reporters for antimicrobial activities can assist in the directed mining of unknown microbial metabolic diversity.

