Background

- **P. aeruginosa** is a common microbial contaminant relating to product recalls in the home and personal care (HPC) industry.
- To prevent product spoilage and potential consumer infection, HPC products incorporate preservative formulations.
- A high tolerance of antimicrobials allows *P. aeruginosa* to survive in the presence of industrial preservatives.
- There is limited knowledge of resistance mechanisms utilised in response to the industrial preservatives benzothiazolinone (BIT) and phenoxethanol (POE).

The aim of this research was to identify genes and metabolic networks involved in industrial *P. aeruginosa* antimicrobial resistance.

Methods

1. Whole genome sequencing of a *P. aeruginosa* strain isolated from an industrial setting:
   - Sequencing was carried out at Cardiff University’s Genome Research Hub.
   - The genome sequence was visualised using the Circular Genome Viewer (CGView).

2. RNA-Seq expression analysis of the industrial strain in response to industry relevant conditions:
   - Industrial RW109 strain was grown for 24-hours in the presence of the following exposure conditions:
     - 1: TSB only
     - 2: BIT at 50% of the MIC
     - 3: POE at 20% of the MIC
     - 4: POE at 50% of the MIC
     - 5: POE and BIT at 20% of the MIC
     - 6: Laundry detergent at 1:100 dilution only
     - 7: Laundry at 1:100 dilution with BIT at 20% of the MIC

3. Genome-scale metabolic network reconstruction (GENRE) of the industrial *P. aeruginosa* strain:
   - Metabolic modelling was carried out in MATLAB using the COnstraint-Based Reconstruction and Analysis (COBRA) Toolbox.

Results

1. Whole genome sequencing:
   - The genome sequence of the industrial strain was a larger average sized genome when compared to a panel of 109 *P. aeruginosa* genome sequences which were isolated from clinical, environmental and industrial sources.
   - Whole genome sequencing revealed the extensive sized genome of *P. aeruginosa* RW109 strain.

2. RNA-Seq gene expression analysis of predicted antimicrobial resistance genes:
   - Gene expression analysis of the predicted RW109 antimicrobial genes was examined when exposed to conditions 2-7, revealed thirty-five which were significantly up or down regulated in at least one condition (including condition 2: BIT at 20% of the MIC where no antimicrobial resistance genes were differentially regulated).
   - Log-2 fold changes and adjusted p-values are shown for conditions 4, 5 and 7 which had the highest numbers of differentially regulated antimicrobial genes.
   - Efflux pumps were important in response to the industrial conditions and the MemOP-OmPol RND efflux system was found to be significantly up regulated when exposed to conditions 2-7.

3. Metabolic modelling of an industrial *P. aeruginosa* strain:
   - Reaction associated with lipid metabolism were the most abundant throughout the industrial strain model and a comparison to PA14 model, RW109 had additional reactions within the lipopolysaccharide biosynthesis and lipid metabolism functional catagory.
   - Exposure to the preservative POE at 10% of the MIC resulted in the highest number of predicted essential reactions with the majority having a functional lipopolysaccharide, a lower number of essential reactions were predicted when the preservative BIT was added, this reflects the mechanism of action of these preservatives.
   - A known pyochelin receptor was not identified in PA14 and PA01, the industrial strain had an additional two-component regulatory system (PA1204-PA0472), which is involved in heces inhibitor uptake.

Conclusions and future steps

- Whole genome sequencing revealed the extensive sized genome of an industrial strain.
- RNA-Seq analysis demonstrated the use of known antimicrobial resistance genes as a mechanisms of survival in the presence of industrial conditions.
- The metabolic model and transcriptomic data integration predicted essential genes during exposure to industrial conditions revealing potential antimicrobial targets.
- Gene expression data will be validated by quantitative PCR and gene essentially predictions confirmed via metabolomics screening.

References