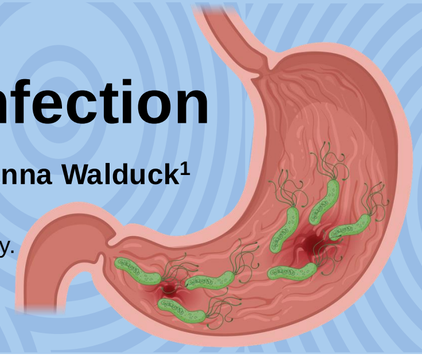
Novel therapeutics to treat *Helicobacter pylori* infection

Sophea Aing[1]*, Bernd Kalinna[1], Thiru Vanniasinkam[2], Jessica Holien[3], Lucy Barr[3], Paul Ramsland[3], Anna Walduck[1]

[1]Rural Health Research Institute [2]School of Dentistry and Medical Sciences, Charles Sturt University [3]School of Science, RMIT University.
***saing@csu.edu.au**



Charles Sturt University
Rural Health Research Institute

Background

H. pylori causes gastric diseases among 50% of the world's population[1]. Empirical therapy with antibiotics is recommended[2]. However, increasing antibiotic resistance prompted WHO to make it a priority for new therapeutics[3]. *H. pylori* glycosylates essential proteins for survival and virulence[4]. Eight glycosyltransferases were selected as novel *H. pylori*-specific drug targets.

Aim

- To identify and test small molecule inhibitors of *H. pylori* glycosyltransferase enzymes as the novel therapeutics

Methods

- *In silico* modelling to identify target enzymes from *H. pylori* genomes;
- *In silico* virtual screening to identify small molecule inhibitors;
- *In vitro* (laboratory) and *in vivo* (mouse model) screening to determine the safety and efficacy of inhibitors (**Figure 1**).

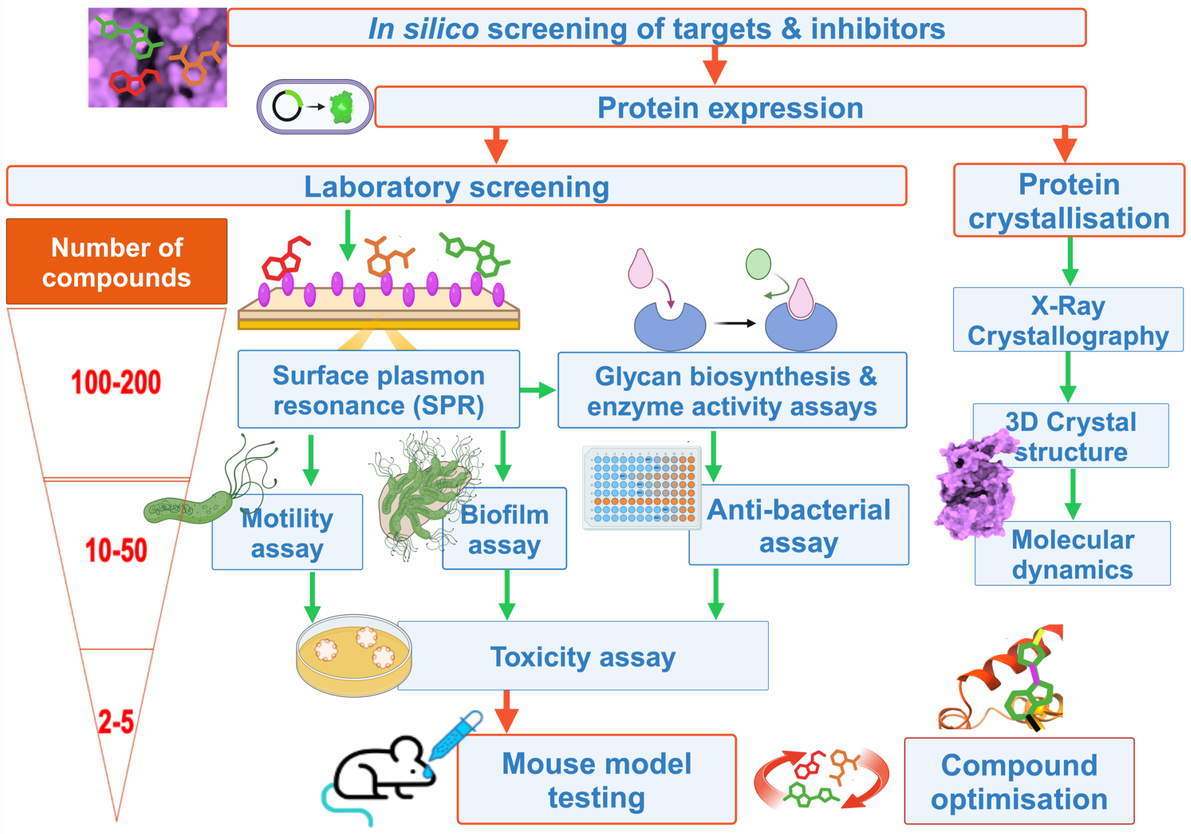
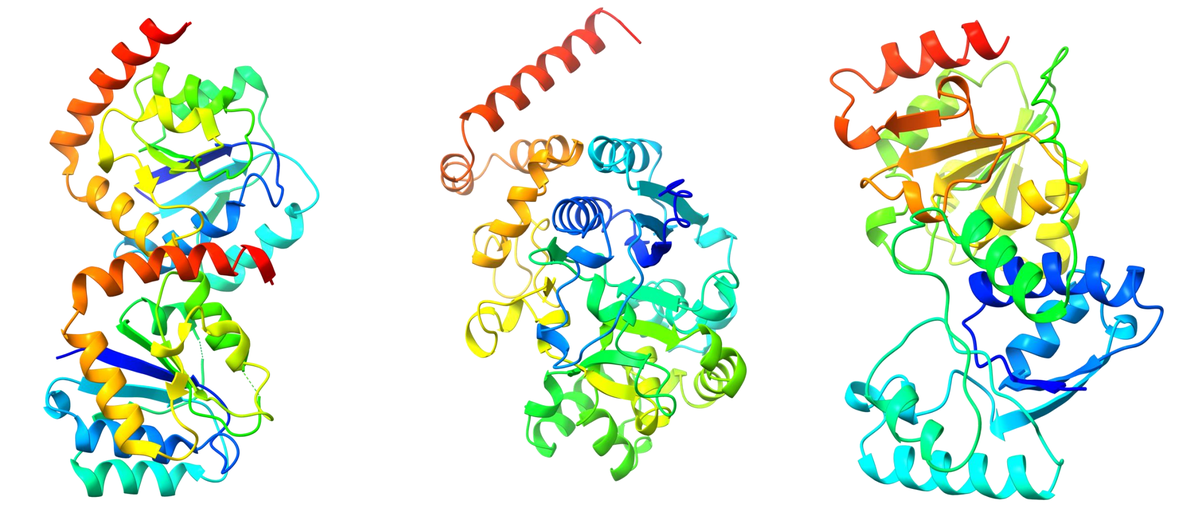


Figure 1. Workflow of *H. pylori* novel drug development pipeline

Result

- As a proof of principle, an *in silico* virtual screening was performed against the predicted structure of the three target enzymes (**Figure 2**). Over 100 hit compounds were identified as small molecule inhibitors.
- Three *H. pylori* glycosyltransferases were expressed in *Escherichia coli* (**Figure 3, 4**) to be tested against inhibitors.



Cholesterol-α-glucosyltransferase, HP0421 | **Lipopolysaccharide1,2-glucosyltransferase, HP0159** | **α-1,2-DD-manno-heptosyltransferase, HP0479**

Figure 2. Protein 3D structures predicted by AlphaFold2

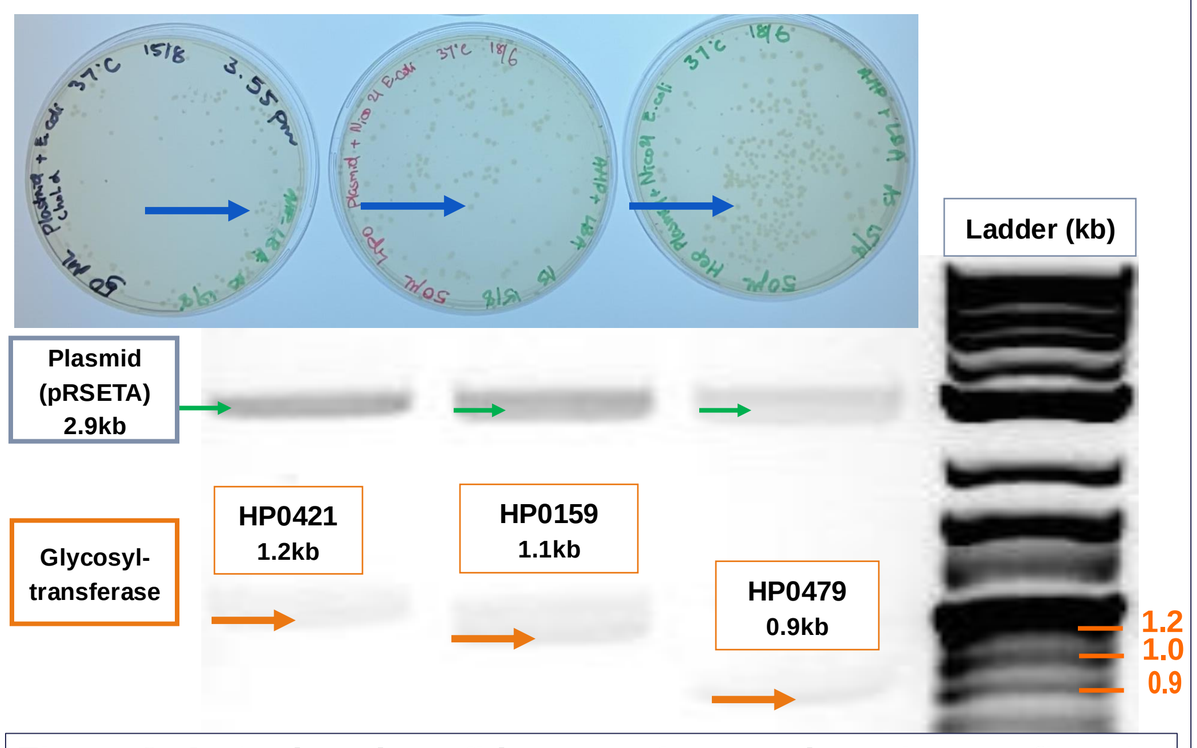


Figure 3. A single colony (blue arrow) was selected to confirm the transformation of *E. coli* with expression plasmids. Electrophoresis confirmed the presence of glycosyltransferase genes in *E. coli* (orange arrow).

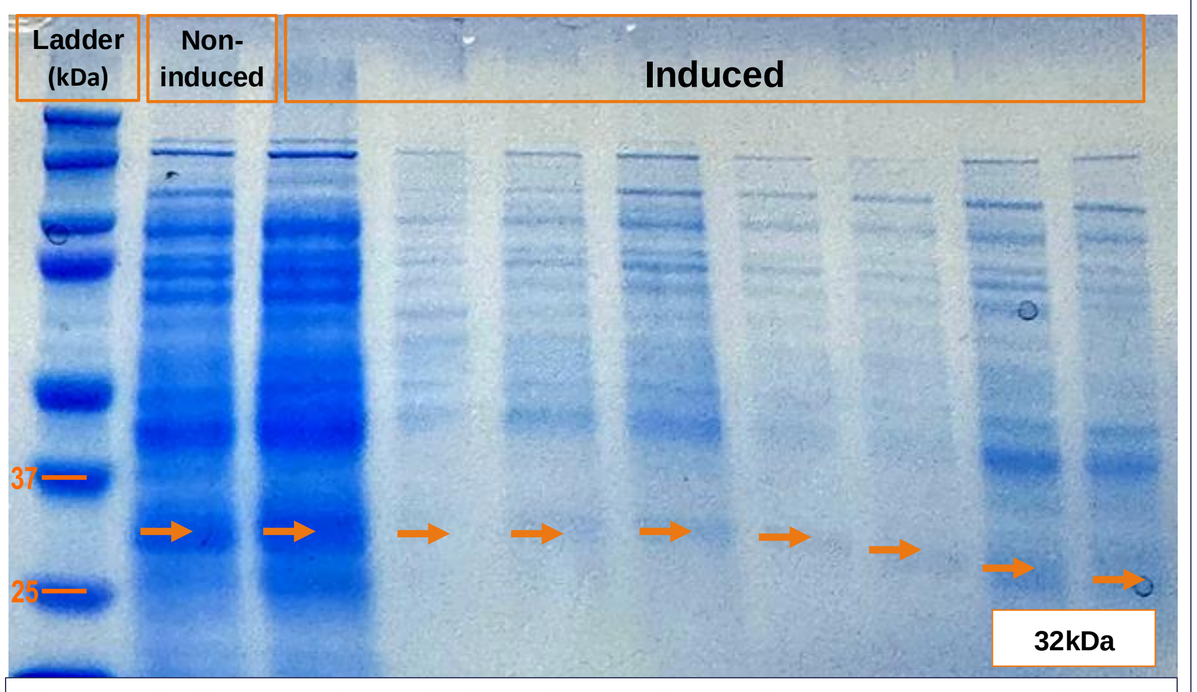


Figure 4. Expression of the glycosyltransferases was confirmed via SDS-PAGE, with bands at the expected molecular weight (HP0479, arrow). HP0421 and HP0159 enzymes were also confirmed (results are not shown).

Conclusion

- Three of the eight potential glycosyltransferases were selected for screening as the proof of principle.
- Over 100 hit compounds were identified from virtual screening against these enzymes.
- Initial work to express the three recombinant proteins has been completed.
- This drug development pipeline is expected to generate 2-5 compounds that can be optimised for pre-clinical testing.

Future work

- To express and purity the glycosyltransferases for:
  - Crystallisation to determine the structure for molecular dynamics studies;
  - Screening against the small molecule inhibitors in a panel of *in vivo* and *in vitro* assays.

References

1. Chen, Y.-C. et al. Global Prevalence of *Helicobacter pylori* Infection and Incidence of Gastric Cancer Between 1980 and 2022. Gastroenterology 166, 605–619 (2024).
2. Graham, D. Y. Illusions regarding *Helicobacter pylori* clinical trials and treatment guidelines. Gut 66, 2043–2046 (2017).
3. WHO. WHO publishes list of bacteria for which new antibiotics are urgently needed.(2017).
4. Teng, K.-W. et al. *Helicobacter pylori* employs a general protein glycosylation system for the modification of outer membrane adhesins. Gut Microbes 14, 2130650 (2022).