1. Introduction

The glycopeptide antibiotics vancomycin and teicoplanin (Fig. 1) are clinically important as a second-line therapy for the treatment of many nosocomial infectious diseases caused by Gram-positive pathogens. After its introduction in the 1980s, teicoplanin quickly became the glycopeptide of choice in many UK hospitals. Although the two glycopeptides share the same common mechanism of action, differences in teicoplanin’s core aglycone, glycosylation pattern and the presence of an acyl chain make teicoplanin a more effective antibiotic, with improved pharmacokinetics and -dynamics, making it a highly valuable antibiotic.

A proposed universal mode of action of glycopeptide antibiotics is to target the terminal residues, D-Ala-(D)-Ala in the cell wall peptidoglycan intermediate lipid II. By complexing with lipid II, the later stages of peptidoglycan biosynthesis are arrested, weakening the bacterial cell wall and eventually causing lysis of the cell. A general resistance mechanism to glycopeptide antibiotics requires a core set of genes, vanRISAH (Fig. 2). The gene products VanRSs are responsible for detecting the presence of a glycopeptide (VanS) and upregulating genes (VanR) which orchestrate the remodelling of D-Ala-D-Ala on lipid II to D-Ala-D-Lactate (VanHAX). Glycopeptide affinity for lipid II is reduced by 1000-fold and biological activity impaired. Teicoplanin acts as a poor inducer of VanS in S. coelicolor (Fig. 2), making this bacterium resistant to vancomycin but highly sensitive to teicoplanin.

2. Comparisons between wt and avatar De Differentially Expressed Genes

Panmap comparisons between the 30- and 60-minute time points only share 1,152 (28%) common differentially expressed (DE) genes between the wt and avatar, showing that there is a lot of variation in the gene expression of the two strains initially after exposure to teicoplanin. Comparisons between the 90- and 30-minute highlight that the wt undergoes little change in gene expression during the later stages of exposure to teicoplanin, but the expression profile of avatar shifts, more closely resembling that of the wt, with both sharing 1,462 (60%) common DE genes.

The 1,557 most significantly differentially expressed genes were hierarchically clustered to make the data analysis more manageable. Seven distinct clusters were generated shown in Fig. 3:

- Cluster 1 is the largest cluster undergoing downregulation over the 90-minutes
- Cluster 3 and 5 both increase in activity within the first 30-minutes for both strains, plateauing at 90-minutes for the wt, but continuing to increase in activity for avatar.
- Cluster 2, 4, 6 and 7 all contain genes that undergo changes in activity in avatar over the time course, but little or no change is seen in the wt.

By grouping genes into clusters, we intended to arrange genes with similar functional roles together. By using a gene ontology (GO) database for S. coelicolor, we were able to identify any functional groups that were over-enriched (P-value < 0.05) in our data. Because teicoplanin targets the cell membrane, particular focus was put into identifying categories with genes that are localised to the cell wall. GO categories that fit these criteria are listed in Table 1.

3. Functional Analysis

4. Comparing teicoplanin dataset with available data on other antibiotics

Our RNA-seq data was scaled alongside previous microarray data on vancomycin, bacitracin and moenomycin to identify differences in the response to teicoplanin for the wt and avatar mutant. Some genes underwent similar changes in activity in all data-sets but we were particularly interested in genes that were specific for either teicoplanin or glycopeptides in general, as these could be genes involved in resistance.

Genes in the penicillin binding protein category (Fig. 4A) are all penicillin binding proteins (PBPs) which are involved in the later stages of peptidoglycan biosynthesis, polymerising and cross-linking nascent chains to strengthen the polymer.

- One PBP (pink) was found to be specifically upregulated in the presence of the antibiotics teicoplanin and vancomycin.

Genes in the response to drug category (Fig. 4B) all belong to either the ATPase binding cassette (ABC) or multidrug and toxic-compound exclusion (MATE) transporter gene families.

- One MATE-transporter (blue) and one ABC-transporter (green) were found to only increase in expression in response to teicoplanin suggesting a specific role in the adaptive response to teicoplanin.

The serine/threonine kinase category (Fig. 4C) contains four serine/threonine kinases (STKs). Similar to those found in eukaryotes, STKs have been implicated in a in a lot of cellular processes including virulence, cell membrane metabolism and antimicrobial resistance.

- One STK gene was found to be upregulated by teicoplanin (pink) but not by other cell wall targeting antibiotics.

5. Conclusions

1. There is a lot of variation between the expression profiles of the wt and avatar immediately after exposure to teicoplanin. By 90-minutes, there is less variation between the expression profiles of both strains.
2. Seven unique clusters were generated by clustering the most significant genes together. Clusters 3 and 5 contained genes that were induced in both strains after exposure to teicoplanin.
3. Within these clusters, their functional groups relating to the cell wall were found to be significantly enriched.
4. Comparing each of these functional groups with previous microarray data showed how S. coelicolor adapts to different cell wall targeting antibiotics, providing evidence for novel genes that may be specific in developing resistance to teicoplanin.