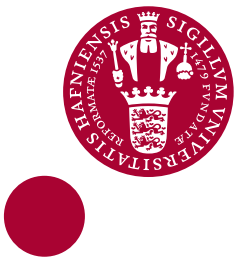

A novel glycopeptide resistance operon in environmental Rhodococcus equi

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A novel glycopeptide resistance operon in environmental *Rhodococcus equi*

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Background

Vancomycin and teicoplanin are last resort glycopeptide drugs for treatment of MRSA and enterococcal infections. They inhibit cell wall formation by binding to the D-Ala-D-Ala terminal residues of peptidoglycan precursors. Resistance is due to synthesis of low affinity precursors that terminate with either D-Ala-D-Lac or D-Ala-D-Ser (1). VanA-type is the most common glycopeptide resistance in enterococci and is characterized by inducible high-level resistance to both vancomycin and teicoplanin (2).

Rhodococcus equi is Gram-positive,soil coco-bacillus that causes severe bronchopneumonia in horses. It can also cause fatal infections in immunocompromised humans (3).Van^R has been reported in human clinical *R.equi* isolates (4), however the resistance mechanism is unknown.

OBJECTIVE: To elucidate the mechanism of glycopeptide resistance in a vancomycin resistant *Rhodococcus equi* isolated from Danish soil.

Methods

Expression of resistance. The Bioscreen^R was used to analyze the growth curves of *R.equi* RE-S7B non-exposed and exposed to 8 mg/l vancomycin (without prior exposure to vancomycin and with pre-exposure to vancomyin) over 2 days at 30˚C. OD_{600} was measured every 30 min. Experiments were done in triplicate. In addition, minimum inhibitory concentration (MIC) was also tested by Etest® on RE-S7B pre-exposed to increasing concentrations of vancomyin.

Whole genome sequencing. RE-S7B was sequenced using Illumina paired-end (PE) technology (500bp library). *De novo* assembly was done using Geneious v6.3, *van*-like genes were annotated using *CLC* genomic work bench and NCBI BLAST.

Results and Conclusions

Resistance in RE-S7B is inducible

RE-S7B had a vancomycin MIC >32µg/mL and teicoplanin MIC = 8µg/mL. At sub inhibitory concentration of vancomycin, vancomycin exposed cells resume growth faster than non-exposed cells (FIG.1). The MIC increased proportionally when exposed to higher vancomycin concentrations (FIG.2).

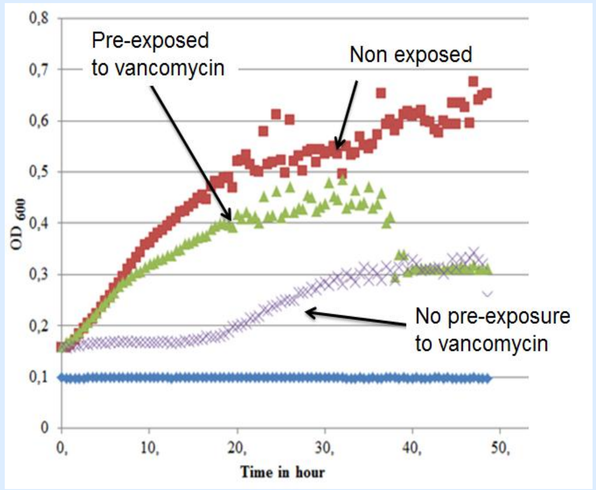

Figure 1: Glycopeptide resistance expression of RE-S7B.

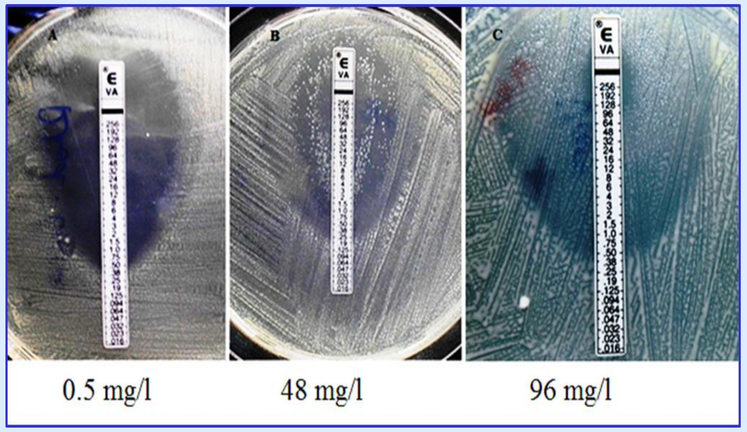

Figure 2: Vancomycin MIC values of RE-S7B recovered from BHI agar plates with no vancomycin (A), 8 mg/l of vancomycin (B) and 20 mg/l of vancomycin (C).

RE-S7B harbors a unique *van* operon

RE-S7B harbors a ***vanA-like* operon** consisting of a ***vanHAX* cluster and a two-component regulatory system,** displaying **60-63% and 39-41%** amino acid identity to the **enterococcal *vanA*** genes, respectively (FIG. 3). The gene **organization is unique** and includes a novel transposase type and additional putative open reading frames. The proposed name for this **novel operon is *van*$_{RE.}$**

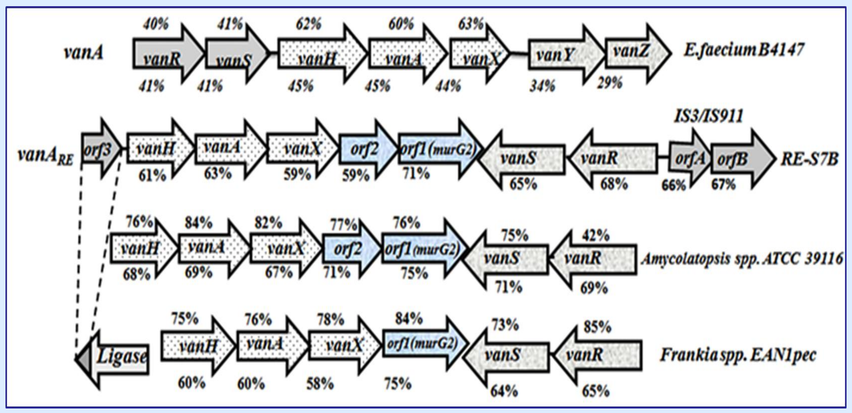

Figure 3: Organization of *vanA* in *E. faecium* , $vanA_{RE}$ in RE-S7B, *van*-like clusters in *Amycolatopsis* spp. *ATCC 39116* (NCBI BLAST) and *Frankia* spp. EAN1pec (NCBI BLAST). Arrows indicate direction of transcription. Percentages below and above the arrows indicate GC content and Nucleotide identity of the corresponding gene to $vanA_{RE}$ operon in RE-S7B.

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References:1) Walsh et al. *Chem. Biol.* 1996, *3*, 21-28. 2). Lebreton et al. *Antimicrob. Agents Chemother.* 2011, *55*, 4606-4612. 3) Weinstock and Brown. *Clinical infectious diseases* 2002, *34*, 1379-1385. 4) Hsueh, et al. *Clinical infectious diseases* 1998, *27*, 370-375.