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Vancomycin variable resistance may occur through creation of a novel promoter after deletion by polymerase slippage

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Background

Vancomycin-variable Enterococci (VVE) are *vanA* PCR positive isolates with glycopeptide susceptible phenotype that can become resistant when exposed to vancomycin. A complex of seven genes aid in acquired VanA-type resistance against vancomycin but only the *vanHAX* genes are necessary to express resistance. We have previously found that a VVE susceptible outbreak strain in Norway had an insertion of an IS*L3* element in the *vanHAX* promoter region. This resulted in a susceptible phenotype due to the separation of VanR activator binding site from the *vanHAX* promoter. When this IS element jumped out, the original *vanA* cluster with the resistant phenotype was restored.[1] In this study, a Swedish susceptible VVE strain (VVE*Swe*-S) became *in vitro* resistant by a different mechanism than the Norwegian outbreak strains.

Materials & methods

The VVE*Swe*-S isolate was detected by *vanA* PCR screening of enrichment broth cultured fecal sample for vancomycin resistant enterococci from a patient after liver transplantation. The genomes of VVE*Swe*-S and the isogenic resistant spontaneous mutant VVE*Swe*-R obtained by growth in 5 ml BHI with 2 mg/L vancomycin were Illumina sequenced to investigate the responsible genetic factors. Assembly was done with Spades 3.6.2.[2] SNP calling was done with GATK.[3] MIC values were obtained using MIC test strips.

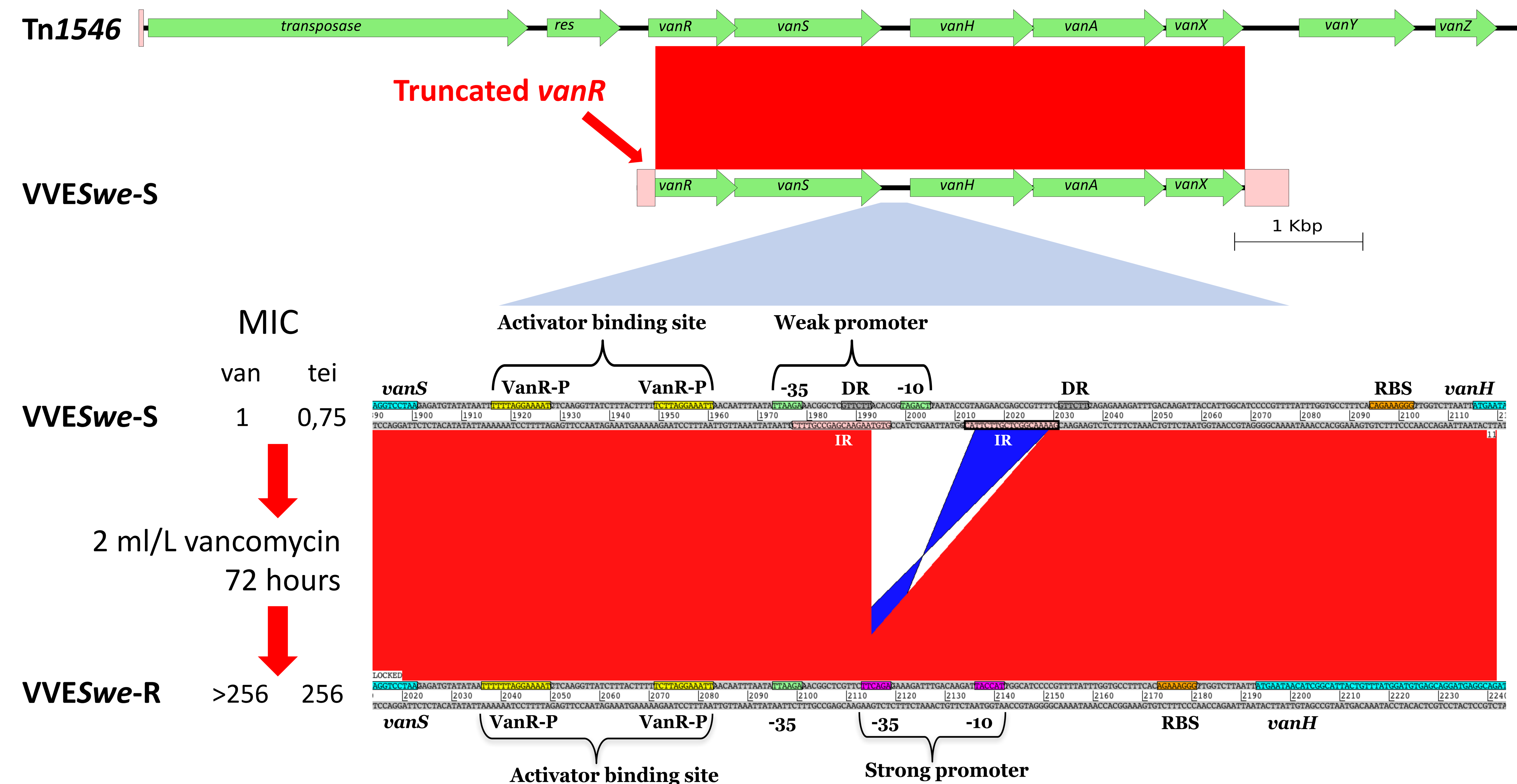

Figure 1. Pairwise alignment comparing the differential region in *vanA* loci between Tn*1546* containing prototype *vanA* cluster and VVE*Swe*-S (upper) and VVE*Swe*-S and VVE*Swe*-R (bottom). Deletion in VVE*Swe*-R creating a new promoter (bottom) is connected to direct (DR) and inverse repeats (IR) which allow polymerase slippage. Other genomic alterations were excluded by contig comparisons and read mapping with subsequent SNP and variation calling (data not shown). van=vancomycin, tei=teicoplanin.

Results

After exposing VVE*Swe*-S to vancomycin for 72 hours, growth of a vancomycin resistant variant (VVE*Swe*-R) could be seen. Both the susceptible isolate (Figure 1 top) and its isogenic resistant counterpart lack an intact *vanR* activator gene. Genome comparisons showed that a 44 bp deletion in the promoter region of the resistant isolate was the only difference between the susceptible and resistant isogenic VVE. This deletion seems to create a new stronger promoter in VVE*Swe*-R that is not dependent on the VanR activator. The deletion occurs in a region with inverted and direct repeats and precisely removes one of the direct repeat sequences as well as the nucleotides between the two direct repeat sequences (Figure 1 bottom).

Conclusion

We hypothesize that the deletion creating a new promoter is the result of polymerase slippage since deletion occurs in a region with inverted and direct repeats and precisely removes one of the direct repeat sequences as well as the nucleotides between them. This VanA phenotype switch mechanism may enable *vanHAX* transcription in *vanA* gene clusters with faulty *vanRS* activation systems.

References

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