Continuous Exposure Of Vibrio Anguillarum To Tropodithietic Acid: Genetic Changes And Influence On Virulence

**Introduction:** The fish pathogen *Vibrio anguillarum* is a major problem in aquaculture causing Vibriosis. Bacteria of the *Roseobacter* clade can antagonize pathogenic vibrios in cultures in live feed such as microalgae, rotifers and *Artemia*, as well as in fish larvae. Therefore, roseobacters could be promising as probiotics in fish rearing. Production of the antibacterial compound tropodithietic acid (TDA) by roseobacters is key in the antagonism of vibrios. However, the effects of continuous exposure to TDA on *V. anguillarum* remain unknown. The purpose of this study was to investigate how prolonged TDA exposure affects *V. anguillarum* focusing on the development of resistance towards TDA and changes in virulence.

**Methods:** Seven lineages of *V. anguillarum* were exposed to increasing TDA concentrations over 300-400 generations and were subsequently genome sequenced. Virulence of the lineages is currently being tested in fish cell infection trials.

**Results:** Following exposure, four lineages reached 1.75 x wild-type MIC and three reached 1.5 x wild-type MIC. Genome sequencing revealed no major changes in the genomes of the lineages. The only virulence-related gene affected was *fliM*, encoding a flagella motor switch protein. However, mutations in this gene were observed in non-exposed controls as well.

**Conclusions:** In conclusion, TDA resistance does not appear to develop, and the virulence genes of *V. anguillarum* are unaffected by TDA exposure, supporting the application of TDA-producing roseobacters as probiotics in aquaculture.

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