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Phaeobacter gallaeciensis cell-to-cell signalling does not influence antagonism in algae cultures

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Background & Aim

Bacterial infections are a major problem in marine larviculture. Using probiotic bacteria to decrease bacterial pathogen concentrations in larvae cultures and in their live feed cultures and presents an alternative to prophylactic use of antibiotics. In an earlier study, we demonstrated that *Phaeobacter gallaeciensis* prevents infections of fish larvae in challenge trials with *V. anguillarum*. Moreover *P. gallaeciensis* was able to colonize cultures of microalgae and rotifers, which are used as first feed for a multitude of species in aquaculture, and significantly reduced concentrations of the introduced pathogen. Production of the antibacterial compound tropodithietic acid (TDA) was suggested as a mechanism of action [1]. In *P. gallaeciensis* N-acyl homoserine lactone-mediated quorum sensing is involved in the regulation of tropodithietic acid production [2]. The aim of this study was to investigate whether quorum sensing (QS) plays a role for the antagonism of *P. gallaeciensis* in cultures of microalgae, which may present an experimental system closer to natural conditions than liquid broth cultures.

Methods & Results

Antagonism in algae cultures. Axenic microalgae (*Tetraselmis suecica*) were grown to mid-log phase in a seawater-based mineral medium (B-medium), and were inoculated with *P.* gallaeciensis BS107 (DSM17395) wild type, a TDA-deficient mutant (Pda8), a mutant deficient in the *lux*R homolog (*pga*R)*, and a mutant deficient in the *lux*I homolog (*pga*I)* at a concentration of 10^2 cfu/ml, or were left axenic as control. All *P. gallaeciensis* strains were plate-counted on Marine Agar (data not shown). *V. anguillarum* NB10 tagged by chromosomal insertion of pNQFlaC4-gfp27 *cat*, *gfp*** was inoculated at 10^3 cfu/ml 2 days after inoculation of *P. gallaeciensis* into all cultures and was plate-counted on Tryptone Soy Agar with 6 mg/l chloramphenicol [1]. Algae concentrations were assessed by photometry of chlorophyll at 665 nm and correlated to counts of reference cultures using a Neubauer-improved counting chamber.

V. anguillarum was inhibited by the QS-deficient mutants, as well as by the wild type, while a TDA-deficient mutant did not have a significant effect (Fig.1). The *P. gallaeciensis strains* grew to 10^7 cfu/ml and growth of the microalgae was not affected by presence of any of the bacterial strains.

TDA production. TDA production of the QS-mutants in Marine Broth was measured indirectly by photometry of the correlated pigment at 398 nm (data not shown) and by inhibition of *V. anguillarum* in a standard agar-diffusion assay (Fig. 2 and 3).

A delay in onset of TDA production was observed in both of the QS-deficient strains under shaken culture conditions (200rpm), but not in static cultures.

* [2], by courtesy of T. Brinkhoff, University of Oldenburg, Germany
** by courtesy of D Milton, University of Umeå, Sweden

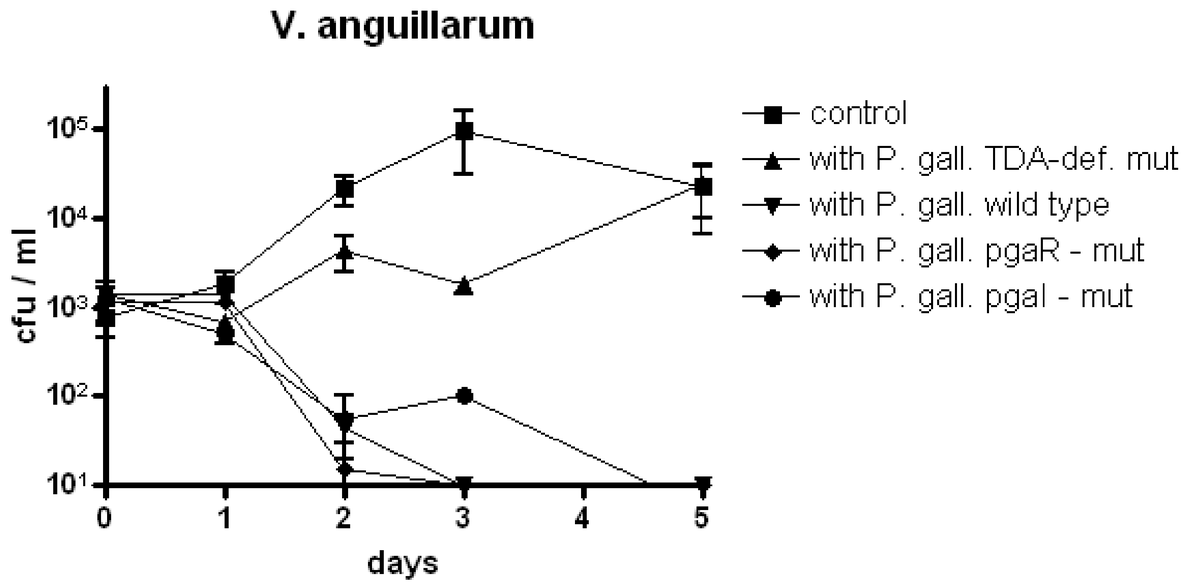

Fig. 1: Effect of *Phaeobacter gallaeciensis* quorum sensing mutants on *Vibrio anguillarum* NB10 in gnotobiotic cultures of the microalga *Tetraselmis suecica*

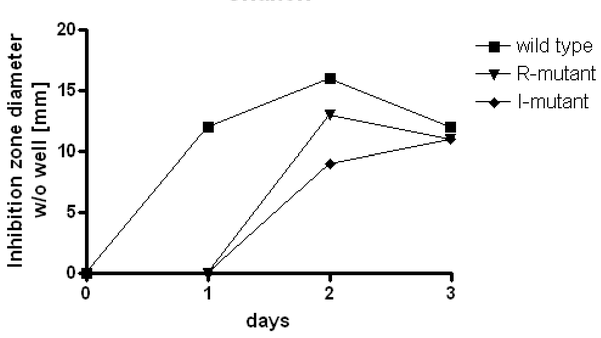

Fig. 2: Inhibition of *V. anguillarum* NB10 in an agar-diffusion assay by supernatants from shaken (200rpm) Marine Broth cultures of *P. gallaeciensis* wild type and quorum-sensing mutants

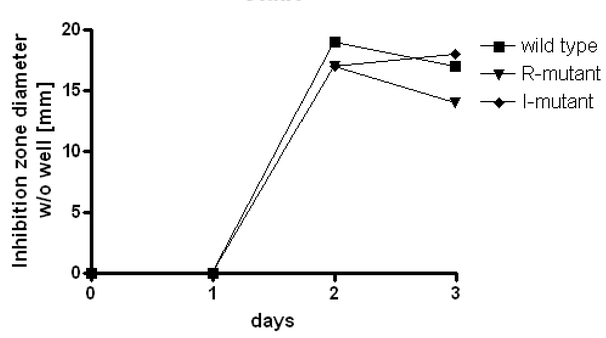

Fig. 3: Inhibition of *V. anguillarum* NB10 in an agar-diffusion assay by supernatants from static (0rpm) Marine Broth cultures of *P. gallaeciensis* wild type and quorum-sensing mutants

Conclusions

AHL-mediated quorum sensing does not influence pathogen inhibition of *P. gallaeciensis* in a model aquaculture environment.

TDA production under laboratory conditions is promoted by quorum sensing in aerated cultures, but not in static cultures. This may be due to an overriding regulation system.

References

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2. Berger M, Neumann A, Schulz S, Simon M, Brinkhoff T (2011) Tropodithietic acid production in *Phaeobacter gallaeciensis* is regulated by N-acyl homoserine lactone-mediated quorum sensing. Journal of Bacteriology 193: 6576-6585.

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