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Effect of virulence factors on survival strategies of *Vibrio cholera*e and *Vibrio mimicus*

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ABSTRACT

Vibrio is a genus of gram-negative bacteria comprising nearly 70 species and they are clinically human pathogens that can cause many infections. This study is mainly based on two species such as *Vibrio cholerae* O1 El Tor, which cause cholera disease and *Vibrio mimicus*, that cause gastroenteritis, open wounds infection, and septicemia. Therefore, this thesis aimed to study the effect of their virulence factors; melanin pigment, LuxO protein, ToxR protein, outer membrane proteins and vesicles on survival strategies; biofilm, rugose morphology and intracellular survival of *V. cholerae* and *V. mimicus* in *Acanthamoeba castellanii* using cell culture, viable cell count, gentamicin assay, vital staining, light-, fluorescent- and electron microscopy, microphotography, spectrophotometry, protein expression and isolation analysis, constructing internal in-frame gene deletion mutants and statistical analysis.

The results showed that *V. cholerae* mutant strain produced more melanin, showed increased UV resistance, expressed more toxin-coregulated pilus and cholera toxin and also increased colonization of infant mouse model compared to the wild-type *V. cholerae*. These findings suggest a possible role of melanin pigment formation in *V. cholerae* virulence factor expression.

V. mimicus had an enhanced growth in presence of *A. castellanii* and showed an intracellular behaviour in the amoeba. The bacteria were localized in the cytoplasm of amoeba trophozoites and the intracellular bacteria were viable for more than 2 weeks. Surprisingly, the *toxR* mutant of *V. cholerae* produced outer membrane protein T, significant biofilm and rugose colonies compared to the wild- type that produced OmpU, which showed decreased biofilm and did not form rugose colonies at 30°C. However, during the association with the amoebae it was observed that *A. castellanii* enhanced the survival of *V. cholerae* wild type compared to *toxR* mutant strain at 37°C. Interestingly, neither the wild- type nor *toxR* mutant strain could form rugose colonies in association with the amoebae. Therefore it shows that ToxR does seem to play some regulatory role in the OmpT/OmpU expression shift, the changes in biofilm, rugosity and survival with *A. castellanii*, suggesting a new role for this regulatory protein in the environments. Interestingly, outer membrane protein A (Omp A) suppressed the survival of alone cultivated wild-type *V. cholerae*, whereas the ompA mutant released more outer membrane vesicles and inhibited the viability of the amoebae. Co-cultivation of bacterial strains with *A. castellanii* enhanced the survival of both wild-type and ompA mutant but OmpA protein has no effect on attachment, engulfment and intracellular growth of *V. cholerae*.

In conclusion, this study based on the role of virulence factors on environmental survival strategies of *V. cholerae* and *V. mimcus* has demonstrated that melanin pigment plays an enhanced role in virulence and virulence factor expression of the bacteria such as resistance for UV- light, expression of toxin-coregulated pilus, cholera toxin and colonization ability.

V. mimicus shows an intracellular growth and survival in *A. castellanii* and neither LuxO regulator nor protease have role on the intracellular behaviour, which is reported for the first time.

The regulatory protein ToxR of *V. cholerae* suggests a new role in the expression of OmpT/OmpU and in the formation of biofilm, switching morphotypes from smooth to rugose colony and in association with the free living amoeba *A. castellanii*.

The outer membrane protein A of *V. cholerae* suppresses the survival of alone cultivated wild-type *V. cholerae* and has no effect on attachment, engulfment and intracellular growth of *V. cholerae* interacted with the amoebae, whereas *ompA* mutant released more outer membrane vesicles, which inhibits the viability of amoebae.

Keywords: *Vibrio cholerae*, *Vibrio mimicus*, melanin, *Acanthamoeba castellanii*, co-cultivation, gentamicin assay, outer membrane proteins, vesicles, rugose, biofilm, intracellular survival

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