Cytotoxicity of phase varying *Flavobacterium psychrophilum* cells

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Abstract

We have previously demonstrated that phase variation can occur in *Flavobacterium psychrophilum*, a Gram-negative pathogenic bacterium that causes bacterial cold water disease in freshwater salmonids [1]. This phase or phenotypic variation is in the laboratory seen as smooth and rough colony phenotypes and physiologically the two cell types have distinct characteristics [2]. For pathogenic bacteria, a switch between two or several phenotypes can enable the cells to evade the immune system of the host or to colonize different sites in the host [3].

Since both smooth and rough cell variants have been found to be virulent for rainbow trout (*Oncorhynchus mykiss*) [2], the aim of the study was to investigate possible differences in *in vitro* cytotoxicity of the smooth and the rough cells to rainbow trout erythrocytes and head kidney macrophages. The *F. psychrophilum* isolates used in the study were previously isolated from internal organs of diseased farmed rainbow trout. All isolates contained cells of smooth and rough colony phenotypic variants that were separated and named after the corresponding colony type as P13-4S/96, P13-4R/96, P6-1S/07, P6-1R/07, P6-3S/07, P6-3R/07, P6-8S/07 and P6-8R/07 [2].

A quantitative micro plate assay was used for measuring the cytotoxicity to erythrocytes, i.e. hemolytic activity, was. Briefly, the isolated rainbow trout erythrocytes were mixed with bacterial cells and the amount of hemoglobin that was released from the lysed erythrocytes was measured spectrophotometrically. The smooth cells showed a high and the rough cells a negligible, concentration dependent, hemolytic activity (Fig. 1) that correlated positively with the cells' hemagglutinating ability. The hemolytic activity of the cells was not regulated by iron availability and cell-free extracellular products did not show any hemolytic activity. Both hemagglutination and hemolytic activity was impaired by treatment of the bacterial cells with sialic acid and the hemolytic activity was furthermore reduced after proteolytic treatment of the bacterial cells. The results suggest that the hemolytic activity in *F. psychrophilum* is highly expressed in the smooth phenotype, and that it is a contact-dependent and two-step mechanism that is initiated by the binding of the bacterial cells to the erythrocytes through sialic acid-binding lectins and then executed by thermolabile proteinaceous hemolysins [4]. The fact that only the smooth cells showed high hemagglutinating and hemolytic activity and that the smooth phenotype can switch into the rough phenotype [2] leads to the hypothesis that the gene encoding for the sialic acid-binding lectin could be affected in the phase variation of *F. psychrophilum*.

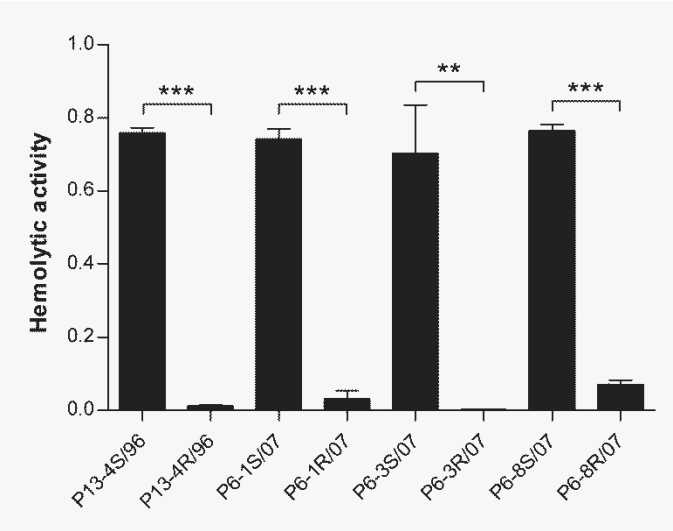

Figure 1. Hemolytic activity (mean ± SD) of cells of smooth (S) and rough (R) phenotypic variants. Statistical differences (T-test) between corresponding smooth and rough cells: **, $P < 0.01$; ***, $P < 0.001$.

The cytotoxic effect of the smooth and the rough cells to rainbow trout head kidney macrophages was measured as the release of lactate dehydrogenase (LDH) from damaged macrophages using Cytotoxicity Detection Kit (LDH, Roche) according to the manufacturer's instructions. The cytotoxicity to macrophages was, in contrast to the hemolytic activity, significantly higher for rough cells compared with the smooth cells (Fig. 2). The cytotoxic activity increased for both cell types with increasing temperature and the cells retained their cytotoxic nature after metabolical inactivation by heat, suggesting a cell-bound cytotoxic mechanism. The cytotoxicity was significantly reduced in both cell types after treatment with sodium (meta)periodate, indicating that the major bacterial structure involved in the cytotoxicity is of carbohydrate nature. Trypsin treatment further reduced the cytotoxicity in smooth cells, while sialic acid treatment reduced the cytotoxicity in rough cells, suggesting different lysing mechanisms for the two phenotypic variants. The results suggest that the cytotoxic activity of *F. psychrophilum* to macrophages is stronger expressed in the rough phenotype and that it is opsonin-independent and initiated by binding of bacterial surface carbohydrates to lectins on the surface of the macrophages. How the lysis of the macrophages is executed is still unclear but it is suggested to function by different mechanisms in the smooth and the rough cells. Since the adhesion mechanism of both smooth and rough cells to macrophages appears to be similar, the synthesis of the surface carbohydrates are therefore not necessary affected in the phase variation. What is causing the actual lysis of the macrophages, and if the bacterial cell structures involved are affected in the phase variation of *F. psychrophilum*, needs however to be further investigated. It appears likely though, that this cytotoxic and antiphagocytic activity could be part of the pathogenic profile of *F. psychrophilum* [5].

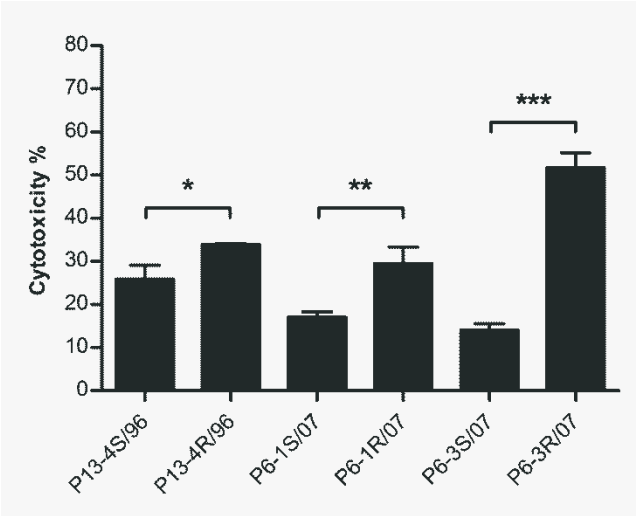

Figure 2. Percentage cytotoxicity (mean ± SD) of cells of smooth (S) and rough (R) phenotypic variants to rainbow trout head kidney macrophages. Statistical differences (independent-samples T test) between corresponding smooth and rough cells: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

References

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