Expression of BilRI-protein in *Aggregatibacter actinomycetemcomitans* in different conditions

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CELL BIOLOGY

Introduction

In this study we experiment with proteins expressed by *Aggregatibacter actinomycetemcomitans* bacteria. The protein of interest is called BilRI (Bacterial Interleukin Receptor 1). BilRI is found out to be a LEA-protein (Late Embryogenesis Abundant) like molecule, which is a stress associated protein. The function of BilRI in *A. actinomycetemcomitans* is yet unknown. It is expressed at a lower than optimal temperature (27 °C instead of optimal 37 °C).

Aim of the study

The aim of the study was to discover the portion of cells in a population that express the BilRI protein and the effects of temperature on that expression.

Materials and methods

The transferred gene was ordered in a plasmid construct and extracted from the plasmid in a linear form.

6His_bilRI_Spe -gene casette (Figure 1) was transformed to the cell via natural transformation, where the bacteria passively accept linear DNA and integrate that DNA into their genome. These mutant cells were then selected on a spectinomycin plate. The correct expression of the gene was ensured via Western blot.

Selected cells where cultivated in biofilms in biofilm bottles and collected. The baceria were then fixed, washed and stained with Atto 488 (figure 2). The excess label were washed off and the cells were analysed via flow cytometry.

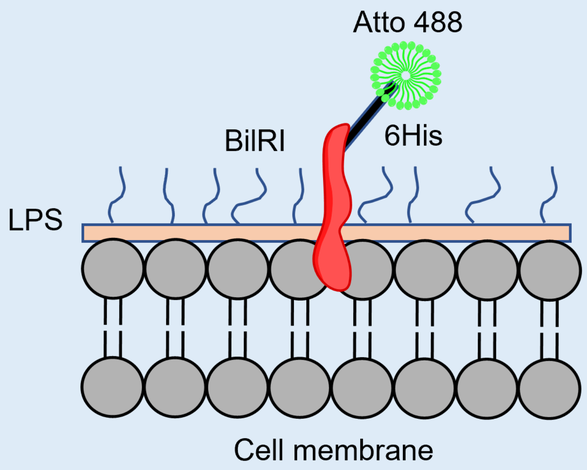

Figure 2 BilRI is a structureless protein located on the surface of the cell. The 6 histine long tail of the protein is labeled with Atto 488. This way the protein could be detected via flow cytometry or fluoresence microscopy. The LPS of the bacteria could affect the ability to label the histidine tail. The N-terminus of the protein contains a lipid part with which the protein attaches to the outer membrane.

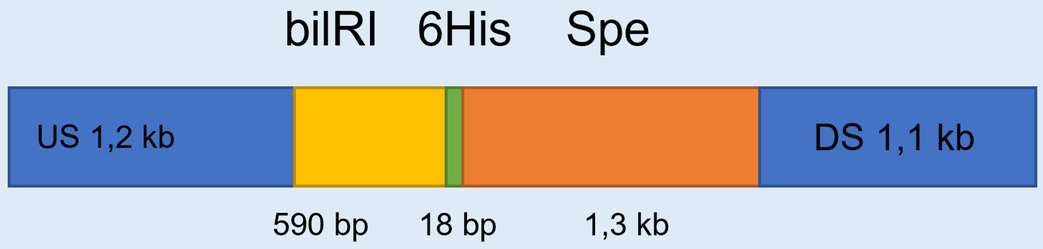

Figure 1 Linear bilRI_6His_Spe DNA that was transformed to *A. actinomycetemcomitans* genome via natural transformation. Histidine tail is labelled as 6His. Spectinomycin resistance is labelled as Spe.

The bacterium

The studied organism *Aggregatibacter actinomycetemcomitans* causes a serious periodontal disease in humans called periodontitis. Periodontitis is the decaying of gum tissue around the teeth. The bacteria form biofilms when growing. The bacterium is classified as a bio safety level 2 organism.

Results

The results we gathered showed that flow cytometry is an inadequate method for studying the singular cells in the population, at least for our methods (Figure 3). Flow cytometer showed no difference between wt and mutant. However, we succeeded in transforming the gene into the cells and producing the protein with the histidine tail.

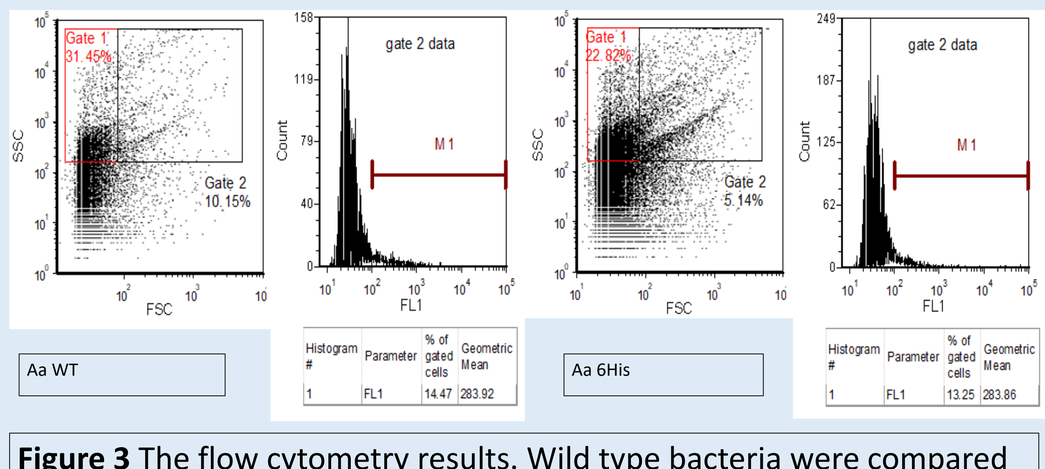

Figure 3 The flow cytometry results. Wild type bacteria were compared to 6His mutant bacteria. The data showed no significant difference between the wild type and the mutant. Fluorescent positive (green fluoresence) cells are shown in the gate M1 (gate 2 data).

Conclusions

The study concluded that flow cytometry is not the best way to study BilRI expression. The subject could be further studied with fluorescent microscopy. The lipopolysacharide layer on the cell surface might hide the histidine tail from the label particles. The fimbria of the cell may also hinder the analysis of the cells.

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