DESIGN AND APPLICATION OF A GENETICALLY MODIFIED *E. COLI* STRAIN FOR ASSESSMENT OF DNA EXTRACTION AND RECOVERY EFFICIENCIES IN *BACTEROIDETES* BASED MST STUDIES

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Introduction

Fecal pollution is a global issue for environmental and human health that affects water quality. To improve target-oriented catchment protection and management of water quality state-of-the-art technologies, such as quantitative PCR (qPCR) are used. This innovative technique allows quantification of fecal pollution and identification of the pollution sources - microbial source tracking (MST). The application of qPCR to quantify genetic fecal markers has been used for a wide range of water samples with contrasting quality characteristics and background matrices. However, comparison of qPCR results assumes comparable recovery and DNA extraction efficacies between samples to avoid an interpretation bias. Therefore appropriate cell spiking systems would be required in order to evaluate and control for a recovery and extraction bias when applying marker-based qPCR methods (Hagedorn et al, 2011).

Objectives

The aim of the present study was to genetically engineer a cell standard, which carries a defined number of a target gene in order to support routine application of a performance control during qPCR based *Bacteroidetes* analysis. The new system was tested for its applicability in an aquatic habitat with a strong gradient of physicochemical quality characteristics. It was hypothesized that these different conditions should have a strong influence on filtration and DNA extraction efficiency and yield. Thus, the application of the newly developed reference system, which has the capability to account for these matrix biases, enhances correct and careful data interpretation and quality control.

Methods

The defined genetic target number cell standard (DeTaCS) was constructed to obtain *E. coli* cells with a single copy of the BacR marker sequence in their chromosome. *E. coli* was used because it is a well-characterized bacterium used in microbiology and genetics, it is much easier to cultivate than an obligate anaerobe such as *Bacteroides* strain (Okabe, 2007). For construction of the DeTaCS, the λ-phage system was used to insert one single copy of the marker fragment into the chromosome of *E. coli*. After genetic construction it was grown under controlled conditions in a fermentation process. For the fermentation, 1 L of LB media was put into the fermenter (RALF Plus-System, Bioengineering AG, Switzerland), and inoculated with 10 ml overnight inoculum at a temperature of 21°C. The stirrer was adjusted to 500 rpm, and the oxygen flow was adjusted to 100 $_N$ L h^{-1}. The samples for the growth curve were taken every hour. The optical density was measured with UV spectrophotometer at a

wavelength of 600 nm. Samples were fixed and SYBR Gold-stained bacteria enumerated by direct fluorescent microscope counting.

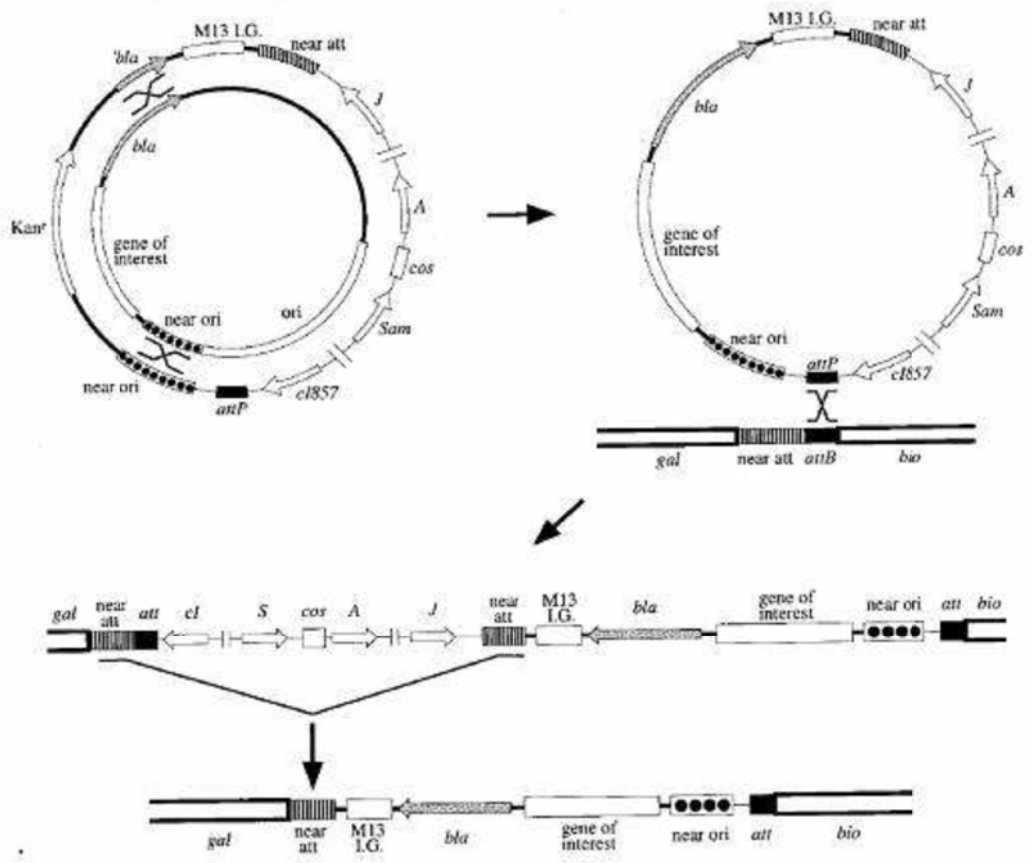

Figure 1. Steps for construction of DeTaCS

The ongoing field study is performed at 7 stations (Lake Neusiedler See [L], reed belt of Lake Neusiedler See [RB], pond [P], groundwater [GW], soda lake pool - Oberer Stinker [OS], untreated wastewater [WW-ut] and treated wastewater [WW-t]), between March and August 2015. Environmental and wastewater samples were spiked with 100 µl of two different concentrations of DeTaCS cells (10^4 and 10^1 cells/µl) in five replicates and quantified by qPCR. In addition samples were analysed and quantified for genetic fecal markers.

Results

The genetic engineering of the DeTaCS strain resulted in the successful and stable insertion of a single copy the BacR marker fragment in the chromosome of an *E. coli* strain. This DeTaCS strain was then successfully produced in larger quantities under tightly controlled conditions in a 2 L batch bioreactor. Various growth conditions were tested to ensure that every DeTaCS cell only carried one chromosome (generation time > replication time). Finally fermentation was performed at 21°C with a generation time of 176 min. Cell concentrations and gene targets in the resulting cell suspension was determined by epifluorescent microscopy and qPCR respectively. Aliquots for the field application study were prepared and stored at -80°C.

The DeTaCS is currently being used in an environmental application study to assess DNA extraction and recovery efficiencies in *Bacteroidetes*-based MST studies. Sampling stations with different physicochemical characteristics were chosen. National park Lake Neusiedler See is located in Eastern Austria and open for recreational activities like swimming and water sports. About 55% of the lake is covered with reeds (*Phragmites australis*), and within this vegetation, extended brown-water areas are found. The Oberer Stinker (OS) is a hypertrophic shallow soda pool with high total salt concentrations and turbidity (Kirschner et al, 2008). Groundwater (GW) represents low microbial contamination whereas wastewater represents high microbial contamination.

Detection of selected MST makers will be done for environmental and wastewater samples and results will be shown in detail at the poster presentation.

Conclusions

Routine application of DeTaCS will allow monitoring and comparing DNA extraction and recovery efficiencies for diverse environmental water matrixes (e.g. surface water, wastewater, ground water, lake water with high turbidity). This is considered a key step for correct data interpretation and quality control regarding MST applications. The application of the DeTaCS will also support a more straightforward determination of the sample limit of detection (SLOD).

References

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