

Selection and characterization of DNA aptamers for health-relevant bacteria in water

Claudia Kolm^{1,2*}, Robert L. Mach³, Regina Sommer⁴, Alexander Kirschner⁴, Maria DeRosa⁵, Georg H. Reischer^{1,7}, Andreas H. Farnleitner^{2,6,7}

¹ TU Wien, Institute of Chemical, Environmental & Bioscience Engineering, Research Group Environmental Microbiology and Molecular Diagnostics, Department IFA-Tulln, Tulln, Austria. ²ICC Interuniversity Cooperation Centre Water & Health, Vienna, Austria. ³ TU Wien, Institute of Chemical, Environmental & Bioscience Engineering, Research Division Biochemical Technology, Research Group Synthetic Biology and Molecular Biotechnology, Vienna, Austria. ⁴ Medical University Vienna, Institute for Hygiene and Applied Immunology, Unit Water Hygiene, Vienna, Austria. ⁵ Carleton University, Chemistry Department, Ottawa, Canada. ⁶ Karl Landsteiner University of Health Sciences, Research Unit Water Quality and Health, Krems, Austria. ⁷ TU Wien, Institute of Chemical, Environmental & Bioscience Engineering, Research Division Biochemical Technology, Research Group of Environmental Microbiology and Molecular Diagnostics, Vienna, Austria.

Health-relevant bacteria in water are a serious threat to public health, in both developing and industrialized countries. However, their detection still represents a challenge. Conventional methods such as culture- and molecular-based methods are time-consuming, expensive or limited in their on-site applicability. Aptamers are an emerging class of molecular recognition agents with the potential to address these major shortcomings of existing methods. Aptamers are short, single-stranded DNA- or RNA molecules with the ability to recognize and bind to a molecular target with high affinity and specificity by folding into complex three-dimensional structures. These functional nucleic acids can be readily produced via chemical synthesis in large quantities and at relative low costs with virtually no batch-to-batch variations. In contrast to their antibody counterparts, aptamers are identified by an iterative *in vitro* selection process, termed "SELEX" (Systematic Evolution of Ligands by Exponential Enrichment), in which aptamers evolve from large random oligonucleotide libraries containing up to 10^{15} unique sequences.

The aim of this project is to select broadly-reactive DNA aptamers that bind to *Enterococcus* spp. by establishing a whole-cell SELEX process and to subsequently characterize them for their applicability in the field of water quality analysis. Such novel biorecognition tools could be vital e.g. for biosensing purposes or for direct cell counting as fluorescently-labelled aptamers that selectively bind to *Enterococcus* spp. could be used in flow cytometry to rapidly test water for fecal contamination. With special focus on this potential application, we perform SELEX experiments with water-associated and frequently encountered target and non-target bacterial strains. Over the course of this project, all aptamer candidates will be thoroughly characterized and tested in terms of binding affinity, inclusivity and exclusivity, as well as their performance in environmental water samples (spiking tests).

* Recipient of the DOC-fellowship of the Austrian Academy of Sciences