In Vivo RNA Labeling and Separation – a Novel Tool for Biofilm Research

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Abstract:

Bacterial stress response to natural and technical stress factors enhances the fitness of bacterial populations such as natural biofilms in hostile environments. These responses range from stress induced gene expression to the secretion of beneficial exoenzymes. Moreover, the intra cellular stabilization of certain groups of stress related mRNAs also seem to play a role in bacterial fitness under adverse conditions.

This *in vivo* RNA labeling and separation technique is based on the incorporation of digoxigenin-11-uridine-5`-triphosphate (DIG-11-UTP) in the RNA of active bacteria. Without any chemical or physical treatment of the populations this nucleotide analog was taken up by bacteria and was incorporated specifically in the *de novo* synthesized RNA in their natural habitat. Depending on the growth conditions, the population's composition and the gene expression activity the assembly rate of DIG-11-UTP ranged from 1.2% to 12.5% of the total intra cellular RNA. The labeling was shown for Gram-positive and Gram-negative reference bacteria as well as for natural biofilms from drinking water, surface water, and lake sediment.

The separation of the DIG-RNA from total RNA extracts was performed with a biotinylated anti-digoxigenin antibody and streptavidin-functionalized magnetic particles. The average yield of separation was about 95% of labeled RNA from total RNA extracts. The unspecific bindings of non-labeled nucleic acids were smaller than 0.2%, evaluated by spiking experiments with an unmarked DNA template. Applicability of the developed method was demonstrated by rRNA-directed PCR-DGGE population analysis of natural biofilms and expression profiling of two stress induced genes (*van*A and *rpo*S) in reference bacteria before and after the DIG-RNA separation. Population analyses demonstrated biofilm fingerprint patterns of active bacteria, which were neither influenced by labeling nor during magnetic bead separation. Even, induction rates of the mentioned stress genes were not altered during labeling and separation procedures.

The presented *in vivo* labeling and separation method is a possible way analyzing the effects of stress response in natural bacterial populations. It could cover four mechanisms of stress response of bacterial populations: gene expression, interspecies interactions, shifts in the population’s composition and the stabilization of certain mRNAs. Therefore, the presented approach could be used as completion or alternative for metatranscriptome analyses putting a tighter focus on stress response of bacteria upon exogenious stimuli in their natural habitat. Analyzing up-regulated and stabilized gene products of active bacteria makes an examination of the entire transcriptome of the population unnecessary.