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Analysis of the growth phase-associated transcriptome of *Streptococcus pyogenes*

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Abstract

Streptococcus pyogenes (group A streptococci, GAS) is a human pathogen which probably varies its multiplication rate and thus, growth phases in association with the type of infection caused in its host. To create a basis for future determinations of such associations, the genome-wide growth phase-related GAS transcriptome was assessed in the present study. Therefore, the published serotype M1 *S. pyogenes* genome sequence as well as the partially sequenced serotype M18 and M49 GAS genomes were used to produce DNA microarrays that carried 2256 oligonucleotide probes matching 3662 open reading frames (ORFs). With these microarrays, the transcriptome of the serotype M49 GAS strain 591 grown to the exponential, transition, and early stationary growth phases was assessed in seven independent experiments. The gained data were compared to real-time RT-PCR assays. Data analysis was refined by a novel approach, i.e. grouping of expressed genes to four classes according to relative transcript abundance and gene functions. At the different growth phases, 86.7%, 79.5% and 55.7% of the at least 1883 ORFs contained in the serotype M49 genome were expressed above the defined detection level. Contrary to the general trend, transcript amounts of genes in the functional groups of transport and membrane proteins as well as stress response factors peaked at the transition phase. The most prominent changes in the transcript abundances were predominantly observed for sugar compound transport and turnover-related ORFs. The majority of known virulence genes had their maximum expression during the transition phase, consistent with the proposed associated change in virulence behavior of the bacteria. With these results, it will now be feasible to assess the in situ growth phase of a given GAS strain during any type of infection by measuring the expression of selected marker genes.

Keywords: *Streptococcus pyogenes*; Batch culture; Growth phase; Microarray; Transcriptome

Introduction

In their natural habitat, bacteria often have access to only limited nutrient supplies which allows them to multiply at a low and constant rate. Under such conditions, the bacterial growth kinetics will be influenced

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either by changes in available nutrients or by changes in the physico-chemical parameters of their immediate environment such as temperature, osmotic pressure, pH or oxygen tension. For pathogenic bacteria, additional environmental parameters affecting the bacterial multiplication rate are defined by the colonized or invaded anatomical compartment and by various host responses.

From this viewpoint, a batch culture and the succession of growth phases, the bacteria going through during this type of handling do not closely resemble the typical bacterial way of life. Yet, batch cultures are frequently used in scientific experiments because they allow the investigation of adaptation mechanisms used by the bacteria to comply with growing cell density, changes in available nutrient amounts, and variation of the pH. In fact, for reasons of simplicity the effects of such principally discernible parameters on gene expression are generally addressed as growth phase-dependent transcriptional regulation. Thus, in *Escherichia coli* as the most extensively studied bacterial species, temporal changes in many metabolic or anabolic pathways and adaptation mechanisms have been examined to great molecular and biochemical details utilizing batch cultures as a way to provide sufficient numbers of bacteria in different growth phases or more correctly, exposed to continuously changing nutritional and environmental conditions as described above.

Recently, the information gained from complete bacterial genome sequences and the application of nucleic acid microarray techniques allowed the assessment of transcriptional changes on a whole bacterial genome level. Quantitative whole-genome transcript measurements have repeatedly been used to compare regulatory gene mutant bacteria to their corresponding wild-type strains (e.g. Schuster et al., 2003; Wagner et al., 2003), specifically stressed bacteria to non-stressed isogenic ones (e.g. Tani et al., 2002), or biofilm-associated bacteria to their planktonic counterparts (e.g. Sauer et al., 2002; Firoved and Deretic, 2003; Beloin et al., 2004).

In Gram-positive bacteria, transcriptome and proteome analyses were used to study the impact of nutrient restriction and the consequences of stress responses as well as spore formation in bacilli and clostridia (Bernhardt et al., 2003; Eichenberger et al., 2003; Tomas et al., 2003). In streptococci, this type of technical approach was applied to study specific stress responses such as the Clp pathways (Robertson et al., 2002), the impact of specific regulators (Dagkessamanskaja et al., 2004; Mascher et al., 2003; McCluskey et al., 2004), and quorum sensing-associated genes (de Saizieu et al., 2000; Peterson et al., 2000, 2004; Joyce et al., 2004). However, an evaluation of the impact of more than two growth phases on the whole-genome gene expression levels has so far been performed only for a few bacterial species such as *Bacillus anthracis* (Liu et al., 2004), *Caulobacter crescentus* (Laub et al., 2000), *Chlamydia trachomatis* (Nicholson et al., 2003), *E. coli* (Selinger et al., 2000; Yoon et al., 2003), *Helicobacter pylori* (Thompson et al., 2003), and *Streptomyces coelicolor* (Huang et al., 2001).

The species *Streptococcus pyogenes* (group A streptococci, GAS) is unique among the streptococcal family. Unlike any other member of the β-hemolytic subgroup, it is completely adapted to humans as its only natural host. Although GAS can persist for several months outside the human body, e.g. in dust or dried blood (Reitmeyer et al., 1993), there is no external habitat for these bacteria. For humans, GAS are of high medical and economical importance, since they frequently cause purulent infections of the skin and mucous membranes and rarely, life-threatening systemic diseases.

Despite their significance for humans, studies on growth phase-associated or temporal changes in GAS functions were predominantly performed before RNA quantification was feasible for this species. After the publication of three complete genome sequences of GAS serotype strains M1 (Ferretti et al., 2001), M3 (Beres et al., 2002), and M18 (Smoot et al., 2002), whole-genome DNA arrays were used to measure the influence of growth temperature and the presence of human leukocytes on the transcriptome of serotype M1 GAS wild-type bacteria (Smoot et al., 2001; Voyich et al., 2003). Employing defined mutants, the impact of the CsrRS and Rgg regulators on the whole-genome gene expression levels in GAS serotype M1 and M49 strains were determined by this technique (Chaussee et al., 2002; Graham et al., 2002). Based upon results from these studies, several growth phase-associated immunogenic factors have been characterized (Reid et al., 2002).

In the present investigation, after partially sequencing the serotype M49 GAS genome, we performed a M49 GAS transcriptome analysis by using for the first time a DNA microarray that carried oligonucleotide probes directed to all open reading frames (ORFs) identified in the serotype M1 GAS genome and probes directed to the majority of ORFs contained in the serotype M18 and M49 GAS genomes. For the first time in epidemiologically important Gram-positive cocci, we measured the growth phase-associated genome-wide gene expression levels in seven completely independent experiments. The resulting data give new insight into the adaptation of transcript steady-state levels in growing GAS as nutritional supplies dwindle and cell density increases.

Materials and methods

Bacterial strain

The serotype M49 GAS strain 591 was provided by R. Lütticken, Aachen, Germany. The strain is a clinical