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Comparative transcriptomics of the response of *Escherichia coli* to the disinfectant monochloramine and to growth conditions inducing monochloramine resistance

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ABSTRACT

Escherichia coli growth in biofilms and growth at a suboptimal temperature of 20 °C have been shown to decrease sensitivity to monochloramine (Berry, D., C. Xi, L. Raskin. 2009. Environ. Sci. Technol. 43, 884–889). In order to better understand why growth conditions affect sensitivity to monochloramine, a comparative transcriptomic approach was used to identify common patterns of differentially-expressed genes under these growth conditions and during monochloramine exposure. This approach revealed a set of differentially-expressed genes shared under multiple conditions (planktonic growth at 20 °C, biofilm growth, and exposure of planktonic cells to monochloramine), with nine genes shared under all three conditions. Functional gene categories enriched in the shared gene sets included: general metabolic inhibition, redox and oxidoreductase response, cell envelope integrity response, control of iron and sulfur transport metabolism and several genes of unknown function. Single gene deletion mutant analyses verified that loss of 15 of the 24 genes up-regulated during monochloramine exposure as well as during other tested conditions increased *E. coli* sensitivity to monochloramine up to two fold. Constitutive expression of down-regulated genes in single gene mutants yielded mixed results, indicating that the expression of some down-regulated genes actually decreases sensitivity to monochloramine. These results contribute to the understanding of the bacterial response to disinfectants by characterizing the overlap between growth condition associated stress responses and monochloramine-associated stress responses. This characterization highlights the bacterial responses responsible for decreased sensitivity to monochloramine under different growth conditions.

1. Introduction

Escherichia coli survival in drinking water supply systems is sometimes observed despite the widespread practice of adding oxidative disinfectants (e.g., free chlorine, chloramines) (Edberg et al., 2000). Free chlorine (hypochlorous acid) is the best studied drinking water disinfectant (Small et al., 2006; Winter et al., 2008), but recent work comparing three

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broad-acting oxidants (peracetic acid, hypochlorous acid and hydrogen peroxide) suggests that the transcriptional response of bacteria is different for different oxidants (Small et al., 2007). Monochloramine is the second most commonly used disinfectant in US drinking water treatment facilities, after free chlorine (Rose et al., 2007). Despite the widespread use of monochloramine, its mode of action is not completely elucidated, although it has been observed that exposure of *E. coli* to levels of monochloramine typically used in drinking water inhibits bacterial transport, respiration, and substrate dehydrogenation, but does not severely damage the cell envelope or nucleic acid functioning (Jacangelo et al., 1991).

It is clear that the sensitivity of bacteria to inactivation with disinfectants is greatly influenced by growth conditions. For example, growth of *E. coli* at suboptimal temperature decreases its sensitivity to chlorine dioxide (at 15 °C) (Berg et al., 1982) and monochloramine (at 20 °C) (Berry et al., 2009). Biofilm growth, which is known to decrease bacterial sensitivity to a number of stress factors as well as antibiotics (Stewart and Costerton, 2001), has also been shown to decrease monochloramine sensitivity of *E. coli* (Berry et al., 2009) and *Pseudomonas aeruginosa* (Tachikawa et al., 2005) compared to planktonic growth. This is particularly relevant since biofilms have been implicated as one of the primary sources of bacteria in drinking water distribution systems (Berry et al., 2006).

It is known that widely varying stress factors can result in a substantial amount of similar differential gene expression, which indicates that non-specific transcriptional responses may be an important physiological phenomenon (López-Maury et al., 2008). The profiling of the transcriptional response of organisms exposed to different stressors has recently been developed as an approach to identify a core set of genes responsible for increased resistance to stresses, termed the "stressome" (Wu et al., 2007). The stressome is the shared commonalities between responses to environmental factors and to disinfectant exposure and it is an indirect mechanism of resistance to disinfection. The present study examines the global gene expression of *E. coli* during exposure to a low dose of monochloramine to determine if such general response systems are expressed during disinfection with monochloramine. Furthermore, this study evaluates the commonalities between the stress responses of planktonic *E. coli* exposed to monochloramine, planktonic *E. coli* cultured at a suboptimal temperature, and *E. coli* grown in biofilms. This was achieved by comparing the transcriptional profiles obtained for growth under these three conditions. Genes that were differentially-expressed during monochloramine exposure and during either one or both of the other growth conditions were further examined for their importance in monochloramine sensitivity and analyzed using functional annotation clustering as well as broad functionality. The goal of this analysis was to identify and characterize a set of genes that may allow cells grown at suboptimal temperatures and in biofilms to exhibit reduced sensitivity to monochloramine in order to understand why certain growth conditions can affect bacterial sensitivity to monochloramine. It is expected that an increased understanding in the underlying response systems will facilitate innovation in new disinfection strategies.

2. Experimental procedures

2.1. Bacterial strains and culture conditions

E. coli K-12 MG 1655 (American Type Culture Collection, ATCC 700926) was used for all microarray experiments and cultured in 1:10 LB medium in chemostat and biofilm annular reactors. The nutrient concentration of the medium was the lowest feasible concentration that yielded sufficient cells for microarray analysis. Chemostat bioreactors (Applikon, Schiedam, The Netherlands) were operated under defined conditions (20 °C or 37 °C, hydraulic residence time of 6.9 h (dilution rate = 0.1 h^{-1}), 200 rpm mixing). Cells were harvested after cell concentrations reached a steady-state (8–10 volume changes) as determined by total cell counts (Improved Neubauer, Hawksley, Lancing, England). Annular bioreactors (37 °C, hydraulic residence time of 6.9 h, 90 rpm) (BioSurfaces Technologies, Bozeman, MT) were used to grow biofilms on removable glass coupons. Biofilms were harvested after 14 days by scraping several coupons with a membrane filter followed by vortexing the filter in phosphate buffered saline (PBS) as described previously (Berry et al., 2009). Each reactor condition was performed in duplicate for downstream microarray analysis. *E. coli* K-12 single gene deletion mutants from the Keio Collection (Baba et al., 2006) were grown in LB medium amended with 25 μg/ml of kanamycin. Strains carrying plasmids with isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible constitutive expression of single genes from of the ASKA library (Kitagawa et al., 2005) were grown in LB medium with 25 μg/ml chloramphenicol, and 0.1 mM IPTG. The mutants and the wild-type strain BW25113 were kindly provided from National Institute of Genetics, Mishima, Japan.

2.2. Monochloramine preparation and exposure for microarray experiments

For each experiment fresh monochloramine was prepared by adding sodium hypochlorite to a well-mixed buffered solution of excess ammonium chloride (Driedger et al., 2001) and quantified using the DPD titrimetric method (Eaton et al., 1995). Planktonic cells cultured in bioreactors at 37 °C were harvested, washed, and re-suspended in PBS (pH 8) to a concentration of 10^9 CFU/mL and exposed to 1.0 mg/L (as Cl_2) monochloramine for 15 min at 20 °C, which previously had been shown to be a sub-lethal condition for *E. coli* (Berry et al., 2009). Sodium thiosulfate (0.12%) was added to neutralize the monochloramine. Control experiments followed the same procedure using PBS in lieu of the monochloramine solution.

2.3. RNA isolation and cDNA synthesis and labeling

Harvested cells were immediately suspended in RNAlater (Ambion, Austin, TX) to stop changes in mRNA profiles, and were subsequently pelleted (12,000 × g, 2 min) and re-suspended in boiling lysis solution (2% SDS, 16 mM EDTA, 200 mM NaCl). RNA was extracted using hot phenol in combination with bead beating, DNA was digested as per manufacturer's suggestions (TurboDNase, Ambion, Austin, TX), and RNA was purified using the RNAeasy kit (Qiagen, Valencia, CA). RNA