Assessing the Effects of Naphthenic Acids Using a Microbial Genome Wide Live Cell Reporter Array System

Xiaowei Zhang[1,2]*, Steve Wiseman[2], Hongxia Yu[1], Honglin Liu[1], John P. Giesy[1,2,3,4], Markus Hecker[2,5]

1 State Key Laboratory of Pollution Control and Resource Reuse & School of the Environment, Nanjing University, Nanjing, China; *2Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada; 3 Dept. Biomedical Veterinary Bioscience, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; 4 Dept. Biology & Chemistry, City University of Hong Kong, Kowloon, Hong Kong, SAR China; 5 ENTRIX Inc. Saskatoon, SK, Canada*

Abstract

Real time gene profiling of time- and concentration-dependent effects of exposure to a commercial naphthenic acid (NA) mixture in live cells for three hours was conducted using a library of 1800 fluorescent transcriptional reporters for *Escherichia coli* growing in 384-well plates. Response patterns obtained after exposure to NAs suggested that the primary cellular responses were up-regulation of the pentose phosphate pathway, processes involved in the molecular function of NADP or NADPH binding, and down-regulation of the ATP-binding cassette (ABC) transporter complex. Transcriptional networks that were significantly modulated by NAs included those that were regulated by transcriptional factors such as *CRP*, *RecA*, and *GadE*. The down-regulation of the SOS response pathway suggested that DNA damage might not be the direct results of NAs within the first three hours of exposure. However, *CRP*-dependent genes modulated by exposure to NAs indicated that the cellular level of cyclic AMP was altered immediately upon exposure of cells to NAs. Furthermore, the linear range of the concentration-response curve of the selected promoter reporters encompassed a range of concentrations between 10 -1000 mg NAs /L, which covers concentrations typically observed in the environment. Thus, this assay system may represent a promising tool for the detection of environmental chemicals such as NAs.

Introduction

Mixtures of NAs, which include cyclopentyl and cyclohexyl carboxylic acids, have been identified as major toxic components in the effluents discharged by the oil sands industry. The present study for the first time demonstrated an application of a high throughput bacterial live cell array in a genome-scale investigation of the toxic mechanisms of environmental chemicals, a commercial NAs technical mixture extracted from crude oil (Sigma).

Methods

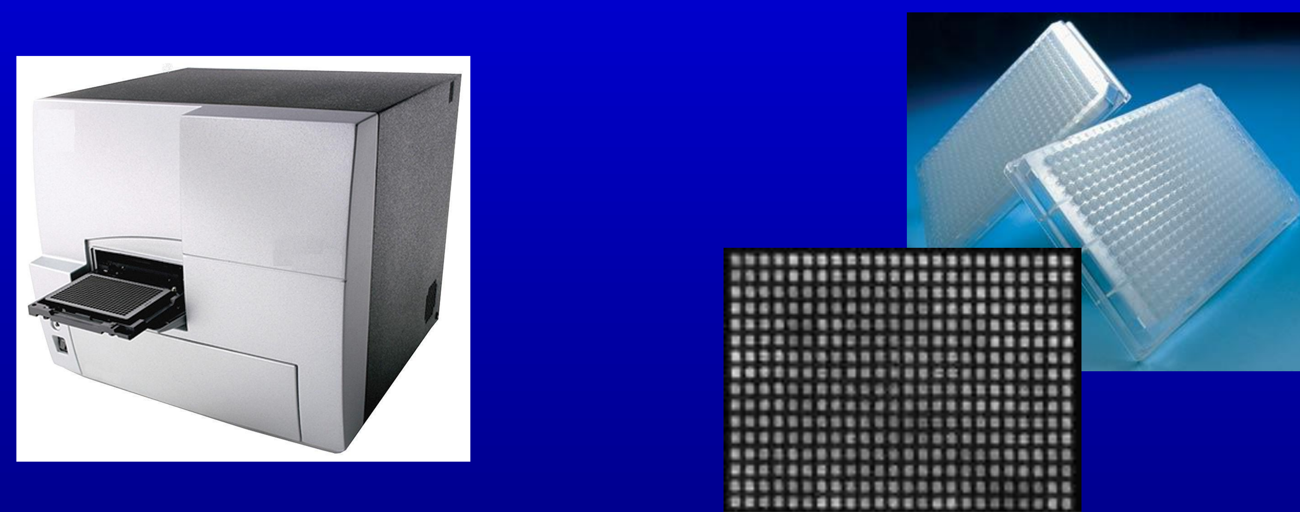


Figure 1. The microbial promoter collection includes more than 1900 promoters for the *E. coli* K12 strain MG1655. Each of the reporter strains has a bright, fast-folding green fluorescent protein (GFP) fused to a full-length copy of an *E. coli* promoter (Zaslaver et al., 2006).

Results

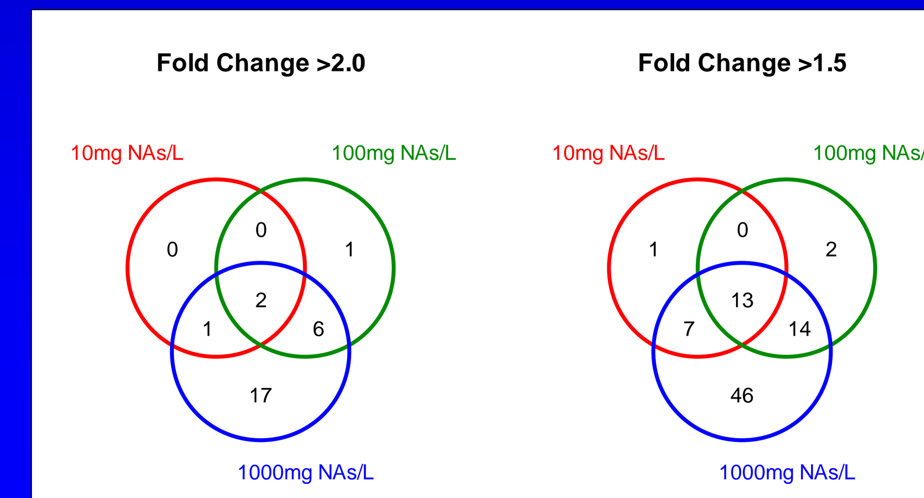


Figure 2 A Venn diagram displaying the differentially expressed genes selected by 1.5 or 2.0 fold change cut-off at three different NAs concentrations, 10, 100, and 1000 mg/L. NAs induced a concentration-dependent response in the number of differentially expressed genes.

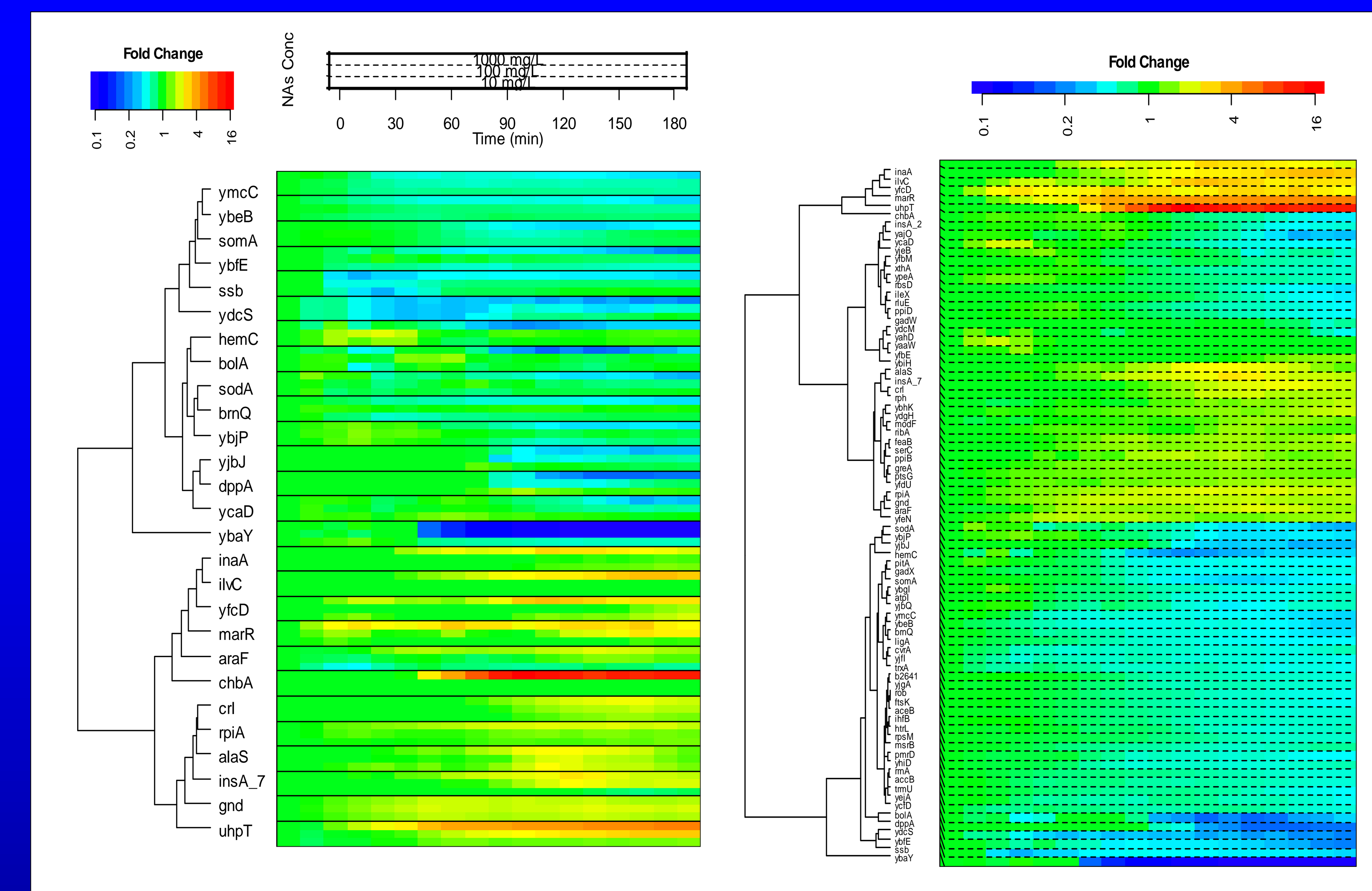
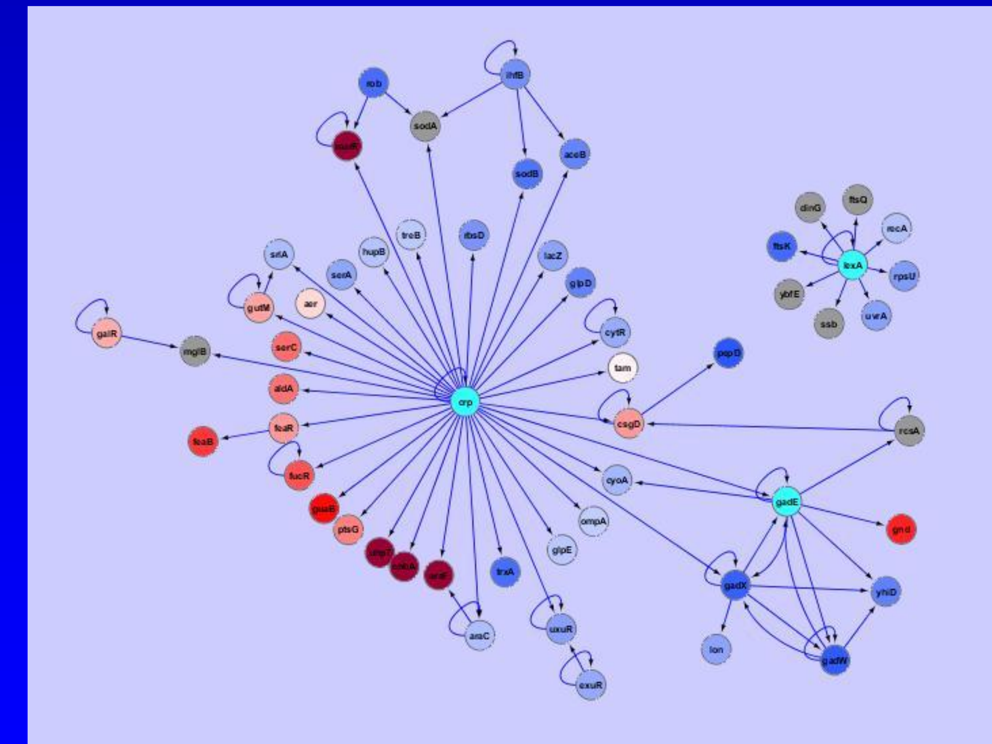


Figure 3. Clustering of genes modulated by NAs. A). Clustering of the concentration- and time-dependent expression of the 27 genes altered at least 2-fold change over background by NAs. B). Clustering of the time-dependent expression of the NAs altered genes selected by1.5-fold change cut-off. Gene expression in cells exposed to 1000 mg NAs/L are displayed. Classification and visualization of the gene expression were derived by use of ToxClust (Zhang et al., 2009).

Figure 4 Active functional modules of a transcriptional network of patters of gene responses in *E coli* exposed to NAs. The level of gene expression in cells exposed to 1000 mg NAs/L is indicated by the color gradient. Brown: >2 fold up regulation; gradient from Red to white: from 2 to1 fold up regulation; gradient from White to blue: from 1 to 2 fold down regulation; Gray: >2 fold down regulation. For the three TFs (crp, lexA and gadE) that displayed no significant change in response to NAs, their roles in the network modules are highlighted by in aquamarine.



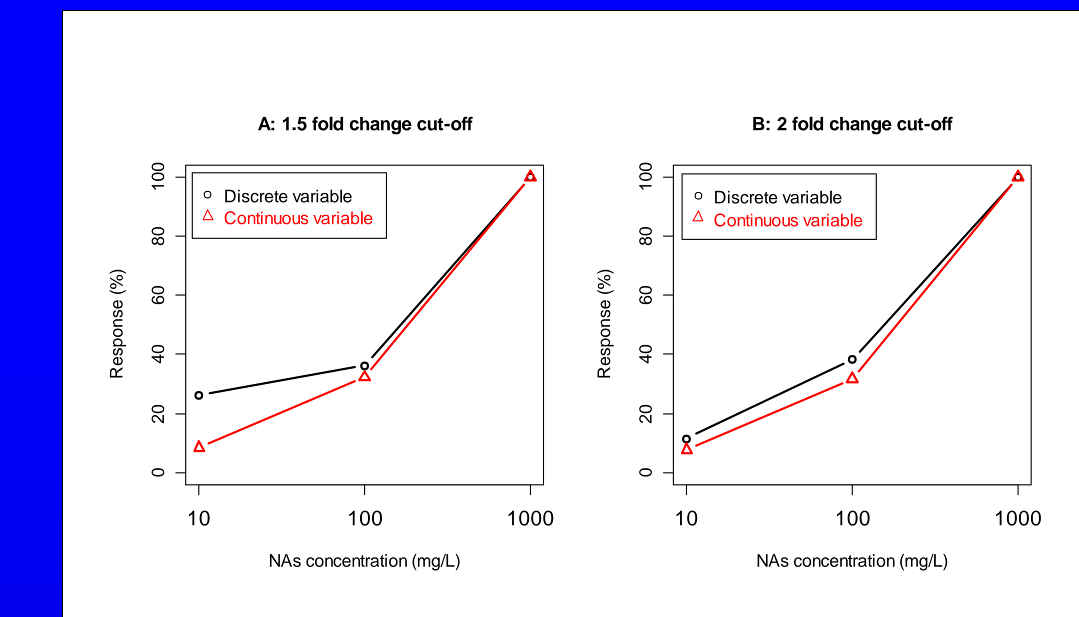


Figure 5. Concentration-dependent transcriptional response to NAs. In the discrete variable approach, the number or the percentage of genes affected was used to describe the degree of chemical-induced effects. In the continuous variable approach, the actual expression level of all the selected genes were integrated to differentiate the degree of effect induced by different concentrations of chemical.

Discussion

1.Biological pathways involved in *NAs effects.*

1) up-regulation of the pentose phosphate pathway, 2) up-regulation of NADP or NADPH binding pathway, 3) down-regulation of the ATP-binding cassette (ABC) transporter complex.

2. Stress responsive pathway affected by NAs exposure.

1) redox-response, 2) SOS-response, 3) osmotic-response

3. Transcriptional networks involved in NAs-induced effects.

Tanscriptional factors: CRP-, RecA-, and GadE

4.Potential biosensors for environmental NAs detection.

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Reference

Zhang et al., *Environ. Sci. Technol.* **2008**, *42* (17), 6762-6769.
Zaslaver et al., *Nat. Methods.* **2006**, *3* (8), 623-628.