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Global Transcriptional Response of *Nitrosomonas europaea* to Chloroform and Chloromethane▽†

Barbara O. Gvakharia,[1] Elizabeth A. Permina,[2] Mikhail S. Gelfand,[3] Peter J. Bottomley,[4] Luis A. Sayavedra-Soto,[1] and Daniel J. Arp[1]*

Department of Botany and Plant Pathology[1] and Department of Microbiology,[4] Oregon State University, Corvallis, Oregon 97331; State Research Institute of Genetics and Selection of Industrial Microorganisms, 1st Dorozhnyj proezd 1, Moscow 113535, Russia[2]; and Kharkevich Institute for Information Transmission Problems RAS, Bolshoi Karetny per. 19, Moscow 127994, Russia[3]

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Upon exposure of *Nitrosomonas europaea* to chloroform (7 μM, 1 h), transcripts for 175 of 2,460 genes were found at higher levels in treated cells than in untreated cells and transcripts for 501 genes were found at lower levels. With chloromethane (3.2 mM, 1 h), transcripts for 67 genes were at higher levels and transcripts for 148 genes were at lower levels. Transcripts for 37 genes were at higher levels following both treatments and included genes for heat shock proteins, σ-factors of the extracytoplasmic function subfamily, and toxin-antitoxin loci. *N. europaea* has higher levels of transcripts for a variety of defense genes when exposed to chloroform or chloromethane.

The ammonia oxidizer *Nitrosomonas europaea* (ATCC 19718) belongs to the β-subdivision of the proteobacteria, derives energy and reductant for growth from the oxidation of ammonia (NH_3) to nitrite (NO_2^-), and can use CO_2 as the sole source of carbon (4). Ammonia-oxidizing bacteria are sensitive to many environmental conditions, including the presence of organic solvents and heavy metals, pH variations, and changes in ammonia concentration (15, 25). Such sensitivity can influence their efficacy in wastewater treatment and bioremediation. The impact of some environmental pollutants, such as chlorinated aliphatic hydrocarbons, on *N. europaea* at the molecular level is of special interest because these compounds can have multiple effects on the cell by serving as cometabolic substrates that drain reductant and can also produce cytotoxic products (15). The cometabolic capability of *N. europaea* provides the basis for its potential to be used in bioremediation.

Rasche et al. (26) grouped 16 chlorinated aliphatic hydrocarbons into three categories based on their impact on ammonia monooxygenase (AMO), the first enzyme in the ammonia oxidation pathway. In the current study, the genome-wide transcriptional responses of *N. europaea* to two chlorinated aliphatic compounds were studied. Chloromethane (CM) is a class 2 compound that is both a substrate for AMO and a noncompetitive inhibitor of ammonia oxidation (16). Although CM drains reductant away from vital cell processes, its oxidation does not lead to irreversible loss of AMO activity (26). Chloroform (CF) is a class 3 compound that is also both a substrate for AMO and inhibitor of ammonia oxidation. However, transformation of CF leads to an irreversible loss of AMO activity (26). Although the responses of ammonia-oxidizing bacteria to chlorinated aliphatic hydrocarbons have been studied at the physiological and biochemical levels (26), little is known about how they respond to these compounds at the transcriptional level.

Conditions for CF and CM treatments. Highly controlled and reproducible conditions were used for production and treatment of cell cultures. *N. europaea* was grown in batch cultures with 25 mM $(NH_4)_2SO_4$ as described previously (33). Cells were harvested by centrifugation at late-exponential phase (optical density at 600 nm [OD_{600}] of ≅0.07), washed, and inoculated into 2 liters of fresh medium with 5 mM $(NH_4)_2SO_4$ to a final OD_{600} of 0.07. The cell suspension was equally divided between two 2.5-liter spinner flasks with side arms (catalog no. 1965-97001; Bellco Biotechnology, Vineland, NJ). The flasks were sealed and shaken (190 rpm at 20°C) for 1 h at which point CF (to 7 μM) was injected as a saturated water solution or CM (to 3.2 mM) was delivered as a gas from an airtight syringe. The concentrations of CF and CM were chosen to decrease AMO activity and NO_2^- production rates to 50% in about 1 h. Cell density, ammonia-dependent O_2 uptake activity, and NO_2^- production rates were monitored at 15-min intervals for 3 h as described previously (15). During this time, no significant cell growth occurred (<0.001 change at OD_{600}), O_2 was not limiting in either treated cells or control cells, and NO_2^- accumulated to 3.5 mM in the control cells.

The rates of ammonia-dependent O_2 uptake in the CF-treated cells decreased throughout the incubation to 50% by 1 h and to 15% by 3 h. In the CM-treated cells, the rates decreased to 50% by 1 h and remained at that level throughout the 3-h incubation. In both treatments, hydroxylamine-dependent O_2 uptake, which requires hydroxylamine oxidoreductase (the second enzyme in the ammonia oxidation pathway) and an intact electron transport pathway, remained unaffected as previously observed (16).

To choose the time point to examine the whole-genome transcriptional stress responses of *N. europaea*, we analyzed changes in protein expression and specific mRNA levels at 1, 2, and 3 h after the addition of CF. Protein expression patterns

* Corresponding author. Mailing address: Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331. Phone: (541) 737-1294. Fax: (541) 737-5310. E-mail: arpd@science.oregonstate.edu.

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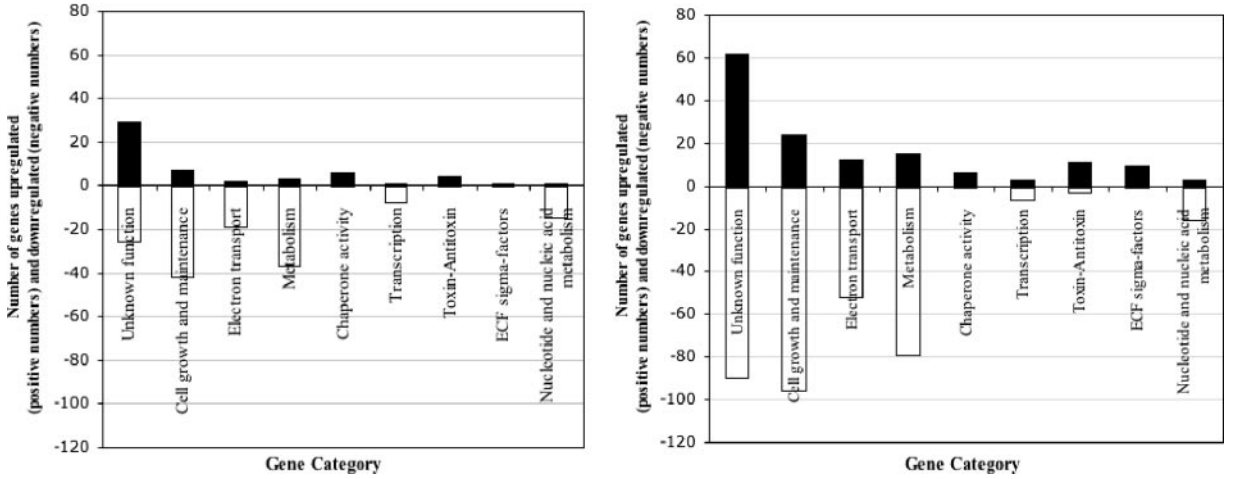

FIG. 1. Selected gene categories of *N. europaea* that showed changes in expression levels when exposed to CM (left) or CF (right). Genes upregulated (black bars) and downregulated (white bars) are those that passed the filtering criteria of 2 or greater change in expression level (treatment/control ratio) and had a P value of <0.05. P values for changes in expression were assessed by Student's t test with GeneSpring software.

were analyzed by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and nano/liquid chromatography tandem mass spectrometry. For isoelectric focusing, 13-cm Immobiline DryStrips (GE Healthcare Bio-Sciences Corp.) were used with a nonlinear pH gradient from 3 to 10. Gel slices with upregulated proteins in the treated samples were excised manually and submitted to Midwest Bio Services, LLC (Overland, KS). Upregulated proteins included putative heat shock proteins (GroEL [NE0028] and DnaK [NE1949]) and two members of the nitrite reductase cluster (cytochrome *c* class IC [NE0925] and multicopper oxidase type 1 [AniA; NE0924]) (4). Analysis of the mRNA levels of *dnaK* and *aniA* by quantitative reverse transcription-PCR (qRT-PCR) showed the highest levels after 1 h of exposure to CF (threefold and twofold, respectively, over untreated cells). Based on these observations, cells for microarray analyses were collected after exposure to CF or CM for 1 h.

Microarray data analysis from treatments with CF and CM. All annotated genes (2,460 total) in the *N. europaea* genome (4) were represented on the high-density Affymetrix GeneChip. Microarray analyses were performed in triplicate, using RNA extracted from three independent experiments (untreated control cells and CF- or CM-treated cells). Data were analyzed using the "log of ratio" mode with GeneSpring software package (Agilent Technologies, Inc.). The results obtained from triplicate experiments were normalized and filtered to identify genes with statistically significant increases (R_i) or decreases (R_d) in the ratios of the transcription levels for CF- or CM-exposed cells (treatment/control ratios of more than twofold). RNA integrity screening, probe synthesis, hybridization, and scanning were conducted by the Center for Genome Research and Biocomputing Core Laboratories at Oregon State University. The microarray data for this study is available at the Gene Expression Omnibus database (http://www.ncbi.nih.gov/geo) under accession numbers GSE6488 and GSE6594. In all cases, we compared the ratios of transcript levels for treated cells to the levels for untreated cells. Therefore, changes in ratios could reflect changes in transcript levels of treated cells, untreated cells, or both. As expected, the percentages of transcripts detected were similar between untreated control cells in the CF experiment (87.3%) and in the CM experiment (84.3%). The CF treatment led to a significant decrease of the percentage of detected transcripts (69.6%), whereas by the CM treatment, no statistically significant change was detected in the percentage of detected transcripts (87.3%).

Many transcripts were found at lower levels in cells treated with CF and CM (see Tables S1 and S2 in the supplemental material). In CF-treated cells, 501 genes were significantly downregulated (R_d of <2). In the CM-treated cells, 148 genes were downregulated. In both treatments, these genes encode proteins involved in a wide range of cellular functions, including cell growth, electron transport, general metabolism, translation, transcription, and nucleic acid metabolism (Fig. 1). Furthermore, several genes encoding ribosomal proteins were downregulated. It is not uncommon for prokaryotes to downregulate proteins of ribosomal biosynthesis under stressful conditions (27).

Analysis of the response of *N. europaea* to CF treatment. Many prokaryotes activate various defense response genes to survive unfavorable conditions (20, 22). Upon CF treatment, *N. europaea* upregulated 175 genes and downregulated 501 genes (see Tables S1 and S3 in the supplemental material). Activated genes included *dnaK* and a variety of genes encoding heat shock proteins, chaperonins, peptidyl-prolyl isomerases (PPIs), and proteases (Table 1). GroEL (NE0028) was shown to be a general stress response protein, which was rapidly produced in activated sludge cultures exposed to environmental stress (8). In *Escherichia coli* this chaperonin with its cofactor GroES promotes protein folding and can interact with as many as 250 different proteins (17).

Prokaryotes and eukaryotes have highly conserved families of PPIs, which catalyze *trans*/*cis* isomerization of the peptide bond immediately preceding proline and can be a rate-limiting step in protein folding (9). In our study, transcripts for two putative PPIs (NE0882 and NE2206) were found at higher levels in CF-treated cells than in control cells and may be required to repair improperly folded proteins. In *E. coli* these isomerases belong to the σ^E regulon, an alternative σ-factor

TABLE 1. Genes in shock response/molecular chaperones/protein modification categories upregulated as a result of CF treatment in *N. europaea*

Locus tag	Description	Gene	Fold change in R_i	*P* value[a]
NE1950	GrpE protein, molecular chaperone	*grpE*	2.10	2.2E−03
NE0528	HflB; ATP-dependent zinc metallopeptidase (cell division FtsH) transmembrane protein	*hflB*	2.11	3.8E−02
NE2206	PpiC-type peptidyl-prolyl *cis-trans* isomerase	*ppiD*	2.26	4.0E−03
NE0882	PpiC-type peptidyl-prolyl *cis-trans* isomerase	*surA*	2.80	6.8E−03
NE1762	Probable HptG; chaperone (heat shock protein HtpG)	*hptG*	2.94	6.8E−03
NE1949	Heat shock protein Hsp70, molecular chaperone	*dnaK*	3.84	4.5E−04
NE2402	ClpB ATPase-dependent protease, chaperonin	*clpB*	4.01	1.3E−03
NE0028	TCP-1 (Tailless complex polypeptide) Cpn60 chaperonin family	*groEL*	4.73	7.5E−03
NE1948	DnaJ molecular chaperone	*dnaJ*	5.51	2.8E−05
NE0027	Chaperonin Cpn10 (10-kDa subunit)	*groES*	8.45	9.0E−04

[a] *P* values for changes in expression were assessed by Student's *t* test with GeneSpring software.

that is induced in response to environmental stress (29) and redirects RNA polymerases to specific promoters.

Proteolysis is another stress defense mechanism that is used by bacteria (14). Transcripts for two putative ATP-dependent proteases, *lonA* (NE1278) and *clpB* (NE2402), were present at higher levels in CF-treated cells than in nontreated cells. Lon can degrade misfolded proteins (13) or specifically degrade RelB antitoxin (5). ClpB and other proteases of this type also mediate ATP-dependent regulation of unfolding or disassembly of protein-protein or protein-DNA complexes (14) or specifically degrade a member of the toxin-antitoxin (TA) protein family, namely, MazE (1).

Upregulation of toxin-antitoxin genes in *N. europaea*. Experimental evidence from a variety of well-studied microorganisms suggests that TA loci are stress response elements that help cells to survive unfavorable conditions by triggering reversible bacteriostatic effects (6). TA loci are grouped in eight families and form operons with the antitoxin gene encoded upstream of toxin, except in the *hig* family where the order is reversed. *N. europaea* has a high number of TA pairs (43 total) belonging to seven known TA families (12).

Transcripts from 11 genes representing four different TA families were found at higher levels in CF-treated cells than in untreated cells (Table 2). Both members of each operon were upregulated with the exception of *higBA-5*. The *parDE-1* and *parDE-2* gene families affect replication by inhibiting DNA gyrase in *E. coli* (12). The *vapBC* gene family members contain a PIN domain and are predicted to be exonucleases involved in nonsense-mediated mRNA decay (6). The *mazEF-2* and *mazEF-3* gene families inhibit translation during unfavorable growth conditions (12). Toxins of MazEF families are identified as sequence-specific RNases that cleave mRNAs stalled on ribosomes, thus releasing ribosomes trapped on truncated mRNAs (23).

The TA-mediated stress response is triggered by bacterial alarmones tetra- and pentaphosphates (p_5G or p_4G), which are synthesized by ppGpp synthase (RelA, or SpoT). This ppGpp synthase is directly activated by uncharged tRNAs during amino acid starvation (12). Transcripts of *spoT* (NE0368) were detected in this study, but their levels did not change upon treatment with CF and CM. Lon and Clp proteases can be directly activated by bacterial alarmones, and they release toxins from the complexes with antitoxins. This release leads to triggering of the TA-induced stress response, which reduces translation rates, causes degradation of ribosomal proteins, and leads to amino acid recycling (12). TA loci may repress their own transcription under normal conditions (12). However, transcripts for 84 of 86 genes annotated as TA genes in the *N. europaea* genome were detected in control samples, albeit at different levels. Similarly, expression of all 22 pairs of *vapBC* TA loci was detected in *Sulfolobus solfataricus* under normal growth conditions (30). Interestingly, we found three genes of the TA family downregulated in CF-treated cells (*relBE-1* [NE0474 and NE0475] and *relBE-7* [NE1563]). The TA system may provide a mechanism for *N. europaea* to constantly monitor environmental conditions for a quick adaptive response to sudden changes in the environment. Toxin efficiency may vary from one TA pair to another, thereby allowing cells to modulate their metabolic activity by activating different TA loci in response to changing conditions.

Expression of σ-70 factors as a result of CF treatment. CF treatment caused upregulation of 9 of the 29 genes annotated as σ-70 in the *N. europaea* genome (Table 3). Eight of them belong to a subfamily of 23 extracytoplasmic function (ECF) σ-factors, and one gene (NE0584) is annotated as a heat shock response factor *rpoH*. In bacteria, redirection of RNA polymerases from synthesis of stable RNA (e.g., rRNA and

TABLE 2. Putative genes of TA family upregulated in CF-treated *N. europaea*

Locus tag	Description	TA loci	Fold change in R_i	*P* value[a]
NE1305	Proteic killer suppression protein	*higBA-5*	2.25	2.4E−02
NE0261	ParE	*parDE-1*	5.46	6.5E−03
NE0262	Hypothetical protein		5.01	4.5E−03
NE0551	Putative YacA	*parDE-2*	4.03	7.7E−03
NE0552	Hypothetical protein		2.85	1.6E−03
NE1349[b]	Hypothetical protein	*vapBC-8*	3.12	1.2E−02
NE1350	Hypothetical protein		2.46	8.3E−03
NE0974	PemK-like protein	*mazEF-2*	2.19	1.9E−02
NE0975	Hypothetical protein		2.30	8.8E−03
NE1181	PemK-like protein	*mazEF-3*	2.59	1.5E−02
NE1182	Helix-turn-helix protein, CopG		2.13	3.3E−02

[a] *P* values for changes in expression were assessed by Student's *t* test with GeneSpring software.

[b] NE1349 encodes a predicted nucleic acid binding protein and contains a PIN domain, COG5611.

TABLE 3. Upregulated σ-factors in CF-treated *N. europaea* cells

Locus tag	Description	Fold change in R_i	*P* value[a]
NE1096	σ-Factor, ECF subfamily	2.48	2.1E−02
NE1617	σ-Factor, ECF subfamily	4.73	1.6E−02
NE0128	σ-Factor, ECF subfamily	2.01	1.1E−02
NE1079	σ-Factor, ECF subfamily	3.18	1.0E−02
NE1217	σ-Factor, ECF subfamily	2.22	9.1E−03
NE0547	σ-Factor, ECF subfamily	2.18	7.1E−03
NE0584	σ-70 factor family RpoH	5.30	3.1E−03
NE1071	σ-Factor, ECF subfamily	5.46	6.4E−04
NE2435	Probably *fecI*	3.99	5.2E−04

[a] *P* values for changes in expression were assessed by Student's *t* test with GeneSpring software.

tRNAs) towards genes required for starvation survival is considered to be a part of the stringent stress response mediated by alternative σ-factors and bacterial alarmones (18). Often the ECF σ-factors control the expression of genes whose products function outside of the cytoplasm (29); however, the expression of these genes can also be an essential part of the stress-fighting strategy within cells (2).

Computational analysis of possible regulons of σ-factors. To identify possible regulons controlled by σ-factors in *N. europaea*, we analyzed its genome based on known regulons for σ-factors in other bacteria. The recognition profiles were constructed using the samples of experimentally verified sites taken from EcoCyc (http://ecocyc.org/) (28). The positional nucleotide weights (W) in profiles were defined as follows:

$$W(b,k) = \log[N(b,k) + 0.5] - 0.25 \sum_{i = \mathrm{A, C, G, T}} \log[N(i,k) + 0.5]$$

where $N(b,k)$ is the count of nucleotide b at position k. The score of a candidate site was calculated as the sum of the respective positional nucleotide weights:

$$Z(b_1 \ldots b_k) = \sum_{\kappa = 1 \ldots \kappa} W(b_k,k)$$

where k is the length of the site. The base of the logarithm was chosen such that Z had a Gaussian distribution with a standard deviation of 1 on random Bernoulli sequences and thus had the meaning of a standard z-score (11). Genomic analyses (genomic scale similarity searches and site searches using profiles) were made using GenomeExplorer (19) and ClustalX 1.5 (31) with default parameters used to do multiple alignment.

We looked specifically for sites that were similar to *E. coli* RpoH and RpoE binding motifs because these transcriptional regulators play a role in a regulatory cascade that responds to envelope stress and heat shock in *E. coli* (28). *N. europaea* has homologs of RpoE (*rpoE1*, NE2331) and RpoH (*rpoH*, NE0584) with sufficiently good similarity to assume that they may play the same role and bind similar signal sequences as their homologs in *E. coli*.

Analysis of upstream regulatory regions of genes upregulated in *N. europaea* by either treatment revealed a possible RpoE1 σ-factor regulon, which includes *rpoH* (Table 4). Regulation of the *rpoH* gene by RpoE is common in proteobacteria that have RpoE orthologs (21). We also found potential binding sites for RpoH upstream of nine genes that were induced by either treatment (Table 5). This potential regulon is typical of proteobacteria, as it contains members of a conserved core of RpoH regulon, such as heat shock proteins and the ClpB protease (24). Thus, RpoH1 likely regulates the expression of heat shock proteins in response to stress in *N. europaea*. The expression of *rpoE1* itself was not affected by either treatment; however, the activation of ECF σ-factors may be the result of ClpB protease activity, which modulates activity of stress response σ-factors by releasing σ-factors from the complexes with anti-σ-factors (34). Thus, upregulation of other genes coding for ECF σ-factors in CF treatment was consistent with upregulation of ClpB protease.

TABLE 4. *N. europaea* genes that belong to RpoE regulon and that were upregulated by either treatment

Locus tag	Treatment	Gene	Site position	Score[a]	Sequence[b]
NE2459	CF	*birA*	−180	5.67	GAACaT-(16)-TCaaA
NE2230	CF, CM		−165	6.07	GAACca-(16)-TCTGA
NE2100	CF, CM		−185	5.95	cAACTT-(16)-cCTGA
NE2092	CF		−114	5.83	GAACTT-(16)-gCgGA
NE1832	CF	*slp*	−71	5.67	GAACaT-(16)-TCcaA
NE1541	CF		−116	6.14	GAACcT-(16)-TCTcA
NE1305	CF	*higA*	−133	6.07	GAAaTg-(16)-TCTGA
NE0922	CF	*cphA*	−152	6.07	aAcCTT-(16)-TCTGA
NE0767	CF, CM	*ccmE*	−56	7.97	GAACTT-(16)-TCTGA
NE0744	CF, CM		−112	5.67	cAACTT-(16)-TaTaA
NE0584	CF, CM	*rpoH*	−75	5.67	GAACTa-(16)-TCTat
NE0512	CF, CM		−155	5.95	GAACaT-(16)-TCaGA

[a] The score of a candidate site was calculated by the equation in the text in "Computational analysis of possible regulons of σ-factors."
[b] A lowercase nucleotide indicates a deviation from the *E. coli* RpoE consensus binding site GAACTT-(16)-TCTGA [(16) means any 16 nucleotides].

Common genes upregulated by CM and CF treatments. CM treatment of *N. europaea* resulted in upregulation of 67 genes (see Table S4 in the supplemental material). Of these, 37 genes, including genes encoding ATP-dependent proteases and two families of TA genes, were also upregulated by CF treatment (see Table S5 in the supplemental material). Only one σ-70 factor, *rpoH* (NE0584), was upregulated by both treatments. Other ECF σ-factors upregulated by the CF treatment (Table 3 and Fig. 1) were not upregulated by CM treatment, suggesting that CM caused less-severe stress.

By both treatments, gene NE2571, which is annotated as a member of metallo-β-lactamase superfamily (NE2571), showed the highest ratio relative to the control cells. Transcripts for NE2570 and NE2569, which are annotated as encoding hypothetical transmembrane proteins, were also much higher in cells treated with CF and CM than in control cells (see Table S5 in the supplemental material). These genes are located upstream of NE2571 and are transcribed in the opposite direction. Possibly, a strong bidirectional promoter is located between NE2571 and genes NE2570-NE2569. Another promoter region with similar bidirectional properties was previously described in *N. europaea* (32). NE2571 showed a 9.7-fold increase (R_i) when the cells were treated with CF and a 114.5-fold increase (R_i) when the cells were treated with CM. The metallo-β-lactamase superfamily is a diverse protein family with a variety of biological functions (7). There are several genes in the *N. europaea* genome annotated as members of this family. Gene NE2571 shows similarity to genes encoding class

TABLE 5. *N. europaea* genes that belong to RpoH regulon and that were upregulated by either treatment

Locus tag	Treatment	Gene	Site position	Score[a]	Sequence[b]
NE2417	CF		19	6.23	gTTGAAA-(11)-CCtgAT
NE2402	CF, CM	*clpB*	−67	6.54	CTTGAAA-(11)-CatCAT
NE2074	CF	*hsp*	−179	6.08	CTTGAAA-(13)-gCttAT
NE1950	CF, CM	*grpE*	−53	6.99	gTTGAAA-(13)-CCCaAT
NE1949	CF, CM	*dnaK*	−65	7.39	CTTGAgA-(13)-CCCCAT
NE1762	CF, CM	*htpG*	−55	6.61	CTTGAAA-(16)-CaCaAT
NE0815	CF		−42	6.16	gTTGAAA-(16)-CCtCgT
NE0028	CF, CM	*groESL*	−158	6.43	CTTGAAA-(14)-CtCtATT

[a] The score of a candidate site was calculated by the equation in the text in "Computational analysis of possible regulons of σ-factors."
[b] A lowercase nucleotide indicates a deviation from the *E. coli* RpoH consensus binding site CTTGAAA-(11–16)-CCCCAT where the number in parentheses is the number of any nucleotides.

B β-lactamases. Genes with similar sequences encode enzymes with glyoxalase II activity, which catalyzes hydrolysis of the thioester of *S*-D-lactoylglutathione D-lactic acid (3). This reaction is part of the mechanism to remove toxic methylglyoxal (35), which is produced from dihydroxyacetone phosphate.

qRT-PCR validation of microarray data. To validate the ratios of transcript levels for a subset of genes identified as upregulated in microarray experiments, we performed qRT-PCR analysis and compared the results with microarray data (Fig. 2). cDNA was synthesized with IScript cDNA synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA) using RNA from treated and untreated cells. qRT-PCR assays were set up with IQ SYBR green supermix (Bio-Rad) and performed on an ICycler instrument (Bio-Rad) according to the manufacturer's recommendations. The nucleotide sequences of the primers used for RT-PCR are given in the supplemental material. The ratios of transcript levels derived from either method followed the same trend (Fig. 2).

Interestingly, the ratios of the transcript levels (R_i) for metallo-β-lactamase (NE2571) when analyzed by qRT-PCR were 14 in CF-treated cells and ~2,000 in CM-treated cells. The ratios (R_i) estimated by microarray analysis were 9.7 and 114, respectively. The dramatic change of expression of this gene suggests that under normal conditions the rate of transcription is low (if any), but it is strongly induced by the stress caused by CF or CM. The large difference in ratios obtained by qRT-PCR and by microarrays probably reflects the difficulty in measuring a very low level of transcript in the untreated cells.

Concluding remarks. The genome-wide analysis of the transcriptional responses of *N. europaea* to CF and CM treatments showed significant changes in gene transcription profiles. Although CF caused more transcript levels to be increased or decreased compared to CM, there were common trends caused by both treatments. Overall, both treatments downregulated genes with essential biosynthetic and cell growth functions, such as translation, transcription, electron transport, general metabolism, nucleotide and nucleic acid metabolism, and cell growth and maintenance. Genes encoding biosynthetic and cell growth functions were also downregulated when *N. europaea* cells were deprived of ammonium and carbonate (33). However, under these conditions, more than 90% of the genes were downregulated and only 10 genes were upregulated (33).

Transcripts for genes that are considered components of the stringent response (10) were found at higher levels in cells treated with CF and CM and included heat shock proteins, chaperonins, Lon and Clp proteases, ECF σ-factors, and TA genes (12). The TA genes are now viewed as an essential part of stress defense and survival mechanisms in prokaryotes, and the expression of TA genes is typically observed during stationary phase, starvation, or oxidative stress (6). The TA genes

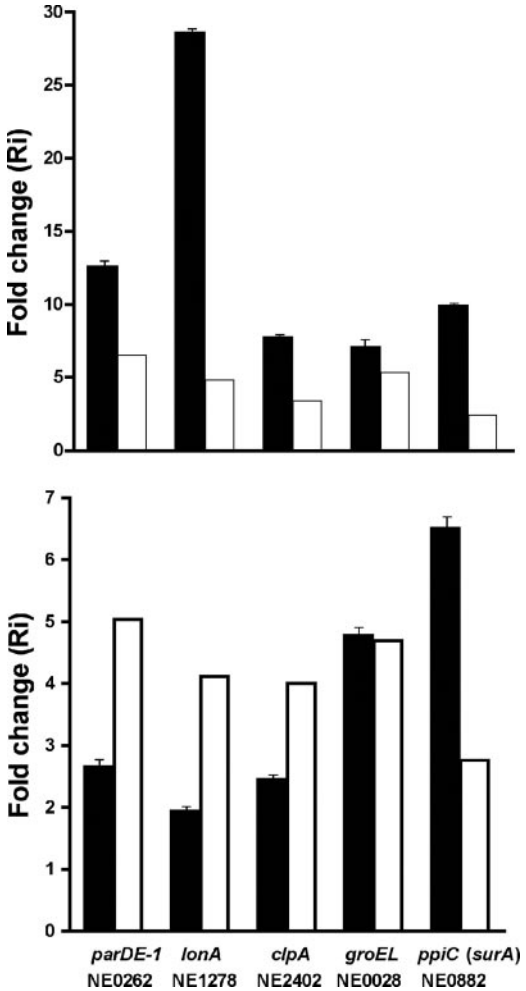

FIG. 2. Comparison of the expression of selected genes by qRT-PCR (black bars) and microarray (white bars) methods. (Top) CM treatment; (bottom) CF treatment. Error bars represent standard errors of the means.

were annotated recently in the *N. europaea* genome by computational analysis (23), and their induction in this study is the first experimental evidence of a response of TA genes to a stress in *N. europaea*. The stringent response in *N. europaea* might be initiated by SpoT causing activation of TA loci, redirecting RNA polymerases, reducing transcription/translation rates, and setting a new metabolic status that can increase stress tolerance. In this respect, *N. europaea* when exposed to chlorinated aliphatic hydrocarbons may behave similarly to other microorganisms under stress, where general repression of transcription, metabolism, cell growth, and nucleic acid metabolism is mediated by the TA system. This is an intriguing hypothesis that we would like to test in future research.

Microarray accession number. The microarray data for this study are available at the Gene Expression Omnibus database (http://www.ncbi.nih.gov/geo) under accession numbers GSE6488 and GSE6594.

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