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Full Length Research Paper

σ^{32} regulation of the *algA* and *pnrB* expression in *Pseudomonas* sp. HK-6 which degrades the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

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***Pseudomonas* sp. HK-6 cells can utilize hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) as a carbon and nitrogen source. HK-6 cells grown in media containing RDX express several genes encoding stress shock proteins (SSPs) and enzymes that function in RDX degradation. The *rpoH* gene (σ^{32}, a stress response sigma factor), *alg* operon (clustered genes for alginate synthesis) and *pnrB* gene (RDX nitroreductase) are included among these expressed genes. To examine whether the transcription of the *algA* and *pnrB* genes are controlled by σ^{32}, their mRNA levels in *rpoH* knock-out cells grown under stress conditions were measured by quantitative real-time polymerase chain reaction (RT-qPCR) and compared with the levels in wild-type HK-6 cells. Expression of *algA* mRNA was approximately 4-7-fold lower in the *rpoH* knock-out cells than in the wild-type cells. Transcription levels of the *pnrB* gene were approximately 3-fold lower in the *rpoH* mutant. These results indicate that σ^{32} production by various environmental stressors, including RDX, is required for the induction of genes encoding SSPs and enzymes for RDX degradation.**

Key words: *Pseudomonas* sp. HK-6, *rpoH, pnrB, alg* operon.

INTRODUCTION

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a nitroaromatic explosive that comprises a great proportion of military waste, giving rise to serious environmental problems with 2,4,6-trinitrotoluene (TNT). RDX, TNT, and their metabolites cause toxic environmental effects, including deleterious effects on certain microorganisms. Public health has also been threatened as a consequence of this type of contamination (Rosenblatt et al., 1991; Hawari et al., 2000). However, several bacteria, including *Pseudomonas* spp. and *Stenotrophomonas* spp., have been reported to metabolize RDX and TNT and use them as a source of nitrogen and carbon (Binks et al., 1995; French et al., 1998; Lee et al., 2002; Chang et al., 2004).

Pseudomonas sp. HK-6 is capable of using RDX as well as TNT as its sole nitrogen and carbon source. HK-6 cells grown in media containing TNT express several stress shock proteins (SSPs) including DnaK and GroEL (Ho et al., 2004). In addition to SSPs, *Pseudomonas* sp. HK-6 cells exposed to TNT also strongly express the *alg* operon. The *alg* operon is a cluster of genes encoding enzymes that catalyze the synthesis of alginate, an exopolysaccharide copolymer composed of *O*-acetylated β-1-4-linked D-mannuronic acid and its C-5 epimer, α-L-guluronic acid (Lee et al., 2008a). The *pnrB* gene is known to be an essential component of the TNT biodegradation pathway in *Pseudomonas* sp. HK-6 (Lee et al., 2008b). The *pnrB* gene encodes a 24 kDa oxygen-insensitive nitroreductase that catalyzes the reduction of nitro groups on aromatic compounds including RDX (Cabrello et al., 2005; Lee et al., 2008b).

Proteins expressed in *Pseudomonas* sp. HK-6 under TNT stress conditions also include σ^{32}, a stress-response sigma factor encoded by the *rpoH* gene (Lee et al., 2008a). In *Pseudomonas*, σ^{32} has been known to

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Table 1. PCR primers used in this study.

Primer	Sequence	Source or reference
dnaK-F	5'- TTC GGT CAT CGA AAT CGC CGA AGT -3'	RT-qPCR/ This study
dnaK-R	5'- TGC CCG ACT CTT TCT TGA ACT CGT -3'	
groEL-F	5'- ATC CGT GCC CAG ATC GAA GAA ACT -3'	
groEL-R	5'- CAA CGC GGG CTT TCT TCT CTT TCA -3'	
algA-F	5'-ACC ACA AGT TCA TCG TCC AGG AG-3'	
algA-R	5'-AGG CAG CAC CAG CAT CAG C-3'	
pnrB-F	5'-CGAGATGACTGAAGAACACCTGAAC-3'	
pnrB-R	5'-GTAGTGACGGCGGCTCTGG-3'	
16S rRNA-F	5'-AAGGAACACCAGTGGCGAAGG-3'	
16S rRNA-R	5'-CCAGGCGGTCAACTTAATGCG-3'	

participate in XylS-dependent transcription of the Pm promoter (Marqués et al., 1999). Molecular characterization of *rpoH* regulation and the structural analysis of its cognate promoter P2 in *Ps. putida* KT2440 were also reported (Manzanera et al., 2001).

In this report, we investigated the involvement of *rpoH* in the regulation of the stress-associated genes *dnaK* and *groEL,* the *alg* operon and *pnrB* gene transcription in HK-6 under stress. To accomplish this, an HK-6 *rpoH* knock-out mutant was constructed. The expression levels of stress-associated genes were detected using western blotting and quantitative real-time polymerase chain reaction (RT-qPCR). Subsequently, the mRNA levels of *algA* and *pnrB* in the wild-type and *rpoH* genetic backgrounds were measured by RT-qPCR. In addition, rates of RDX degradation by the *rpoH* mutant were compared to those of the wild-type strain.

MATERIALS AND METHODS

Bacterial strain and cultural conditions

Pseudomonas sp. HK-6 was collected from RDX-contaminated soils. Cells were grown in LB or liquid basal salt media composed of the desired RDX concentration (0.01 to 0.1 mM), 10 mM K_2HPO_4, 5 mM NaH_2PO_4, 1 mM $MgSO_4.H_2O$, 0.07 mM $CaCl_2.2H_2O$, 0.04 mM $FeCl_3.6H_2O$, 0.0005 mM $MnCl_2.4H_2O$, and 0.00035 mM $ZnSO_4.7H_2O$, along with several trace metals and 2 mM fructose as a supplemental carbon source under aerobic conditions. The cultivation and maintenance conditions of *Pseudomonas* strains have been described in detail previously (Chang et al., 2004).

Construction of the *rpoH* knock-out mutant

To construct the *rpoH* knock-out mutant, a 540 bp internal *rpoH* DNA fragment was amplified with two primers complementary to the internal sequences of the gene (5′-ATGAATTCAGCGCTTCGTGCAGGTTGC-3′ and 5′-ATCCAAGCTTATTATGAGCAGGATGTCGAGG-3′), digested with *Eco*RI and *Hin*dIII (sites underlined) and then inserted into a pBGS18 vector (a kanamycin-resistant vector analogous to the plasmid pUC18, generously provided by B. G. Spratt, Imperial College of London).

Stress treatment with RDX

The HK-6 wild-type cells and *rpoH* mutant cells were grown in LB and harvested by centrifugation at 2000 *g* for 10 min. These cells were washed 3 times with 10 mM phosphate buffer (pH 7.0) and then incubated at approximately 10^8 cells/ml in 50 ml basal salts media in 250 ml Erlenmeyer flasks containing 0.1 mM RDX.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis

RDX-treated cells were analyzed for the expression of SSPs using western blotting with anti-DnaK and anti-GroEL monoclonal antibodies (StressGen Biotechnologies Corp., Canada). Isolation of the SSPs from the HK-6 wild-type and mutant strains was performed using 12% acrylamide for the separating gel and 5% acrylamide for the stacking gel according to the method described by Bollag et al. (1992). The gels were transferred to Hybond^{TM}-polyvinylidene fluoride (PVDF) membrane (GE Healthcare, USA) with the Bio-Rad tank blot system. The blots were incubated overnight with blocking solution (5% skim milk) at 4°C and then for 1.5 h with primary antibody (diluted 1:5000 in PBS-0.08% Tween 20). Subsequently, the blots were washed three times with phosphate-buffered saline (PBS)-0.08% Tween 20, incubated with a 1:5000 dilution of horseradish peroxidase (HRP)-conjugated secondary antibody (StressGen Biotechnologies Corp., Canada) for 1.5 h at room temperature, rewashed three times with PBS-0.08% Tween 20, and developed using an ECL detection kit (GE Healthcare, USA).

RNA isolation and real-time qPCR analysis

To determine whether stress-associated genes (*dnaK* and *groEL*), *algA* and *pnrB* expression is regulated by σ^{32}, stress-associated genes, *algA* and *pnrB* mRNA levels in wild-type and *rpoH* mutant HK-6 cells were measured by RT-qPCR. Total RNA was extracted from the wild-type and *rpoH* mutant cells with an RNA extraction kit (Macherey-Nagel Inc., Düren, Germany) according to the manufacturer's instructions. Total RNA was treated with RNase-free DNase and quantified at 260 nm with a Tecan Multi-Reader spectrometer (Männedorf, Switzerland).

For RT-qPCR analysis, *dnaK*-F/*dnaK*-R, *groEL*-F/*groEL*-R, *algA*-F/*algA*-R, *pnrB*-F/*pnrB*-R and internal control primers 16S rRNA-F/16S rRNA-R primers were designed (Table 1). RT-qPCR analysis

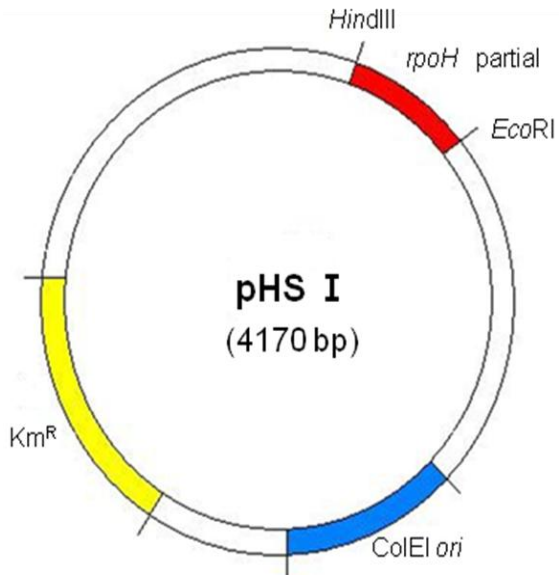

Figure 1. Plasmid map of pHSI used in the construction of the *Pseudomonas* sp. HK-6 *rpoH* mutant. An approximately 540-bp internal DNA fragment of the *rpoH* gene of HK-6 was inserted into the pBGS18 plasmid. Because pHSI does not have a *Pseudomonas* replication origin, transformants were able to attain kanamycin resistance only through the integration of this construct into the original *rpoH* locus, generating two nonfunctional partial *rpoH* gene fragments.

was performed using an iScript™ One-Step RT-qPCR kit with SYBR Green (Bio-Rad, Hercules, CA, USA) in a Bio-Rad CFX 96 RT-PCR System. Each 50 µl of reaction mixture contained 25 µl of 2×SYBR Green RT-qPCR reaction mix, 1.5 µl of gene-specific forward and reverse primers, 1 µl of iScript® Reverse Transcriptase, and 100 ng of total RNA template. The cDNA synthesis was performed for 10 min at 50°C. Thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 45 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 30 s. RT-qPCR samples were run in triplicate, and the data were analyzed using the Bio-Rad CFX Manager software.

Analytical methodology

Residual RDX was analyzed using high performance liquid chromatography (HPLC). The HPLC system consisted of a pump (Shimazu LC-10A, Kyoto, Japan), an injector fitted with a 20 µm loop, a UV detector, and an integrator. Analytes were detected using a commercial Zorbax ODS reverse column (C_{18}, 250 mm × 4.6 mm, particle size 5 µm) and a mobile phase of a mixture of 40% (v/v) acetonitrile and 80% water at a flow rate of 1.0 ml/min. The procedures for sample preparation and the analytical methodology for RDX have been previously described in detail (Chang et al., 2004).

RESULTS AND DISCUSSION

Strain HK-6 was isolated from the RDX-degrading enriched culture because of its ability to degrade RDX under aerobic conditions. Physiological and biochemical characterization of strain HK-6 has been previously described in detail (Chang et al., 2004). Based on the results of the Biolog system and 16S rRNA sequence analysis, the isolate could be assigned and designated as *Pseudomonas* sp. HK-6 and was registered in GenBank as [DQ163021].

To investigate the role of *rpoH* in the expression of stress-associated genes (*dnaK* and *groEL*), the *alg* operon and *pnrB* under RDX stress conditions, an *rpoH* mutant was constructed (Figure 1). To accomplish this, a partial 540 bp internal *rpoH* gene fragment lacking both initiation and stop codons was amplified from HK-6 genomic DNA and integrated into the original *rpoH* locus by single crossover homologous recombination, resulting in the generation of two partial non-functional *rpoH* genes. To verify proper integration, two primers, one for pBGS18 and another for the *rpoH* gene, were synthesized to amplify an approximately 700 bp-long DNA fragment with a junction of the pBGS18 vector and *rpoH* gene. The PCR results showed that this 700 bp DNA fragment was amplified in the *rpoH* knock-out mutant but not in the wild-type strain, confirming exact integration into the original site (Figure 2).

The HK-6 *rpoH* knock-out mutant, unlike the wild-type

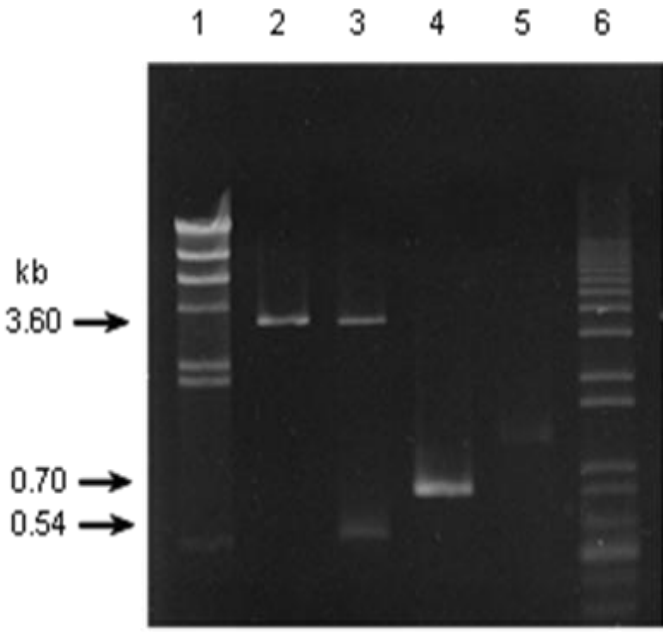

Figure 2. Agarose gel electrophoresis verifying the construction of pHSI and integration of the partial *rpoH* gene into the original locus to generate its knock-out mutation. Lane 1, lambda bacteriophage digested with *Hin*dIII DNA molecular markers; lane 2, pBGS18 (3.6 kb) digested with *Eco*RI and *Hin*dIII; lane 3, pHSI digested with *Eco*RI and *Hin*dIII, showing the approximately 540-bp partial *rpoH* gene; lane 4, the approximately 700 bp PCR product from the *rpoH* knock-out strain; lane 5, a 700 bp DNA band was not amplified from the wild-type strain; lane 6, 1-kb DNA ladder molecular marker.

Table 2. Growth comparisons of *Pseudomonas* sp. HK-6 and the *rpoH* mutant under different stress conditions.

Stress condition		Wild type	Mutant strain
Temperature (°C)	30[a]	+	+
	37	+	+/ -
	40	+	+/ -
RDX (mM)	0.01	+	+/-
	0.03	+	+/-
	0.05	+	+/-
	0.1	+	+/-
TNT (mM)	0.05	+	+/ -
	0.1	+	+/ -
	0.2	+	+/ -

[a], Normal growth temperature (30°C); +, growth; +/-, slow growth.

strain, showed very slow growth at above 37°C, or in media containing 0.01 to 0.1 mM RDX, or 0.05 to 0.2 mM TNT at 30°C (Table 2). Production of the DnaK protein was reduced in the *rpoH* mutant compared with the wild-type strain (Figure 3A). The transcription of the *dnaK* gene, which encodes the DnaK chaperone protein, is induced by its σ^{32}-specific promoter. Many reports have described σ^{32} and its mutations, and it has been

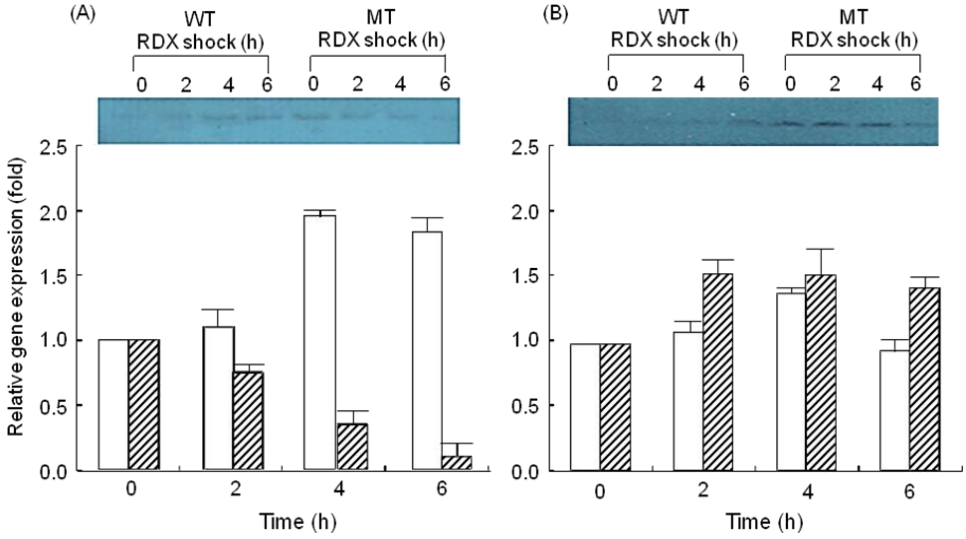

Figure 3. Analysis of *dnaK* and *groEL* gene expression under RDX stress condition. (A) Induction of the DnaK protein in the wild-type strain and *rpoH* mutant treated with 0.1 mM RDX. The DnaK concentrations were analyzed using western blotting with an anti-DnaK monoclonal antibody, and *dnaK* gene expression was analyzed using RT-qPCR of HK-6 cells (□) and *rpoH* mutant cells (▨) treated with 0.1 mM RDX. (B) Induction of the GroEL protein in the wild-type strain and *rpoH* mutant treated with 0.1 mM RDX. The GroEL concentrations were analyzed using western blotting with an anti-GroEL monoclonal antibody, and *groEL* gene expression was analyzed using RT-qPCR of HK-6 cells (□) and *rpoH* mutant cells (▨) treated with 0.1 mM RDX. The relative expression ratios were calculated as fold increases in *dnaK* and *groEL* gene expression levels above those at time zero in the wild type and *rpoH* mutant. The numbers on x-axis of RT-qPCR graph and above the western blot photos indicate the time points for samples collected. The numbers on y-axis represent relative gene expression. Concentration values of the DnaK and GroEL proteins measured by densitometer were not incorporated in these figures.

suggested that σ^{32} acts as a built-in RNA thermosensor (Morita et al., 1999). However, it was interesting that the levels of GroEL proteins were not reduced as expected (Figure 3B).

To understand whether transcription levels of the *alg* operon and *pnrB* gene were affected by *rpoH* mutation, their mRNA concentrations were measured with RT-qPCR. The results showed that both *algA* and *pnrB* mRNA levels were much lower in the *rpoH* mutant than those in the wild-type strain (Figures 4A and 4B). Transcription of *algA* in the wild-type strain increased 6-7-fold after 2 to 6 h of exposure to 0.1 mM RDX, and *pnrB* was induced by 3.5-fold after 30 days of exposure to 0.05 mM RDX. However, expression of neither *algA* nor *pnrB* was observed in the *rpoH* mutant grown in LB liquid broth supplemented with 0.05 mM RDX or in basal salts media supplemented with 0.1 mM RDX (Figures 4A and 4B). These results strongly suggest that σ^{32} is absolutely required for the induction of *algA* and *pnrB* transcription in HK-6 cells grown under stress conditions.

Many studies have investigated the regulation of alginate biosynthesis. Spontaneous *algT* mutations in the mucoid *Ps. aeruginosa* strain cause a conversion to the non-mucoid strain. AlgT, a predicted 22 kDa protein in *Ps. aeruginosa*, shows strong homology with several alternate sigma factors in bacteria and interacts directly with the RNA polymerase core to activate the alginate gene promoters of *Ps. aeruginosa* (DeVries and Ohman, 1994). In the phytopathogen *Ps. syringae*, alginate production is controlled by *algT*, which encodes the alternate sigma factor ς^{22} (Keith. and Bender, 1999). The regulation of exopolysaccharide (mainly alginate) synthesis in *Rhizobium* sp. strain TAL1145 involves an alternative sigma factor gene, *rpoH2*, and its product shows significant similarity to RpoH-like sigma factors in other gram-negative bacteria (Kaufusi et al., 2004). These previous reports and our results indicate that σ^{32} is likely a common regulatory sigma factor for alginate biosynthesis in gram-negative bacteria.

Several reports have addressed the regulation of nitroreductase expression. In *Escherichia coli*, nitroreductase appears to be induced by exposure to paraquat in a manner that depends on *soxR* expression (Liochev et al., 1999). In the photosynthetic bacterium *Rhodobacter capsulatus*, the nitroreductase NprA is under the control of ammonium, whereas the nitroreductase NprB is not. Furthermore, *nprB* gene expression appears to be constitutive, whereas *nprA*

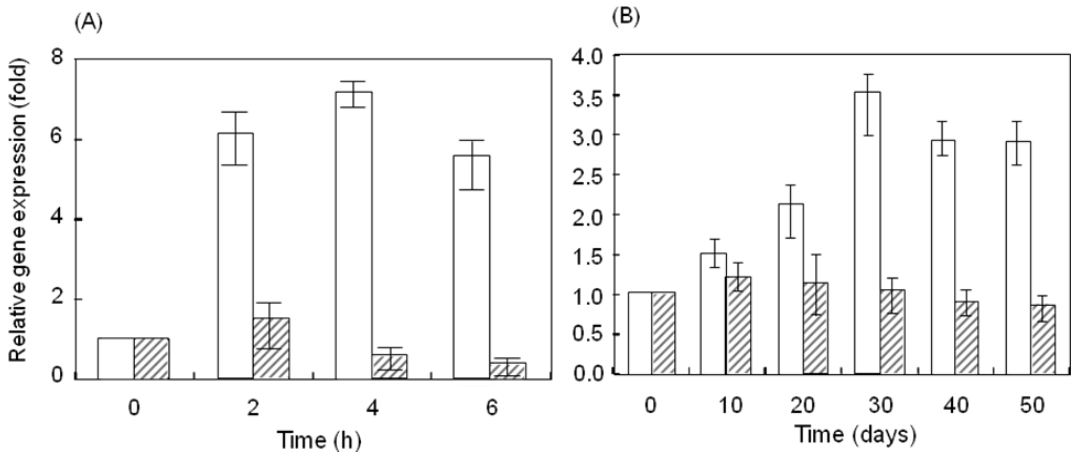

Figure 4. Analysis of *algA* gene and *pnrB* gene expression by RT-qPCR. (A) HK-6 (□) and *rpoH* mutant cells (▨) grown in LB broth supplemented with 0.1 mM RDX. The mRNA levels of *algA* were measured at different timepoints. (B) Nitroreductase (*pnrB*) mRNA levels in HK-6 cells (□) and *rpoH* mutant cells (▨) during the biodegradation of 0.05 mM RDX. The relative expression ratios were calculated as fold increases in *algA* and *pnrB* gene expression levels above those at time zero in the wild type and *rpoH* mutant.

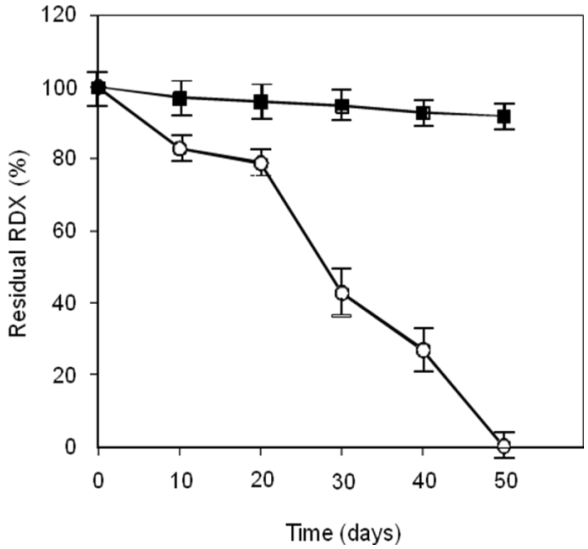

Figure 5. RDX degradation by the *rpoH* mutant. Residual RDX concentrations in the supernatants of HK-6 wild-type cells (○) and *rpoH* mutant cells (■) grown aerobically in liquid salt media supplemented with 0.05 mM RDX were measured. The wild-type strain was able to degrade all of the RDX present during 50 days of incubation, but the *rpoH* mutant degraded less than 10% of this amount.

gene transcription is inducible by a wide range of nitroaromatic and heterocyclic compounds (Pérez-Reinado et al., 2005). Caballero et al. (2005) reported that both *pnrA* and *pnrB* mRNAs were expressed constitutively in TNT-degrading *Ps. putida* JLR11 cells grown on different nitrogen sources and that the levels of these genes were relatively constant regardless of the growth substrate. Therefore, it was interesting that *pnrB* gene expression in *Pseudomonas* sp. HK-6 was highly inducible when RDX was added to the culture medium.

To determine whether the *rpoH* mutation significantly affects RDX degradation, the rates of RDX degradation in wild-type HK-6 and *rpoH* knock-out strains were assessed via measurement of the residual RDX in the supernatants following culture. HK-6 completely degraded 0.05 mM RDX within 50 days, whereas the *rpoH* mutant achieved less than 10% of that level of degradation in the same time period (Figure 5). This result indicated that PnrB (reductase) production was insufficient for RDX degradation in the *rpoH* mutant. Therefore, functional σ^{32} plays a pivotal role in the RDX degradation pathway of the HK-6 strain. To our knowledge, this is the first report that the *rpoH* gene controls the induction of the *algA* and *pnrB* genes in response to explosive RDX in *Pseudomonas* sp.

To gain additional information regarding σ^{32} regulation of transcription of the *alg* operon and *pnrB* gene, it may be necessary to analyze their promoter regions and characterize other phenotypes under different stress conditions in the future.

Conclusions

Pseudomonas sp. HK-6 was able to completely degrade 0.05 mM RDX within 50 days, whereas an *rpoH* knock-out mutant only achieved approximately 10% within the same time period. In HK-6, there is an increase in the transcription of the *dnaK* and *groEL* genes encoding HSP 70 and HSP 60 upon exposure to RDX. In contrast, the

rpoH mutant showed that the transcription of *dnaK* was not observed and that the transcription of *groEL* was still partially induced by RDX stress. The results of this study indicate that σ^{32} production induced by RDX as an environmental stressor is required for the induction of genes encoding SSPs and enzymes for RDX degradation.

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REFERENCES

Binks PR, Nicklin S, Bruce NC (1995). Degradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by *Stenotrophomonoas maltophilia* PB1. Appl. Environ. Microbiol., 61: 309-317.

Bollag DM, Rozycki MD, Edelstein SJ (1992). Protein methods, 2nd ed. New York: Wiley-Liss.

Caballero A, Lazaro JJ, Ramos JL, Esteve-Nunez A (2005). PnrA, a new nitroreductase-family enzyme in the TNT-degrading strain *Pseudomonas putida* JRL11. Environ. Microbiol., 7: 1211–1219.

Chang HW, Kahng HY, Kim SI, Chun JW, Oh KH (2004). Characterization of *Pseudomonas* sp. HK-6 cells responding to explosive RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine). Appl. Microbiol. Biotechnol., 65: 323-329.

Cho YS, Lee BU, Oh KH (2008). Simultaneous degradation of nitroaromatic compounds TNT, RDX, atrazine, and simazine by *Pseudomonas* sp. HK-6 in bench-scale bioreactors. J. Chem. Technol. Biotechnol., 83: 1211-1217.

DeVries CA, Ohman DE (1994). Mucoid-to-nonmucoid conversion in alginate-producing *Pseudomonas aeruginosa* often results from spontaneous mutations in *algT*, encoding a putative alternate sigma factor, and shows evidence for autoregulation. J. Bacteriol., 176: 6677–6687.

French CE, Nicklin SS, Bruce NC (1998). Aerobic degradation of 2,4,6-trinitrotoluene by *Enterobacter cloacae* PB2 and by pentaerythritol tetranitrate reductase. Appl. Environ. Microbiol., 64: 2864-2868.

Hawari J, Paquat L, Zhou E, Halasz A, Zilber B (2000). Characterization of metabolites during biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) with municipal anaerobic sludge. Appl. Environ. Microbiol., 66: 2652-2657.

Ho EM, Chang HW, Kim SI, Kahng HY, Oh KH (2004). Analysis of TNT (2,4,6-trinitrotoluene)-inducible cellular responses and stress shock proteome in S*tenotrophomonas* sp. OK-5. Curr. Microbiol., 49: 346-352.

Kaufusi PH, Forsberg LS, Tittabutr P, Borthakur D (2004). Regulation of exopolysaccharide synthesis in *Rhizobium* sp. strain TAL1145 involves an alternative sigma factor gene, *rpoH2*. Microbiology, 150: 3473–3482.

Keith LM, Bender CL (1999). AlgT (ς^{22}) controls alginate production and tolerance to environmental stress in *Pseudomonas syringae*. J. Bacteriol., 181: 7176-7184.

Lee BU, Park SC, Cho YS, Kahng HY, Oh KH (2008b). Expression and characterization of the TNT nitroreductase of *Pseudomonas* sp. HK-6 in *Escherichia coli*. Curr. Microbiol., 56: 386-390.

Lee BU, Park SC, Cho YS, Oh KH (2008a). Exopolymer biosynthesis and proteomic changes of *Pseudomonas* sp. HK-6 under stress TNT (2,4,6-trinitrotoluene). Curr. Microbiol., 57: 477-483.

Lee MS, Chang HW, Kahng HY, So JS, Oh KH (2002). Biological removal of explosive 2,4,6-trinitrotoluene by *Stenotrophomonas* sp. OK-5 in bench-scale bioreactors. Biotechnol. Bioprocess Eng., 7: 105-111.

Liochev SI, Hausladen A, Fridovich I (1999). Nitroreductase A is regulated as a member of the *soxRS* regulon of *Escherichia coli*. Proc. Natl. Acad. Sci. USA, 96: 3537-3539.

Manzanera M, Aranda-Olmedo I, Ramos JL, Marqués S (2001). Molecular characterization of *Pseudomonas putida* KT2440 *rpoH* gene regulation. Microbiology, 147: 1323–1330.

Marqués S, Manzanera M, González-Pérez MM, Gallegos MT, Ramos JL (1999). The XylS-dependent Pm promoter is transcribed *in vivo* by RNA polymerase with sigma 32 or sigma 38 depending on the growth phase. Mol. Microbiol., 31: 1105-1113.

Morita MT, Tanaka Y, Kodama TS, Kyogoku Y, Yanagi H, Yura T (1999). Translational induction of heat shock transcription factor sigma^{32}: evidence for a built-in RNA thermosensor. Genes Dev., 13: 655-665.

Pérez-Reinado E, Blasco R, Castillo F, Moreno-Vivián C, Roldán MD (2005). Regulation and characterization of two nitroreductase genes, *nprA* and *nprB*, of *Rhodobacter capsulatus*. Appl. Environ. Microbiol., 71: 7643-7649.

Rosenblatt DH, Burrows EP, Mitchell WR, Parme DL (1991). Organic explosive and related compounds. In: Hutzinger O (ed) The Handbook of Environmental Chemistry, Springer-Verlag, Berlin, Germany, pp. 196-225.