NOTE

Comparative Analysis of 2,4,6-Trinitrotoluene (TNT)-Induced Cellular Responses and Proteomes in *Pseudomonas* sp. HK-6 in Two Types of Media

Yun-Seok Cho¹, Bheong-Uk Lee², Hyung-Yeel Kahng³, and Kye-Heon Oh¹*

¹Department of Biotechnology, Soochunhyang University, Asan 336-600, Republic of Korea
²Division of Biological Sciences, Kosin University, Busan 606-701, Republic of Korea
³Department of Environmental Education, Sunchon National University, Suncheon 540-742, Republic of Korea

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TNT-induced cellular responses and proteomes in *Pseudomonas* sp. HK-6 were comparatively analyzed in two different media: basal salts (BS) and Luria broth (LB). HK-6 cells could not degrade more than 0.5 mM TNT with BS medium, while in LB medium, they exhibited the enhanced capability to degrade as much as 3.0 mM TNT. Analysis of total cellular fatty acids in HK-6 cells suggested that the relative abundance of several saturated or unsaturated fatty acids is altered under TNT-mediated stress conditions. Scanning electron microscopy showed the presence of perforations, irregular rod formations, and wrinkled extracellular surfaces in cells under TNT stress. Proteomic analysis of soluble protein fractions from HK-6 cultures grown with TNT as a substrate revealed 11 protein spots induced by TNT. Among these, seven proteins (including Alg8, AlgB, NirB, and the AbpC/TsA family) were detected only in LB medium containing TNT. The proteins AspS, Tsf, and assimilatory nitrate reductase were increasingly expressed only in BS medium containing TNT. The protein dGTPase was found to be induced and expressed when cells were grown in either type of TNT-containing media. These results provide a better understanding of the cytotoxicity and survival mechanism used by *Pseudomonas* sp. HK-6 when placed under TNT stress conditions.

Keywords: cellular responses, proteomics, alginate, *Pseudomonas* sp. HK-6, 2,4,6-trinitrotoluene

Microbial degradation of 2,4,6-trinitrotoluene (TNT) has been extensively studied (Fiorella and Spain, 1997; French et al., 1998; Oh and Kim, 1998; Pak et al., 2000; Lee et al., 2002), but information on TNT-induced stress shock proteins (SSPs) has been reported for only a few microorganisms (Chang et al., 2004; Ho et al., 2004). Proteome analysis is a powerful tool for investigating global changes in prokaryotic gene expression (Jungblut et al., 1999, 2000). Because 2-dimensional electrophoresis (2-DE) displays on a gel all bacterial soluble proteins expressed under specific culture conditions, high-throughput screening of induced proteins is possible. To date, proteome analysis has been performed on several bacteria including *Acinetobacter* sp. KS-1 (Kim et al., 2003), *Acinetobacter lwofii* K24 (Kim et al., 2001, 2002), *Pseudomonas putida* KT2442 (Lupi et al., 1995), and *Stenotrophomonas* sp. OK-5 (Ho et al., 2004). Recently, enzymes and related proteins responsible for the degradation of aromatic compounds such as aniline, benzoate, 2,4-D, and TNT have been extensively studied by proteomic analysis. The results of such studies have provided environmental microbiologists with valuable information on the degradation of aromatic compounds (Park et al., 2001; Cho et al., 2002; Ho et al., 2004). In order to expand our knowledge of TNT-responsive SSPs capable of TNT degradation in *Pseudomonas* sp. HK-6 and increase our understanding of TNT-mediated toxicity and cellular responses, this study analyzed TNT-inducible stress shock proteomes in HK-6 cells.

*Pseudomonas* sp. HK-6 was cultivated in two different media: Luria broth (LB) containing TNT and basal salts (BS) medium containing TNT. The conditions of cultivation and maintenance of the HK-6 strain have been described previously (Chang et al., 2004). To monitor TNT degradation, residual TNT concentration was determined by reverse-phase HPLC as previously described (Ho et al., 2004). *Pseudomonas* sp. HK-6 can degrade TNT in BS medium and in LB medium under aerobic conditions (Fig. 1). With BS medium, 0.5 mM TNT was completely degraded within 144 h of incubation, and the culture pH changed from 7.2 to 7.1 (Fig. 1A). Surprisingly, in HK-6 cultures grown on LB medium containing 0.5 mM TNT, complete degradation was achieved within just 18 h (Fig. 1B). To further evaluate the capability of *Pseudomonas* sp. HK-6 in LB medium to degrade TNT, various concentrations of TNT were tested (Fig. 1C). Strain HK-6 grown on LB media was able to completely degrade TNT at all the concentrations tested, including the highest concentration of 3.0 mM TNT.

To examine fatty acids that shifted in response to TNT,
cells grown on tryptic soy broth (TSB) at 37°C for 24 h were collected, washed twice in potassium phosphate buffer (pH 7.0), and incubated in LB medium, BS medium containing 0.5 mM TNT, or LB medium containing 0.5 mM TNT. After 24 h, the fatty acid compositions were analyzed as described previously (Cho et al., 2002). As shown in Fig. 2, the dominant lipids in LB-grown HK-6 cells were 16:1 \(\omega_7c/15:0\) iso 2OH, 16:0 and 18:1 \(\omega_7c/\omega_9t/\omega_{12t}\), which decreased severely in cells exposed to TNT. Fatty acids 10:0 iso, 14:1 \(\omega_5c/\omega_5t\) and 17:0 cyclo were 10%, 8%, and 3% of the total cellular fatty acids in LB-grown cells, respectively, while in BS medium containing TNT, they accounted for 22%, 21%, and 8% of total cellular fatty acids, respectively. Some fatty acids exhibited similar shift patterns in HK-6 cells regardless of the medium used. The relative abundance of fatty acids 16:0, 16:1 \(\omega_7c/15:0\) iso 2OH, and 18:1 \(\omega_7c/\omega_9t/\omega_{12t}\) increased severely in cells exposed to TNT. Fatty acids 10:0 iso, 14:1 \(\omega_5c/\omega_5t\) and 17:0 cyclo were 10%, 8%, and 3% of the total cellular fatty acids in LB-grown cells, respectively, while in BS medium containing TNT, they accounted for 22%, 21%, and 8% of total cellular fatty acids, respectively. Some fatty acids exhibited similar shift patterns in HK-6 cells regardless of the medium used. The relative abundance of fatty acids 16:0, 16:1 \(\omega_7c/15:0\) iso 2OH, and 18:1 \(\omega_7c/\omega_9t/\omega_{12t}\)

![Fig. 1. Rate of TNT degradation by *Pseudomonas* sp. HK-6. HK-6 cells were grown with 0.5 mM TNT in BS medium (A) or with 0.5 mM TNT in LB (B), and then the cell density was measured at 660 nm (●). The rate of TNT degradation (□), and pH change (♦) over the incubation time course were determined in 250 ml flasks. Rates of TNT degradation in LB medium containing 0.5 mM (○), 1.0 mM (●), 1.5 mM (□), 2.0 mM (■), 2.5 mM (▲), and 3.0 mM (▲) of TNT were similarly determined (C).](image1)

![Fig. 2. Fatty acid profiles of *Pseudomonas* sp. HK-6 cells grown on LB, BS medium with TNT, or LB medium with TNT. Fatty acids were identified based on the retention of authentic references.](image2)