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Identification of detoxification pathways in plants that are regulated in response to treatment with organic compounds isolated from oil sands process-affected water

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HIGHLIGHTS

- Transcript profiling of Arabidopsis plants exposed to the AEO fraction of OSPW.
- AEO treatment resulted in the upregulation of xenobiotic detoxification pathway genes.
- Several β-oxidation and stress responsive pathway genes were also activated.
- The AEO induced genes are targets for biosensing and phytoremediation strategies.

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ABSTRACT

Bitumen mining in the Athabasca oil sands region of northern Alberta results in the accumulation of large volumes of oil sands process-affected water (OSPW). The acid-extractable organic (AEO) fraction of OSPW contains a variety of compounds, including naphthenic acids, aromatics, and sulfur- and nitrogen-containing compounds that are toxic to aquatic and terrestrial organisms. We have studied the effect of AEO treatment on the transcriptome of root and shoot tissues in seedlings of the model plant, *Arabidopsis thaliana*. Several genes encoding enzymes involved in the xenobiotic detoxification pathway were upregulated, including cytochrome P450s (CYPs), UDP-dependent glycosyltransferases (UGTs), glutathione-S-transferases (GSTs), and membrane transporters. In addition, gene products involved in oxidative stress, β-oxidation, and glucosinolate degradation were also upregulated, indicating other potential mechanisms of the adaptive response to AEO exposure. These results provide insight into the pathways that plants use to detoxify the organic acid component of OSPW. Moreover, this study advances our understanding of genes that could be exploited to potentially develop phytoremediation and biosensing strategies for AEO contaminants resulting from oil sands mining.

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1. Introduction

The Alberta oil sands cover approximately 140,000 km^2 of area in the northern boreal plain ecozone. The region contains the third largest oil reserve in the world, with an estimated 170 billion barrels of recoverable bitumen. In the open pit (surface) mining process, crushed ore is subjected to an alkaline hot water extraction, which facilitates the release of the bitumen from the solid particles (Oil-sands Advisory Panel; OAP, 2010). This process generates large volumes of oil sands process-affected water (OSPW) that accumulate in tailings ponds. OSPW contains residual bitumen, solids (sand and clay), salts, heavy metals and organic compounds. The acid-extractable organic (AEO) fraction of OSPW consists of several types of compounds, including naphthenic acids (NAs). NAs are a diverse group of aliphatic and alicyclic carboxylic acids that are classically defined by the general formula $C_nH_{2n+z}O_2$, where n indicates the carbon number and z refers to the hydrogen deficiency that signifies the number of rings or double bonds (Clemente and Fedorak, 2005). Recent work has identified specific NAs that are found in OSPW, including tricyclic (diamondoid), tetracyclic, and pentacyclic acids (Rowland et al., 2011a,b). Other components of AEOs include dicarboxylic acids, various nitrogen- and sulfur-containing compounds, and aromatics (Grewer et al., 2010; Jones et al., 2012; West et al., 2014).

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OSPW toxicity studies have mainly focused on NAs that exhibit acute and chronic toxic effects in a variety of organisms (Whitby, 2010). NA exposure causes detrimental effects on several plant species, such as jack pine, aspen, cattail, common reed, and the model plant *Arabidopsis thaliana* (Arabidopsis) (Apostol et al., 2004; Armstrong et al., 2009; Crowe et al., 2001; Kamaluddin and Zwiazek, 2002; Leishman et al., 2013). In addition to NAs, other organic compounds in the AEO fraction of OSPW may also contribute to toxicity, but knowledge in this area is limited. Indigenous aerobic bacteria isolated from OSPW have been shown to degrade NAs, and the biodegradation efficiency is dependent on chemical structure (Biryukova et al., 2007; Clemente et al., 2004; Del Rio et al., 2006; Lai et al., 1996). In addition, certain algal species have also been shown to degrade NAs by β-oxidation (Quesnel et al., 2011). Although less explored, plants have the metabolic capacity to degrade a range of organic compounds and thus can be considered in remediation strategies. Degradation of xenobiotic pollutants in plants is often divided into three different phases: transformation, conjugation, and compartmentalization. It is through these phases that plants modify pollutants into a less toxic product and compartmentalize them in the vacuole and cell walls (Eapen et al., 2007; Abhilash et al., 2009). Further breakdown and detoxification can then take place while the toxins are safely sequestered away from the cytosol.

Our previous study revealed that Arabidopsis displayed germination defects, a decrease in primary root growth and detrimental shoot phenotypes in response to OSPW, AEO, and model NA treatment (Leishman et al., 2013). In the present study, we have mapped the global transcriptional response of Arabidopsis root and shoot tissues treated with AEOs isolated from OSPW. In both roots and shoots there was an enrichment of genes encoding common xenobiotic detoxification enzymes. In addition, roots also showed upregulation of genes that function in β-oxidation and glucosinolate degradation and may be involved in the biodegradation of AEOs. The identification of biochemical pathways and molecular responses involved in AEO degradation and detoxification in Arabidopsis will be useful in the development of phytoremediation and biosensing strategies for improved management of OSPW.

2. Materials and methods

2.1. Sources of OSPW and extraction of AEOs

OSPW samples that were used in this study were from an aged oil sands tailings pond on a mine site in the Athabasca region of northern Alberta. The AEO fraction of OSPW was isolated using a liquid–liquid organic extraction with dichloromethane (DCM) (Sigma–Aldrich), as described previously (Leishman et al., 2013). Yields ranged from 22 to 32 mg AEOs/L of OSPW. The AEOs were resuspended in DMSO at a stock concentration of 100 mg/mL.

2.2. Plant material, seed sterilization, media preparation and growth

Wild-type (Columbia-0) Arabidopsis seeds were surface sterilized with 70% ethanol and placed in a horizontal line on culture plates using sterile forceps. The solid culture plate medium consisted of half-strength Murashige and Skoog (MS) salts (Sigma–Aldrich) along with 1% sucrose and 0.7% phytoagar (Phytotechnology Laboratories) at a pH of 5.7. The solution was autoclaved and 30 mL was poured into each 8.5 cm diameter petri plate. The seeded culture plates were placed at 4 °C and the seeds were stratified in the dark for 3–4 days. Plates were then oriented vertically in a growth chamber (Percival Scientific) at 22 °C with a 12 h light/12 h dark photoperiod. After nine days of growth, the seedlings were transplanted into individual compartments of a 38-well (5.5 cm diameter each) tray with Promix BX Mycorrhizae general purpose growing medium (Premier Tech Horticulture). After two weeks of growth, the seedlings were transplanted into the hydroponic system (described in the Supplementary Methods).

2.3. Transcript profiling and quantification real-time PCR (qPCR) analysis

Total RNA was extracted from control and AEO-treated root and shoot tissues using Trizol (Life Technologies, Carlsbad, CA). For half of the root samples (one replicate) and all shoot samples, poly-A mRNA was isolated from total RNA and converted to cDNA as described previously (Chua, 2009). For the remainder of the root samples (one replicate), cDNA was synthesized directly from total RNA rather than mRNA. Selected differentially expressed genes from microarray data were validated by qPCR. For root tissues, the same RNA samples used in the microarray experiments served as template RNA. Due to limited availability of RNA used in the shoot microarray experiments, template RNA from shoot tissues was extracted independently using the Plant RNA kit (Omega Bio-Tek). Details of the microarray and qPCR methodologies are described in the Supplementary Methods.

3. Results

3.1. Transcript profiling of root tissue from hydroponically grown plants treated with OSPW AEOs

RNA from root tissues of control seedlings and seedlings treated with 50 mg/L AEOs for 8 h was prepared and hybridized to Arabidopsis 4 × 44 K Agilent expression microarrays. The results showed that 243 and 168 genes were upregulated and downregulated, respectively, at least twofold (p-value <0.005) in their level of expression (Table S1). Examination of the ten most highly induced genes revealed that half of these genes encoded proteins that are implicated in xenobiotic stress responses and adaptation (Table S2). These gene products included glutathione S-transferases (GSTs), a UDP-dependent glycosyltransferase (UGT), an ABC transporter (PDR12) and a raffinose synthase. In contrast, the ten most highly downregulated genes possess diverse functions.

The entire list of differentially-expressed genes was next analyzed for enrichment in gene ontology using the Princeton GO Term Finder (http://go.princeton.edu/cgi-bin/GOTermFinder). The upregulated root genes were highly enriched for many GO Process and Function categories, whereas the downregulated genes were not enriched for any relevant GO categories (Table S3). The most notable of the 104 upregulated GO Process categories were toxin metabolic process, response to cyclopentanone, response to organic cyclic compound, response to oxidative stress, and lipid modification (Table 1). The toxin metabolic process category contained 31 genes that encoded several GSTs, UGTs and two NAC family transcription factors (TFs). Similarly, the response to cyclopentanone and organic cyclic compound categories also contained GST and UGT genes. The response to oxidative stress category included genes that encoded a GST, an ATP-Binding Cassette (ABC) transporter (*PDR12*), a heat shock protein (*HSP70B*), and two heat shock-like proteins (AT2G29500 and AT1G53540). The lipid modification category included genes involved in fatty acid β-oxidation, such as *SDRB*, *CSY2*, *KAT2*, and *LACS7*. When the upregulated genes were examined for enrichment in the GO Function categories, the top three of 17 categories were GST activity, UGT activity and oxidoreductase activity (Table 1). In total, there were nine GST and seven UGT genes that were upregulated in roots in response to AEO treatment (Table 2). Other common xenobiotic

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