LIPIDOMICS PROFILING OF ZERO-VALENT IRON NANOPARTICLE-TREATED CYANOBACTERIA

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OVERVIEW

We present fatty acid methyl ester (FAME) and polar lipids profiles of nZVIs-treated *Fremyella. diplosiphon* strains.

INTRODUCTION

Increased global concerns on food security, the energy crisis, and pollution caused by fossil fuels have generated significant interest in cyanobacteria-derived fuels as a sustainable third-generation biofuel agent.[1,2]

For biofuel production, *F. diplosiphon*, is an ideal model cyanobacterium due to its lipid producing capacity, fast growth rate, and ability to adapt to varying wavelengths and intensities of light.[3,4]

Nanotechnology has been applied to cyanobacteria and microalgae to enhance lipid accumulation by stimulating metabolism and cell growth.[5,6] In particular, the zero-valent iron nanoparticles (nZVIs) can inertly penetrate cells to form reactive oxygen species (ROS), which induce cell oxidative stress.[7]

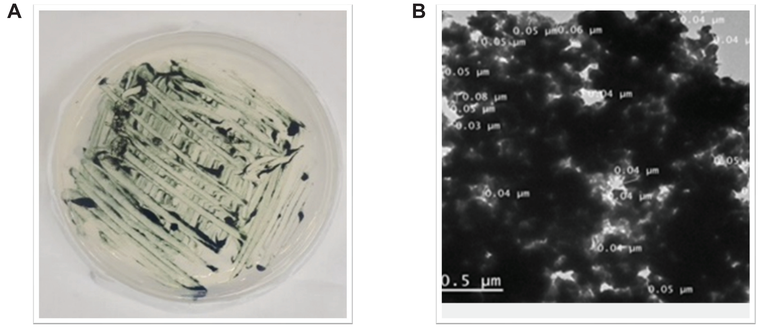

Figure 1. (A) *Fremyella diplosiphon* B481-SD strain grown on an agar plate **(B)** Nanofer 25s zerovalent iron nanoparticles visualized using scanning electron microscopy.

METHODS

Total lipids in nZVI-treated and untreated *F. diplosiphon* were extracted and measured using gravimetric analysis.[8] Extracted lipids were converted to FAMEs via direct transesterification using a commercial multimode scientific microwave.[9] Detailed characterization and quantitation of FAMEs and other volatile organic matter such as alkanes and olefins were characterized using comprehensive two-dimensional gas chromatography- time-of-flight mass spectrometry (GC×GC-TOFMS) from LECO. The polar lipids from the strains were extracted, followed by reverse-phase liquid chromatography coupled with positive and negative electrospray ionization (ESI) orbitrap and FT-ICR mass spectrometry. Data-dependent collision-induced dissociation MS/MS was acquired to obtain additional structural information.

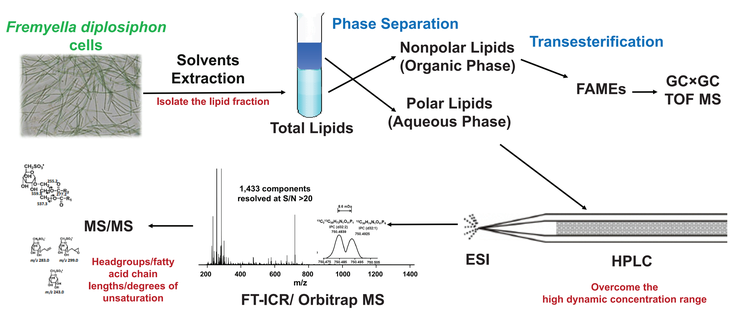

Figure 2. Schematic diagram for extraction and analysis of lipids from nano-treated cyanobacterial cells.

RESULTS AND DISCUSSION

Iron nanoparticle-induced ROS can affect lipid content and composition through lipid peroxidation. Such changes have an impact on the production and properties of fatty acid-derivatives such as FAMEs. The impact of nZVIs on lipid profiles of *F. diplosiphon* strains was investigated. A significant increase in *F. diplosiphon* lipids yield and FAMEs production were observed due to oxidative stress induced by the optimal nZVI concentrations (0.2-1.6 mg L^{-1}) (Fig.3).

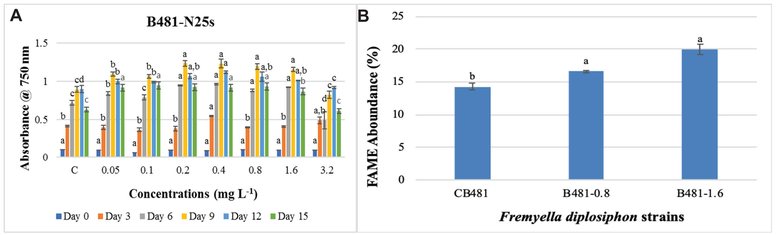

Figure 3. (A) Impact of zero-valent iron nanoparticle (Nanofer 25s) on *Fremyella diplosiphon* B481 growth. **(B)** Fatty acid methyl ester (FAME) abundance in transesterified extractable lipids of *F. diplosiphon* B481 control (CB481) and 0.8 and 1.6 mg L^{-1} Nanofer 25s-treated cells (B481, 0.8 and B481, 1.6) determined by GC×GC−TOF MS. Different letters above bars indicate significance among treatment means (P<0.05)

The polar lipid compositions in nZVI-treated stains were studied by efficient on-line LC separation with FT-ICR MS. The reverse-phase phenylhexyl column separates polar lipids according to the length and degree of unsaturation of hydrocarbon side changes and polar headgroup (Fig.4).

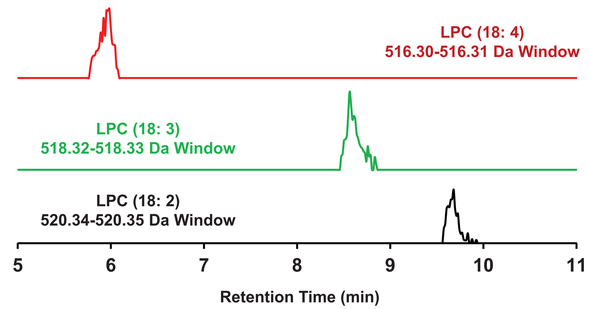

Figure 4. Selected mass chromatograms based on nano-LC (+) ESI FT-ICR MS spectra for three representative lysophosphatidylcholine (LPC) from *F. diplosiphon B481*. Order of retention times for LPC (18:2) > LPC (18.3) > LPC (18.4), as the more double bonds, the less hydrophobic and result in earlier elution.

The complexity of bacterial lipid extracts requires ultra-high mass resolving power available to FT-ICR MS (Fig. 5). The combination of accurate mass measurement (<1ppm) and MS/MS spectrum allowed quick identification of polar lipids with high fidelity. FT-ICR MS data were used to identify trends in lipid class distribution, changes in individual lipid species and novel lipid classes.

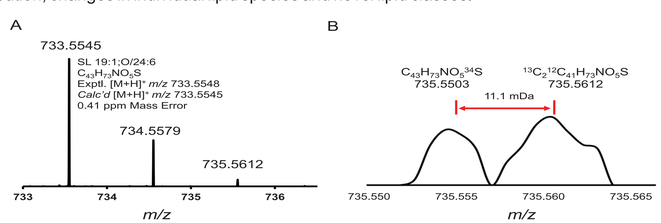

Figure 5. (A) Mass scale expanded segment from the broadband mass spectra **(B)** isotopic fine structure for the protonated molecular ion of Saccharolipids (SL) 12:1;O/32:3;O. The isotopic fine structure shows a resolution of ions differing in elemental composition by $^{12}C_2{}^{34}S$ and $^{13}C_2{}^{32}S$, resulting in a mass difference of 11.1 mDa.

More polar lipids were identified from Cells treated with Nanoparticles (nZVIs), with 36% assigned to SL class compared to the 25% in the control (Fig. 6). The resulting lipid profiles provide a detailed compositional comparison of the nanoparticle-treated and non-treated cyanobacterial cells (Fig. 7).

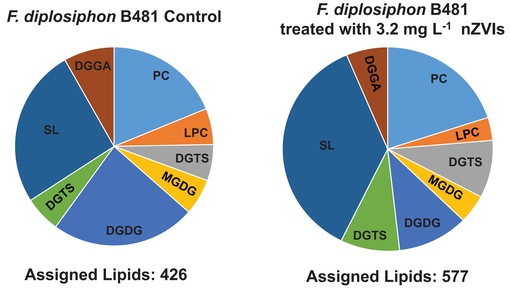

Figure 6. Identified lipid class for *Fremyella diplosiphon* B481 control and cells treated with 3.2 mg L^{-1} Nanofer 25s. The area of each color represents the percentage of the number of assigned lipids in each class over the total number of assigned lipids.

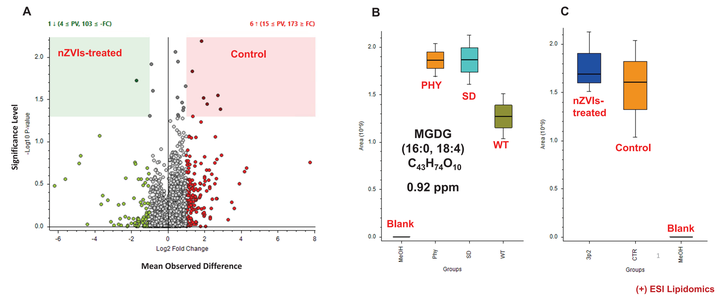

Figure 7. Volcano plot showing the statistical significance (P=0.05) versus magnitude of change when comparing the compounds detected in nZVI-treated and control samples (A). An example of lipid MGDG (16:0, 18:4) detected in samples is shown in box whisker charts. The lipid is present in higher amounts in genetically transformed strains PHY and SD compared to the wild-type strain WT (B). The lipid amounts between the nZVI-treated cells and the control are not statistically different (P>0.01)(C).

CONCLUSION

Nanofer 25s nZVIs at optimal concentrations enhance total lipid content. Molecular characterization of lipids profiles provides understandings of how iron nanoparticle-induced oxidative stress affects lipid domains in a model cyanobacterium. Polar lipid profiles of *F. diplosiphon* B481 before and after nanoparticle treatment have been established. Ultrahigh-resolution FT-ICR and Orbitrap MS allowed the identification of polar lipids and characterization of lipid compositions.

ACKNOWLEDGMENTS

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