README

Characterization of triacylglycerol secretion with shikonin derivatives in *Lithospermum erythrorhizon*

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Abstract

This dataset contains data from electron microscopy and biochemical analyses described in the paper: “Tatsumi, K., et al. Excretion of triacylglycerol as a matrix lipid facilitating apoplastic accumulation of a lipophilic metabolite shikonin” submitted to Journal of Experimental Botany. The raw data underlying the paper are given as separate excel files, which are deposited to ‘Data’. The Excel file ‘result03\_positive\_181119\_Yazaki\_Lab\_5.xlsx’ is the data of LC-MS for Figure 3 of the above paper. Another Excel file ‘GC-FID\_rawdata-processed.xlsx’ is the raw data for the quantitative analysis of fatty acids by GC-FID as well as the processed data shown in Figure 5 and Supporting Figure S7. The third Excel file ‘lipidome\_data\_processed.xlsx’ is the data of lipidome analysis shown in Supporting Figures S3, S5, and S6 . Followings are explanations to those Excel files.

**Excel file name: result03\_positive\_181119\_Yazaki\_Lab\_5**

Analysis of TAG molecules secreted by *L. erythrorhizon* cells cultured under three different conditions; LS medium (dark), M9 medium (dark), and M9 medium (light). Lipids of plant cells were extracted into three parts, *i.e.*, medium, cell surface, and the rest of the cell fraction.

Abbreviations of each lipid molecule is as follows. PC, phosphatidylcholine; lysoPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; lysoPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; TAG, triacylglycerol; DAG, diacylglycerol; Acyl-ACP, acyl-acyl carrier protein; FAS, fatty acid synthase.

**Excel file name: GC-FID\_rawdata-processed.xlsx**

Quantitative analysis of TAG in three fractions of *L. erythrorhizon* cells (T-TOM): medium, cell surface, and inside cells. *L. erythrorhizon* cells were cultured under three different conditions, i. e., LS medium (dark), M9 medium (dark) and M9 medium (light). Quantitative analysis of TAG in hairy root of *L. erythrorhizon* (hairy root), tobacco cultured cells (BY-2) and intact tobacco plant (tabacum) are given in separate sheets. Fatty acid composition of TAG recovered from three fractions (medium, cell surface, and inside cells) of *L. erythrorhizon* cells is also shown.

**Excel file name: lipidome\_data\_processed.xlsx**

Data of lipidome analysis by LC-MS are summarized. Three fractions of *L. erythrorhizon* cells (T-TOM): medium, cell surface, and inside cells were analyzed, and those cells were cultured under three different conditions; LS medium (dark), M9 medium (dark), and M9 medium (light). The data of each lipid molecule are given in separate sheets.

**Keywords:**

apoplastic accumulation, lipid secretion secondary metabolites, *Lithospermum erythrorhizon*, shikonin, triacylglycerol

**Methods:**

Transmission electron microscopy

The method of transmission electron microscopy is as follows. Cultured cells were treated with 5 mM aluminum chloride for 3 h before chemical fixation of shikonin derivatives, as described (Tatsumiet al*.,* 2016). High-pressure freezing and the freeze substitution method (HPF/FS) were performed as follows. Cells cultured in M9 or LS medium containing 3% sucrose were added to flat specimen carriers and frozen using a high-pressure freezing machine (Leica EM PACT, Leica Microsystems, Wetzlar, Germany). The frozen samples were transferred to 2% osmium tetroxide in anhydrous acetone at -80 °C and incubated at -80 °C for 6 days (120 h). These samples were warmed gradually from -80 to -30 °C over 5 h, warmed again from -30 to 4 °C over 3.4 h, and held at 4 °C for 2 h (Cryo Porter CS-80CP, Scinics Corporation, Tokyo, Japan). Subsequently, the samples were washed with acetone, and embedded in Epon812 resin (TAAB, Aldermaston, England). Ultrathin sections (60-80 nm) were cut with a diamond knife on an ultramicrotome (Leica EM UC7, Leica Microsystems) and placed on formvar-coated copper grids. The ultrathin sections were stained with 4% uranyl acetate followed by lead citrate solution and observed with a JEM-1400 (JEOL Ltd., Tokyo, Japan) transmission electron microscope at 80 kV. Some sections were not stained with lead citrate to prevent excess staining.

LC-MS analysis

The liquid cultures were filtered through Miracloth (Merck Millipore) to separate the culture medium (30 ml) from the cells or root tissues. The cultured medium was partitioned with 15 ml of 2 : 1 (v/v) chloroform : methanol to yield organic phase (medium fraction). The harvested wet cells/tissues were rinsed with 15 ml of 2 :1 (v/v) chloroform-methanol and 30 ml distilled water by prompt everting of the glass vessel to recover the cell surface lipids (surface fraction). The remaining cells/tissues were completely dried and the lipids extracted with 2 ml of 2 :1 (v/v) chloroform-methanol to yield the cell fraction. Each fraction was evaporated under nitrogen stream before chromatographic analyses. Before LC-MS analysis, lipid extracts were roughly separated into polar and non-polar lipids by thin-layer chromatography (TLC) using silica plates (TLC silica gel 60, Merck Millipore) developed with chloroform because the high amount of shikonin derivatives hampered the chemical analysis of TAG and polar lipids by LC-MS. Lipid samples, except for shikonin derivatives that could be recognized by their red color, were recovered from TLC plates and extracted with chloroform or methanol from the silica gel. The lipids were subjected to LC-q-TOF-MS (Waters, Boston, MA) analysis with an Acquity UPLC HSS T3 column (Waters), as described (Okazaki et al., 2013; Okazaki and Saito, 2018). Lipidomic analysis was performed using the data set recorded in the positive ion mode. Levels of lipid species were normalized relative to the intensity of the internal standard PC (20:0). To compare the amount of each lipid class among samples, the level of each lipid class, which is the sum of individual lipid species belonging to the class, was standardized by using the mean of cell fraction in LS Dark cultured cells.

GC-FID analysis

To quantify TAG, TAG was purified by preparative TLC developed with 6 : 4 (v/v) n-hexane : diethyl ether. Following derivatization to fatty acid methyl esters using a fatty acid methylation kit (Nacalai Tesque), TAG was quantified by capillary gas chromatography GC-2014 (Shimadzu, Kyoto, Japan) with a J&W DB-23 capillary column (GL Science, Tokyo, Japan) as described (Kajikawa et al., 2015; 2016), with heptadecanoic acid (C17:0) used as the internal standard.