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## Data Article

# Dataset of the phospholipidome and transcriptome of *Campylobacter jejuni* under different growth conditions



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## ABSTRACT

The membrane phospholipid composition is not a stable bacterial characteristic but can change in response to altered environmental conditions. Here we provide the dataset of the phospholipidome and transcriptome of the microaerophilic human pathogen *Campylobacter jejuni* under different environmental conditions. These data have been used in Cao (2020), The unique phospholipidome of the enteric pathogen *C. jejuni*: Lysolipids are required for motility at low oxygen availability. Here the abundance of each phospholipid is shown during the growth of *C. jejuni* for 0–108 h under low and high oxygen conditions (0.3 vs 10% O<sub>2</sub>). The phospholipid data were obtained by applying high performance liquid chromatography tandem-mass spectrometry (LC-MS/MS). The transcriptomic data obtained by RNA-seq show the differential expressed genes between logarithmic and stationary grown bacteria. In addition, our data might serve as a reference information for further in-depth investigation to

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understand the relation between specific phospholipids and the activity of membrane associated proteins.

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## Specifications Table

Subject	Microbiology
Specific subject area	Adaptation of the phospholipids composition and gene transcription of bacteria towards different environmental conditions to understand the relation between specific phospholipids and membrane associated proteins.
Type of data	Table
How data were acquired	Bacterial samples were analysed by; high performance liquid chromatography tandem-mass spectrometry (LTQ-XL mass spectrometer) and software XCMS version 1.52.0 and RNA -seq was acquired with MiSeq instrument (Illumina) and software Geneious 10.1.
Data format	Data are in raw format and have been analyzed. The raw Illumina cDNA reads were submitted to NCBI SRA database with accession SRP118838 and are part of the BioProject PRJNA412095.
Parameters for data collection	Samples were taken from six independent <i>C. jejuni</i> cultures grown for 0, 4, 8, 24, 36, 60 and 108 h in Heart Infusion (HI) under high oxygen (10% O <sub>2</sub> , 10% CO <sub>2</sub> , 70% N <sub>2</sub> , 10% H <sub>2</sub> ) or under oxygen-limited conditions (0.3% O <sub>2</sub> , 10% CO <sub>2</sub> , 79.7% N <sub>2</sub> , 10% H <sub>2</sub> ) at 42°C.
Description of data collection	Six fresh HI medium cultures were inoculated with <i>C. jejuni</i> strain 81116 at grown for 0–108 h at 42°C under high (10% O <sub>2</sub> ) or under oxygen-limited conditions (0.3% O <sub>2</sub> ). Samples (500 µl) were taken at 0, 4, 8, 24, 36, 60 and 108 h of growth and centrifuged. Approximately 2.10 <sup>8</sup> bacteria were transferred to glass coated 96-well plates with a conical bottom, and the plates were centrifuged. Pellet were resuspended in 150 µl of chloroform/methanol (1:1 v/v), extracted for 1 h at 4°C, and centrifuged. Supernatants were added to a glass-coated 96-well plate that was covered with aluminium foil and placed in the autosampler. Phospholipid extract were separated using a HILIC column at a flow rate of 1 ml/min to resolve different phospholipid classes. For RNA-seq, <i>C. jejuni</i> strain 81116 overnight cultures were diluted in HI broth (OD <sub>550</sub> ~0.05) and grown under microaerophilic conditions at 42°C until the logarithmic (6 h) and stationary phase (12 h) was reached. RNA was extracted from <i>C. jejuni</i> using RNA-Bee kit. RNA samples were treated with RNase-free DNase I according to the manufacturer's manual.
Data source location	Institution: Veterinary Medicine, Utrecht University City/Town/Region: Utrecht Country: The Netherlands
Data accessibility	With the article and the raw Illumina cDNA reads are available at the NCBI SRA database accession SRP118838 that are part of the BioProject PRJNA412095. ( <a href="https://trace.ncbi.nlm.nih.gov/Traces/sra/?study=SRP118838">https://trace.ncbi.nlm.nih.gov/Traces/sra/?study=SRP118838</a> )
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## Value of the Data

- This is the description of the complete phospholipidome of *C. jejuni* and how it changes due to different environmental conditions. These data will help us to understand the relation between specific phospholipids and the activity of membrane associated proteins.
- Researchers, teachers, policy makers and all institutions involved in infectious diseases can take advantage of our data because they can see how the pathogenic bacterium *C. jejuni* adapts to a different environment in order to survive.
- These data can be (re)used to compare them with data of other bacteria and they can be used to obtain insight how bacteria adapt to their environment which may help us to find novel prevention strategies.
- These data provide insight that like the bacterial proteome, transcriptome and metabolome the phospholipidome is continue changing in order to be optimal adapt to the environment
- These data may help to explain why *C. jejuni* is a leading causes of bacterial foodborne illness.

## 1. Data Description

**Table 1** reveals gene transcripts difference between logarithmic and stationary grown *C. jejuni* bacteria. **Table 2** presents the complete list of phospholipid species and abundancy of *C. jejuni* under different environmental conditions.

**Table 3** presents the phospholipidome of the wt and *cfa* and *pldA* mutants.

## 2. Experimental Design, Materials and Methods

To investigate whether the annotated phospholipid biosynthesis genes are regulated by the growth phase of the bacterial culture, we performed RNA-seq analysis on the complete mRNA content of logarithmic (6 h) and stationary phase (12 h) cultures of *C. jejuni*, as described by Stel et al., 2018 [1] (**table 1**). Hereto, a pre-culture of *C. jejuni* strain 81116 was grown in Hearth Infusion medium (HI-medium, Biotrading, The Netherlands) for 16 h at 42°C under microaerophilic atmosphere (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 10% H<sub>2</sub> and 75% N<sub>2</sub>). Subsequently the bacteria were diluted in HI until OD<sub>550</sub> of 0.05 and growth was prolonged for 6 h or 12 h at 42°C. *C. jejuni* RNA was immediately extracted using the RNA-Bee kit (Tel-Test) according to the manufacturer's manual and treated with RNase-free DNase I (Invitrogen) and then stored at -80°C. Total RNA was quantified with a Nanodrop ND 1000 spectrophotometer (Thermo Scientific) and examined for quality on an Agilent Bioanalyzer according to manufacturer's instructions (Agilent Technologies, Santa Clara, CA). Prior to sequencing, the total RNA was treated with Ribozero Magnetic Kit for Gram-negative bacteria (Illumina) to remove rRNA from RNA samples according to manufacturer's instructions. Before and after Ribozero treatment, samples were examined for RNA using the Agilent Pico 6000 RNA kit with Prokaryotic Total RNA Pico assay on an Agilent Bio-Analyzer to determine the amounts of total RNA and to visualize the 5 s, 16 s, 23 s RNA peaks. After-Ribozero, the 16 s RNA and 23 s RNA peaks were only 0.3–0.8% of the total area of the RNA peak, as compared to about 50% before treatment. The Illumina MiSeq sequencing libraries were prepared using the KAPA stranded RNA-seq kit (Kapa Biosystems, Wilmington, MA), following manufacturer's instructions with the following changes: 1) 159–400 ng RNA was sheared for 6 min at 85°C; 2) based on starting RNA amount standard desalted TruSeq LT primers (Integrated DNA Technologies, Coralville, IA) were used at 50–100 nM final concentration and 3) the PCR step was reduced to 6 cycles. The sequencing libraries were quantified using the KAPA Library Quantification Kit (Kapa), with less volume (10 µl) and 90 s annealing/extension time during the PCR. The pooled and normalized libraries (4 nM) were quantified by ddPCR on a QX200 Droplet Digital PCR system (Bio-Rad, Hercules, CA) using the Illumina TruSeq ddPCR Library Quantification Kit according to the manufacturer's protocol. The libraries were sequenced on a Miseq

instrument at 13.5 pM (Illumina) in two  $2 \times 76$  bp paired end v3 runs. Fastq files were generated for each sample by the MiSeq Instrument software and the sequences were trimmed to remove poor quality bases and assembled to the reference genome CP000814 (*Campylobacter jejuni* strain 81116) using Bowtie within Geneious 11.1 software. Within Geneious software, normalized transcript levels per million (TPM) from the resulting assemblies were calculated using the “Calculate Expression Levels” function, and normalized for comparison of expression levels between the different growth phases using the “Compare Expression Levels” function with the DESeq2 method and parametric fit type.

The phospholipid composition of *C. jejuni* wildtype 81116 was determined after growth for 0–108 h under high oxygen (10% O<sub>2</sub>, 10% CO<sub>2</sub>, 70% N<sub>2</sub>, 10% H<sub>2</sub>) or under oxygen-limited conditions (0.3% O<sub>2</sub>, 10% CO<sub>2</sub>, 79.7% N<sub>2</sub>, 10% H<sub>2</sub>) at 42°C (table 2) [2]. The phospholipid composition was also determined of *C. jejuni* wildtype 81116 and its isogenic mutants, *cfa* and *pldA* after growing the strains in HI for 16 h at 42°C under microaerophilic conditions (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 75% N<sub>2</sub>, 10% H<sub>2</sub>) (table 3). At each time point a 500 µl culture sample was taken and immediately frozen at -80°C. After all samples were obtained, they were thawed and centrifuged at 20,000 g for 5 min at 4°C. The pellets were washed once with PBS and taken up in PBS to an OD<sub>550</sub> of ~0.2 corresponding to  $\sim 1 \times 10^9$  bacteria. Approximately  $2 \times 10^8$  bacteria were transferred to glass coated 96-well polypropylene plates with a conical bottom (ThermoFisher Scientific, Waltham, MA), and the plates were centrifuged at 1,800 g for 20 min at 4°C. The pelleted cells were resuspended in 150 µL chloroform/methanol (1:1 v/v) and the lipids were allowed to extract for 1 h at 4°C, followed by centrifugation (1,800 g, 20 min, 4°C) to remove proteins and other insoluble macromolecules. The lipid containing supernatant was transferred to a new plate which was covered with aluminium foil to prevent evaporation of the organic solvent. Chromatography of 10 µl of each of these lipid extracts was performed on a hydrophilic interaction liquid chromatography (HILIC) column (2.6 µm HILIC 100 Å, 50 × 4.6 mm, Phenomenex, Torrance, CA). Lipid classes were separated by gradient elution on a Dionex Ultimate 3000 RS UPLC (ThermoFisher Scientific, Waltham, MA). Solvent A consisted of acetonitrile/acetone (9:1, v/v) whereas solvent B consisted of acetonitrile/H<sub>2</sub>O (7:3, v/v) with 10 mM ammonium formate in addition, both solvents contained 0.1% (v/v) formic acid. Flow rate was constant at 1 mL/min, and the gradient was as follows (time in min, %B): (0, 0), (1, 50), (3, 50), (3.1, 100), (4, 100). Subsequent samples were injected without re-equilibration of the column. The outlet of the LC column was connected to a heated electrospray ionization (HESI) source of an LTQ XL mass spectrometer (ThermoFisher Scientific, Waltham, MA) operated in negative ionization mode. Source- and capillary temperatures were set to 450°C and 400°C, respectively and the ionization voltage to -2.5 kV. Full scan spectra were collected in the range from 450–1150 amu at a scan speed of 3 scans/s. The resulting LTQ-XL-raw mass spectrometer files were converted to the mzML data-format and analysed in R version 3.4.2 (R Development Core Team, 2016) using the XCMS package (version 1.52.0).

## Declaration of Competing Interest

Authors declare no conflict of interest.

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None.

## Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.dib.2020.106349](https://doi.org/10.1016/j.dib.2020.106349).

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- [2] X Cao, JF Brouwers, L van Dijk, CH van de Lest, CT Parker, S Huynh, et al., The Unique phospholipidome of the enteric pathogen *Campylobacter jejuni*: Lysophospholipids are required for motility at low oxygen availability, *J Mol Biol* (2020 Jul 22) S0022-2836(20)30459-9.