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Data Article

Total mRNA sequence dataset from *Pectobacterium atrosepticum* colonising potato or radish roots

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ABSTRACT

Pectobacterium atrosepticum (Pba) is a gram-negative bacterium that causes blackleg and tuber soft rot of potato but can also asymptomatically colonise other (non-host) plant species. The aim of this study was to investigate the molecular processes and responses involved in Pba-host (potato) and Pba-non-host (radish) interactions, under laboratory conditions. To achieve this, we used total mRNA-sequencing to measure the gene expression patterns from all three species: Pba, potato and radish. We employed an end-point dual transcriptome approach. We used hydroponically grown potato (*Solanum tuberosum* var. Estima) and oil radish (*Raphanus sativa* var. Bento) roots inoculated with Pba SCRI1039 for 14 days compared to un-inoculated control plants or cultured bacteria. Total RNA was extracted from replicates of the two plant species and the bacterium using a Macherey-Nagel Nucleospin Plant RNA kit. The RNA from the 17 samples was then subjected to total mRNA-sequencing (paired-end) on an Illumina NovaSeq 6000™ sequencing platform. This gave between 39.2-58.1M reads per sample. The high-quality reads obtained were mapped to the corresponding reference genomes using Bowtie2 and the percentages of bacterium and plant transcripts calculated. This dataset constitutes the raw read fastq files and can be used to inform on genes active in plant rhizosphere-microbe interactions.

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Specifications Table

Subject	Plant Science: Plant Microbe Interaction
Specific subject area	Molecular basis to host-microbe interactions
Type of data	RNA sequencing (RNA-seq) data
How the data were acquired	Illumina NovaSeq 6000 sequencing platform performed at Novogene UK
Data format	Raw RNA sequence data (fastq)
Description of data collection	We employed an end-point whole transcriptome approach of hydroponically grown potato (*Solanum tuberosum* var. Estima) and oil radish (*Raphanus sativa* var. Bento) roots inoculated with *Pectobacterium atrosepticum* SCRI1039 for 14 days compared to un-inoculated control plants or cultured bacteria.
Data source location	Laboratory experiment, total RNA extraction, sequence data analysis The James Hutton Institute Invergowrie, Dundee, DD2 5DA United Kingdom Illumina library preparation and sequencing was carried out by Novogene (UK) Company Limited 25 Cambridge Science Park, Milton Road, Cambridge, CB4 0FW United Kingdom
Data accessibility	Repository name: BioStudies: Array Express Data identification number: E-MTAB-13434 Direct URL to data::https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-13434?key=c8dd799b-77d0-4d51-a10b-88b6b08086ac

1. Value of the Data

- This dataset contributes to our understanding of the molecular mechanisms involved in *Pectobacterium atrosepticum* colonisation of plant hosts. This information has the potential to be applied to other soft rot *Pectobacteriaceae*, and other plant associated bacteria (phytopathogens or beneficial).
- This dataset contributes to our understanding of plant root responses, both host and non-host associated, to plant-associated bacteria
- The dataset is of benefit to researchers in plant-microbe interactions, environmental microbiology and plant responses to biotic stress
- This dataset will enable the identification of enriched sets of contributing to Pba-plant- adherence mechanisms, plant bacterial recruitment and plant stress responses.

2. Objective

Pectobacterium atrosepticum (Pba) is a gram-negative bacterium that causes blackleg and tuber soft rot of potato. Potato is a host plant for Pba but *Pectobacterium* spp can also asymptomatically colonise other (non-host) plant species [1], such as cover crops used in agricultural rotations to improve soil health [2]. Oil radish are commonly planted before potato in crop rotations and is well characterized for its biofumigant properties and anti-nematode effects [3]. Therefore, there is the need to understand the molecular processes that the bacteria use to colonise these non-host plants, and the reciprocal plant response to bacterial challenge. Using dual RNA-seq, whole transcriptome data from Pba SCRI1039 (Pba) infected potato and radish roots was collected (along with uninfected plant and Pba-only controls) to enable us to compare the transcriptional responses to pathogen challenges in both host and non-host plants.

3. Data Description

This dataset comprises 17 raw (unprocessed) paired-end RNA-sequence files in fastq format. Table 1 describes the raw RNA-seq data files for all seventeen samples. For radish, an average of

Table 1
Details of total mRNA sequence dataset from *Pectobacterium atrosepticum* colonising potato or radish roots.Column descriptors as follows. 1. Sample identifier. 2. ENA ERS accession number, 3. File name for forward (_1) and reverse (_2) reads uploaded to ENA, 4. description of the sample. 5. number of raw reads, Number of trimmed reads mapped to reference genome (6. Pba, 7. Radish. 8. Potato).

1	2	3	4	5	6	7	8
Sample ID	ENA Sample ID	Fastq Files	Sample Description	# Raw Reads (M)	# Pba Trimmed Reads	# Radish Trimmed Reads	# Potato Trimmed Reads
a1	ERS16480351	a1_1.fq, a1_2.fq	Radish	42.2	–	24,435,249	–
a2	ERS16480360	a2_1.fq, a2_2.fq	Radish	44.6	–	20,999,282	–
a3	ERS16480361	a3_1.fq, a3_2.fq	Radish	39.2	–	19,466,286	–
a4	ERS16480362	a4_1.fq, a4_2.fq	Radish + Pba	45.1	62,233	19,887,153	–
a5	ERS16480363	a5_1.fq, a5_2.fq	Radish + Pba	44.1	127,048	25,088,077	–
a6	ERS16480364	a6_1.fq, a6_2.fq	Radish + Pba	42.5	349,230	24,664,747	–
a7	ERS16480365	a7_1.fq, a7_2.fq	Radish + Pba	55.2	572,664	28,382,989	–
a8	ERS16480366	a8_1.fq, a8_2.fq	Potato	42.7	–	–	25,365,379
a9	ERS16480367	a9_1.fq, a9_2.fq	Potato	42.8	–	–	25,007,773
a10	ERS16480352	a10_1.fq, a10_2.fq	Potato	54.1	–	–	29,286,374
a11	ERS16480353	a11_1.fq, a11_2.fq	Potato + Pba	46.8	2,484,255	–	28,096,406
a12	ERS16480354	a12_1.fq, a12_2.fq	Potato + Pba	58.1	8,014,042	–	27,027,991
a13	ERS16480355	a13_1.fq, a13_2.fq	Potato + Pba	47.0	81,268	–	29,234,951
a14	ERS16480356	a14_1.fq, a14_2.fq	Potato + Pba	46.8	232,649	–	22,743,708
a15	ERS16480357	a15_1.fq, a15_2.fq	Pba	41.2	37,685,903	–	–
a16	ERS16480358	a16_1.fq, a16_2.fq	Pba	47.5	44,636,973	–	–
a17	ERS16480359	a17_1.fq, a17_2.fq	Pba	43.3	40,940,095	–	–

65% of reads mapped to reference GCA_019705865.1 [4]. For potato, an average of 73.8% of reads mapped to the reference GCF_000226075.1 [5]). For the Pba-only control samples, an average of 97.2% of reads mapped to the reference GCF_000011605.1([6]).

4. Experimental Design, Materials and Methods

4.1. Plant propagation for Pectobacterium colonisation

Radish (*Raphanus sativus* L. var. *oleiformis* Pers. cv. Bento; Kings Crops, UK) were soaked in sterile distilled water for two hours before being surface sterilised in 2% calcium hypochlorite

solution (10ml) for 15 minutes. The seeds were washed vigorously six times with sterile distilled water and germinated on distilled water agar (0.5% w/v) in the dark for 5-7 days, at ~20°C. Potato (*Solanum tuberosum*) var Estima microplants (Gentech Propagation, UK) were cut at the internode and transplanted onto MS + 20% (w/v) sucrose (MS20) agar, incubated for 14 days to establish new microplants. Seedlings, or microplants, were transplanted into 175ml pots (Greiner, UK) containing autoclaved perlite and sterile 0.5 x Murashige and Skoog (MS) medium (Merck, USA). Seedlings were grown in a cabinet with a light intensity of 150 umol m^2s^{-2} (16 hour photoperiod) for a further 7 (potato) or 21 days (radish) at 20°C before bacterial inoculation.

4.2. Bacterial challenge and RNA preparation

Pba SCRI_1039 was grown in lysogeny broth (LB) at 27°C, with shaking (180rpm) for 16 hours. Bacterial cells were collected by centrifugation at 4000 rpm and resuspended in 0.5 x MS media to wash the cells. The OD_{600} was measured and adjusted to 0.02 with 0.5 x MS to provide a suspension of 10^7 cfu/ml. This was serially diluted in 0.5 x MS to reach a final concentration of 10^4 cfu/ml for inoculating the plant culture medium with a resulting concentration of 10^3 cells per plant. Fourteen days after inoculation, plants were removed from the pots and the roots aseptically removed from the stem with an ethanol sterilised scalpel. Perlite, and loosely attached bacteria, was removed from the roots by rinsing with sterile distilled water. Roots from three plants were pooled per radish replicate and two plants pooled per potato replicate before flash freezing in liquid nitrogen. Uninoculated plant controls were sampled in parallel. Total RNA was extracted from four biological replicates per plant species challenged with bacteria and, three biological replicates of plant only controls using Macherey-Nagel Nucleospin Plant RNA kit following the manufacturers guidelines with RAP buffer. For the Pba culture only samples, three biological replicates were prepared as described above but adjusted to 10^8 cfu/ml in 0.5 x MS and incubated for 3 hours at 20°C before bacterial cell collection by centrifugation. Bacterial pellets were flash frozen in liquid nitrogen, resuspended in 100ul TE buffer + 1mg/ml lysozyme and incubated at 37°C for 10 minutes before total RNA was extracted using Macherey-Nagel Nucleospin plant RNA kit with RAI buffer and treated with Turbo DNase-free kit (Invitrogen, AM1906) to remove bacterial gDNA carryover. RNA was quantified with a Qubit RNA BR kit (Invitrogen, UK) and the quality and integrity checked using a 2100 BioAnalyser system (Agilent).

4.3. Illumina library preparation and total RNA sequencing

NovaSeq 6000 platform was used to produce paired end reads 150 base pairs in length at Novogene (UK) Company Limited (Cambridge, United Kingdom).

4.4. Illumina raw read analysis and mapping to genomes

The raw reads were quality assessed using fastqc (v0.11.9) [7]. Trimmomatic (v0.39) [8] was used to trim poor quality and short reads (using options LEADING:20 TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:30). Bowtie2 (v2.4.2) [9] was used with default settings to map reads to reference genomes for Pba (reference NZ_CP009125.1), radish (reference GCA_019705865.1) and potato (reference GCF_000226075.1).

Data Availability

Total mRNA sequence dataset from Pectobacterium atrosepticum colonising potato or radish roots (Original data) (ArrayExpress).

CRediT Author Statement

Ashleigh Holmes: Conceptualization, Funding acquisition, Methodology, Investigation, Writing – original draft; **Sonia Humphris:** Conceptualization, Funding acquisition, Writing – review & editing; **Susan Jones:** Funding acquisition, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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