

Transcriptome analysis of EcN incubated with T4 phages for 2 h

Material and methods

Extracted RNA was depleted of ribosomal RNA using the Ribo-Zero rRNA Removal Kit for bacteria (Illumina) according to the manual. Depleted RNA was fragmented for 3 min at 94°C using the NEBNext Magnesium RNA Fragmentation Module. The RNA ends were repaired with two consecutive T4 PNK incubations (-/+ ATP) and a RppH treatment. Library preparation was performed according to the NEBNext Multiplex Small RNA Library Preparation Guide for Illumina. All adapters and primers were diluted 1:4 and 15 and 16 cycles of PCR were used, respectively. No size selection was performed at the end of the protocol. 12 libraries were pooled and sequenced on a NextSeq 500 with a read length of 75 nt.

Analysis of deep-sequencing data

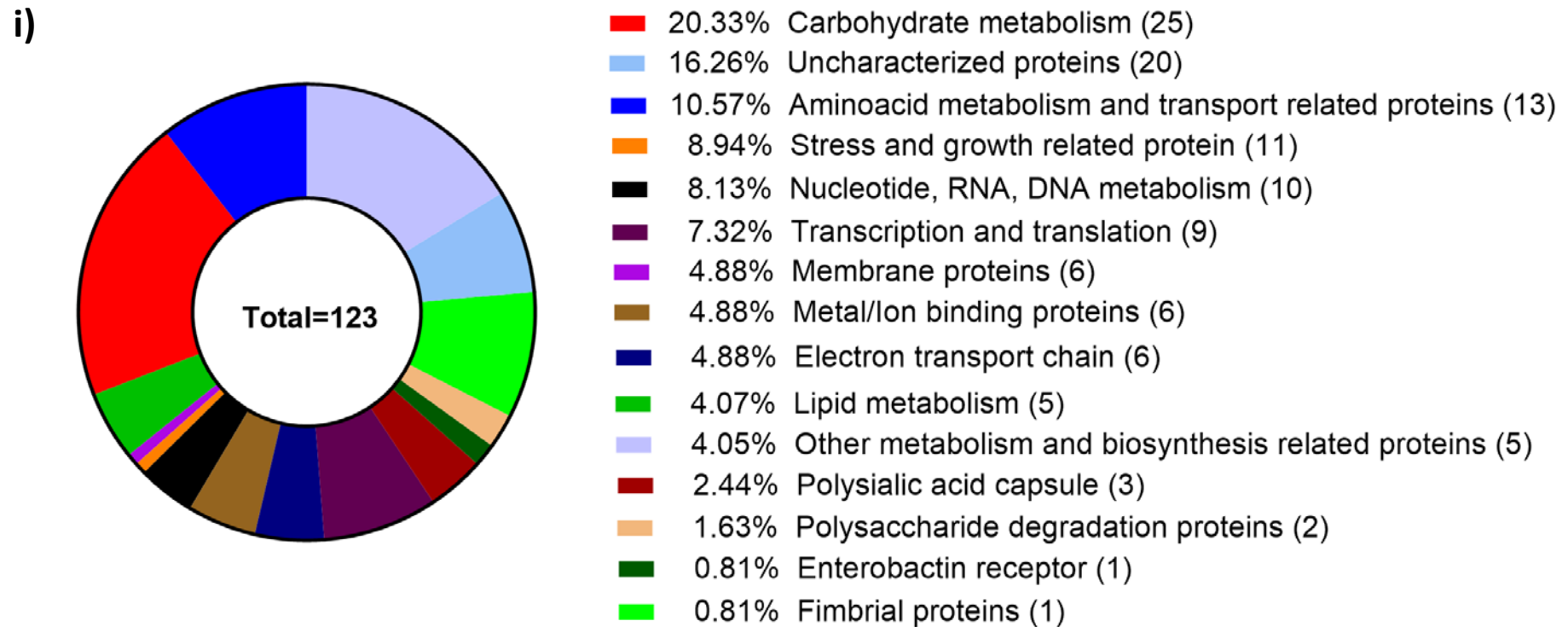
The quality of raw reads (Phred scores, number of duplicates and adapter) were assessed using FastQC (version-0.11.31) (Andrews, 2010). In order to assure a high sequence quality, the Illumina reads in FASTQ format were trimmed with a cut-off phred score of 20 by cutadapt (version-1.15) (Martin, 2011) that also was used to remove the sequencing adapter sequences. The following steps were performed using the subcommand "create", "align" and "coverage" of the tool READemption (version-0.4.3)(Forstner et al., 2014) with default parameters. Reads with a length below 20nt were removed and the remaining reads were mapped to the reference genome sequences (NCBI Reference Sequence: NZ_CP007799.1, [27 June 2014]) using segemehl (Hoffmann et al., 2009). Coverage plots in wiggle format representing the number of aligned reads per nucleotide were generated based on the aligned reads and visualized in the Integrated Genome Browser (Freese et al., 2016). Each graph was normalized to the total number of reads that could be aligned from the respective library. To restore the original data range and prevent rounding of small error to zero by genome browsers, each graph was then multiplied by the minimum number of mapped reads calculated over all libraries. The differentially expressed genes were identified using DESeq2 (version-1.16.1)(Love et al., 2014). In all cases, only genes with maximum Benjamini-Hochberg corrected p-value (padj) of 0.05, were classified as significantly differentially expressed. The data were represented as MA plots using R. The RNA-Seq data presented in this work has been deposited at the NCBI Gene Expression Omnibus (Edgar et al., 2002) and can be accessed through GEO series accession number GSE135946 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE135946>).

The results of the transcriptome analysis are presented here in the sections table S1, figure S2, table S2 and table S3

Table S1: Details of gene regulation in EcN incubated with T4 phages after 2 h when compared to EcN incubated in LB medium for 2 h

Genes downregulated ($\log_2\text{foldchange} < -2$, $\text{padj} < 0.05$)	123 genes
Genes upregulated ($\log_2\text{foldchange} > 2$, $\text{padj} < 0.05$)	33 genes

Figure S2: Donut graphs representing the functional grouping of the genes that were downregulated (i) with $\log_2\text{foldchange} < -2$, $\text{padj} < 0.05$ and upregulated (ii) with $\log_2\text{foldchange} > 2$, $\text{padj} < 0.05$ in EcN when incubated with T4 phages after 2 h. In the legend of the graph, the numbers in the bracket after the functional groups represents number of genes down or upregulated in the respective group.



ii)

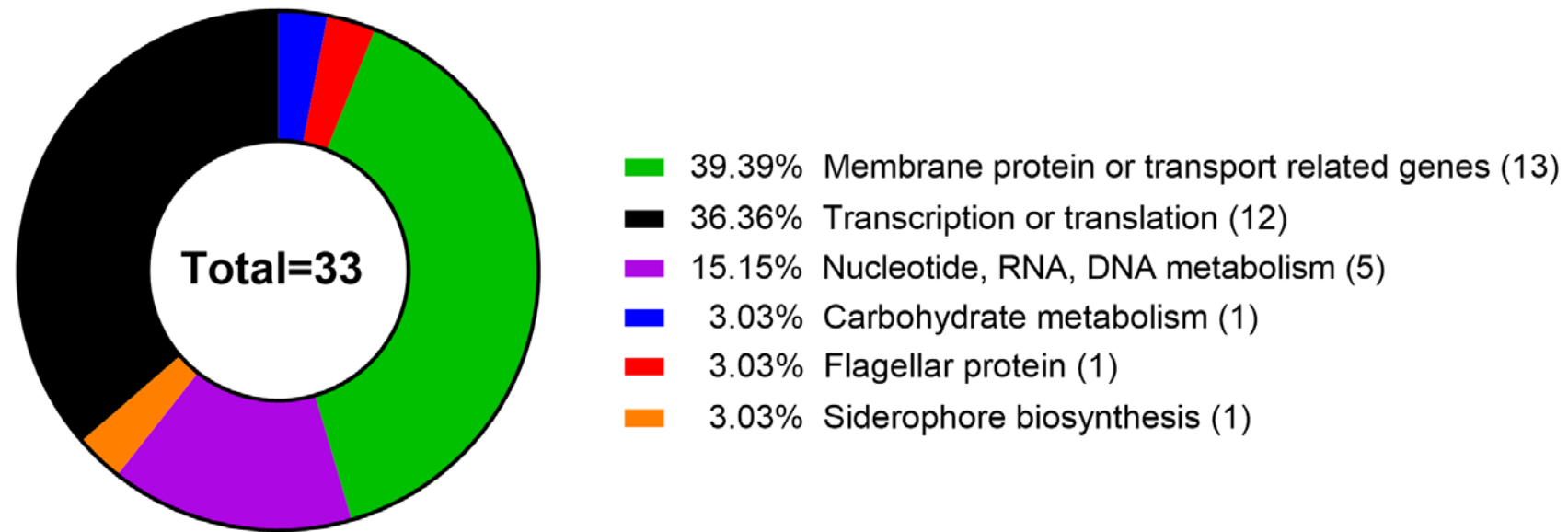


Table S2: Details of the genes downregulated in EcN when incubated with T4 phages after 2 h ($\log_2\text{foldchange} < -2$, $\text{padj} < 0.05$)

Downregulated genes	$\log_2\text{foldchange}$	Functional prediction	Functional group
EcN1917_4157	-5,074	Tryptophan production	Aminoacid metabolism and transport related
EcN1917_3127	-5,035	Aspartate/ornithine carbamoyl transferase family protein protein YgeW,	Nucleotide, RNA, DNA metabolism
EcN1917_1644	-4,859	N-acetylgalactosamine 6-sulfate sulfatase (GALNS), polysaccharide degradation protein	Polysaccharide degradation proteins
EcN1917_4791	-4,850	Uncharachetrised protein	Uncharachetrised proteins
EcN1917_4158	-4,557	Tryptophan-specific transport protein	Aminoacid metabolism and transport related genes

EcN1917_4896	-4,312	N-acetylneuraminic acid outer membrane channel protein NanC	Membrane proteins
EcN1917_3113	-4,098	Diaminopimelate decarboxylase (EC 4.1.1.20), amino acid biosynthesis	Aminoacid metabolism and transport related genes
EcN1917_3447	-4,033	Glycogen synthesis protein GlgS , glycogen synthesis	Carbohydrate metabolism
EcN1917_3135	-3,953	Predicted oxidoreductase 2C Fe-S subunit, membrane proteins	Membrane proteins
EcN1917_3128	-3,945	Diaminopropionate ammonia-lyase (EC 4.3.1.15), amino acid metabolism	Aminoacid metabolism and transport related genes
EcN1917_4920	-3,898	Uncharacterised protein	Uncharacterised proteins
EcN1917_4483	-3,787	Zinc resistance associated protein, stress related protein	Stress related proteins
EcN1917_2521	-3,762	Acetyl-CoA:acetoacetyl-CoA transferase 2C alpha subunit (EC 2.8.3.8), lipid/fatty acid metabolism	Lipid metabolism
EcN1917_3136	-3,752	SsnA protein, metal/ion binding	Metal/ion binding
EcN1917_3523	-3,710	Uncharacterised protein	Uncharacterised proteins
EcN1917_2441	-3,598	Pseudouridine 5'-phosphate glycosidase, nucleotide metabolism	Nucleotide, RNA, DNA metabolism
EcN1917_1574	-3,590	YdcH protein , uncharacterised protein	Uncharacterised proteins
EcN1917_0001	-3,586	Aspartokinase (EC 2.7.2.4) / homoserine dehydrogenase (EC 1.1.1.3), homoserine biosynthetic process	Aminoacid metabolism and transport related genes
EcN1917_2442	-3,512	Pseudouridine kinase (EC 2.7.1.83), nucleotide metabolism	Nucleotide, RNA, DNA metabolism
EcN1917_3129	-3,436	Putative deacetylase YgeY, metal/ion binding	Metal/ion binding
EcN1917_2543	-3,428	Uncharacterised protein	Uncharacterised protein

EcN1917_3133	-3,386	Accessory protein YqeC in selenium-dependent molybdenum hydroxylase maturation, transcription and translation	Transcription and translation
EcN1917_3137	-3,344	Uncharacterised protein YgfM	Uncharacterised protein
EcN1917_2868	-3,250	Ribosome hibernation protein YfiA, translation	Transcription and translation
EcN1917_3072	-3,210	Type 6 secretion system tube protein	Membrane proteins
EcN1917_2542	-3,175	Anaerobic glycerol-3-phosphate dehydrogenase subunit C (EC 1.1.5.3), electron transport chain	Electron transport chain
EcN1917_4488	-3,074	Translation elongation factor Tu	Transcription and translation
EcN1917_4482	-3,045	Zinc resistance-associated protein, stress related protein	Stress related proteins
EcN1917_4723	-3,040	Possible membrane protein	Membrane proteins
EcN1917_1544	-3,032	Universal stress protein F	Stress related proteins
EcN1917_2652	-3,012	D-serine permease DsdX, D-serine transport	Aminoacid metabolism and transport related genes
EcN1917_1383	-2,981	Uncharacterised protein	Uncharacterised protein
EcN1917_0003	-2,971	Threonine synthase (EC 4.2.3.1), threonine biosynthetic process	Aminoacid metabolism and transport related genes
EcN1917_3134	-2,966	CTP:molybdopterin cytidylyltransferase , translation	Transcription and translation
EcN1917_2538	-2,940	Glycerophosphoryl diester phosphodiesterase 2C periplasmic, lipid metabolism	Lipid metabolism
EcN1917_4895	-2,931	Sialic acid-induced transmembrane protein YjhT(NanM) 2C possible mutarotase, polysialicacid capsule metabolism	Polysialicacid capsule
EcN1917_2426	-2,924	Galactose/methyl galactoside ABC transport system 2C D-galactose-binding periplasmic protein MglB (TC 3.A.1.2.3), galactose transport	Carbohydrate metabolism

EcN1917_2540	-2,904	Anaerobic glycerol-3-phosphate dehydrogenase subunit A (EC 1.1.5.3), lipid metabolism	Lipid metabolism
EcN1917_3610	-2,898	Putative sugar isomerase involved in processing of exogenous sialic acid, polysialicacid capsule metabolim	Polysialicacid capsule
EcN1917_2423	-2,891	Uncharacterised protein	Uncharachterised protein
EcN1917_0906	-2,883	Cold shock protein CspD	Stress related protein
EcN1917_1643	-2,881	GALNS arylsulfatase regulator (Fe-S oxidoreductase), metal/ion binding	Metal/ion binding
EcN1917_3138	-2,857	Possible hypoxanthine oxidase XdhD, purine nucleobase metabolic process	Nucleotide, RNA, DNA metabolism
EcN1917_0002	-2,839	Homoserine kinase (EC 2.7.1.39), threonine biosynthetic process	Aminoacid metabolism and transport related genes
EcN1917_0616	-2,808	Enterobactin receptor protein	Enterobactin receptor
EcN1917_0651	-2,795	Citrate [pro-3S]-lyase] ligase (EC 6.2.1.22), involved in biosynthetic process	Other metabolism and biosynthesis related proteins
EcN1917_3664	-2,784	Uncharacterised protein	Uncharachterised protein
EcN1917_4722	-2,772	Putative Uncharacterised protein YjfN	Uncharachterised protein
EcN1917_4790	-2,735	Arginine deiminase (EC 3.5.3.6), arginine metabolic process	Aminoacid metabolism and transport related genes
EcN1917_1048	-2,730	Galactosamine-6-phosphate isomerase, glycolysis	Carbohydrate metabolism and transport related genes
EcN1917_2440	-2,729	Inner membrane transport protein YeiM 2C in cluster with pseudouridine metabolism operon, RNA metabolism	Nucleotide, RNA, DNA metabolism
EcN1917_1014	-2,658	Uncharacterised protein	Uncharachterised protein

EcN1917_4789	-2,634	Carbamate kinase (EC 2.7.2.2), arginine metabolic process	Aminoacid metabolism and transport related genes
EcN1917_2539	-2,629	Glycerol-3-phosphate transporter, glycolysis	Carbohydrate metabolism and transport related genes
EcN1917_2541	-2,624	Anaerobic glycerol-3-phosphate dehydrogenase subunit B (EC 1.1.5.3), lipid metabolism	Lipid metabolism
EcN1917_0062	-2,617	L-ribulose-5-phosphate 4-epimerase (EC 5.1.3.4), carbohydrate metabolism	Carbohydrate metabolism and transport related genes
EcN1917_2421	-2,616	Pyridine nucleotide-disulphide oxidoreductase family protein, electron transport chain	Electron transport chain
EcN1917_0407	-2,593	Threonine efflux protein,	Aminoacid metabolism and transport related genes
EcN1917_0417	-2,592	Galactoside O-acetyltransferase, lactose biosynthesis	Carbohydrate metabolism
EcN1917_0035	-2,577	Transcriptional activatory protein CaiF, transcription	Transcription and translation
EcN1917_2813	-2,577	Stationary phase inducible protein CsiE, stress/ growth related protein	Stress related protein
EcN1917_4398	-2,554	Glycerol uptake facilitator protein, glycerol transport	Carbohydrate metabolism
EcN1917_2653	-2,552	D-serine dehydratase (EC 4.3.1.18), D-serine metabolism	Aminoacid metabolism and transport related genes
EcN1917_2422	-2,528	Dihydropyrimidine dehydrogenase [NADP+] (EC 1.3.1.2), nucleotide metabolism	Nucleotide, RNA, DNA metabolism
EcN1917_2537	-2,521	Protein inaA, stress related protein	Stress related protein
EcN1917_0753	-2,518	Uncharacterised protein	Uncharacterised protein
EcN1917_4134	-2,512	Heat shock protein, stress related protein	Stress related protein
EcN1917_0275	-2,483	Uncharacterised protein	Uncharacterised protein

EcN1917_2958	-2,479	tRNA metabolism, translation	Transcription and translation
EcN1917_3520	-2,474	D-glucarate catabolic process	Carbohydrate metabolism
EcN1917_3521	-2,464	2-hydroxy-3-oxopropionate reductase (EC 1.1.1.60), glyoxylate metabolic process	Carbohydrate metabolism
EcN1917_3132	-2,446	Selenium-dependent molybdenum hydroxylase system protein YqeB, transcription	Transcription and translation
EcN1917_3019	-2,445	Uncharacterised protein	Uncharacterised protein
EcN1917_3517	-2,419	Threonine catabolic operon transcriptional activator TdcA, transcription	Transcription and translation
EcN1917_0631	-2,417	Carbon starvation protein A, stress related protein	Stress related protein
EcN1917_2252	-2,414	Uncharacterised protein	Uncharacterised protein
EcN1917_2519	-2,398	Sensory histidine kinase AtoS, membrane protein	Membrane protein
EcN1917_1013	-2,392	Glucose-1-phosphatase (EC 3.1.3.10), glycolysis	Carbohydrate metabolism and transport related genes
EcN1917_0063	-2,374	L-arabinose isomerase (EC 5.3.1.4), carbohydrate metabolism a	Carbohydrate metabolism and transport related genes
EcN1917_0675	-2,373	Uncharacterised protein	Uncharacterised protein
EcN1917_4649	-2,349	Melibiose operon regulatory protein, transcription	Transcription and translation
EcN1917_2271	-2,340	Uncharacterised protein	Uncharacterised protein
EcN1917_2741	-2,337	Ethanolamine utilization polyhedral-body-like protein EutS, polysaccharide degradation	Polysaccharide degradation proteins
EcN1917_3131	-2,320	Carbamate kinase-like protein YqeA	Aminoacid metabolism and transport related genes
EcN1917_4397	-2,314	Glycerol kinase (EC 2.7.1.30)	Lipid metabolism
EcN1917_1015	-2,307	Flavoprotein WrbA, stress related protein	Stress related protein

EcN1917_3613	-2,306	Sialic acid transporter (permease) NanT, polysialicacid capsule metabolism	Polysialicacid capsule metabolism
EcN1917_1387	-2,304	Phosphoenolpyruvate-dihydroxyacetone phosphotransferase (EC 2.7.1.121), glycolysis	Carbohydrate metabolism
EcN1917_2438	-2,303	Inosine-uridine preferring nucleoside hydrolase (EC 3.2.2.1), metal binding in metabolic process	Metal/ion binding
EcN1917_0298	-2,300	putative membrane protein	Membrane protein
EcN1917_0646	-2,292	2-(5"-triphosphoribosyl)-3'-dephosphocoenzyme- A synthase (EC 2.7.8.25), prosthetic group biosynthetic process	Other metabolism and biosynthesis related proteins
EcN1917_2096	-2,271	Uncharacterised protein	Uncharacterised protein
EcN1917_1786	-2,266	putative oxidoreductase 2C Fe-S subunit, metal/ion binding	Metal/ion binding
EcN1917_3528	-2,260	Tagatose-6-phosphate kinase AgaZ (EC 2.7.1.144), galactitol metabolic process	Carbohydrate metabolism
EcN1917_1839	-2,255	Ribulosamine/erythrulosamine 3-kinase potentially involved in protein deglycation	Carbohydrate metabolism
EcN1917_3144	-2,246	Xanthine permease, involved in metabolic processes	Other metabolism and biosynthesis related proteins
EcN1917_3524	-2,228	D-galactarate dehydratase (EC 4.2.1.42), carbohydrate metabolism	Carbohydrate metabolism
EcN1917_3126	-2,227	Uncharacterised sigma-54-dependent transcriptional regulator YgeV, transcription	Transcription and translation
EcN1917_3614	-2,224	N-acetylneuraminate lyase (EC 4.1.3.3), carbohydrate metabolism	Carbohydrate metabolism
EcN1917_1597	-2,202	Transcriptional regulator 2C GntR family, transcription	Transcription and translation

EcN1917_3048	-2,201	L-fucose phosphate aldolase (EC 4.1.2.17), carbohydrate metabolism	Carbohydrate metabolism
EcN1917_3124	-2,188	Xanthine dehydrogenase 2C FAD binding subunit (EC 1.17.1.4), nucleotide metabolism	Nucleotide, RNA, DNA metabolism
EcN1917_3388	-2,176	Predicted oxidoreductases (related to aryl-alcohol dehydrogenases), electron transport chain	Electron transport chain
EcN1917_2073	-2,166	Ferritin-like protein 2, metal binding	Metal/ion binding
EcN1917_3612	-2,158	N-acetylmannosamine-6-phosphate 2-epimerase (EC 5.1.3.9), carbohydrate metabolism	Carbohydrate metabolism
EcN1917_2072	-2,148	L-arabinose-binding periplasmic protein precursor AraF (TC 3.A.1.2.2), L-arabinose transport	Carbohydrate metabolism
EcN1917_3935	-2,137	Cytochrome c551 peroxidase (EC 1.11.1.5), electron transport chain	Electron transport chain
EcN1917_1556	-2,121	Aldo-keto reductase, electron transport chain	Electron transport chain
EcN1917_3925	-2,120	Chaperone HdeB, acid stress	Stress related protein
EcN1917_3926	-2,115	Chaperone HdeA, acid stress	Stress related protein
EcN1917_3130	-2,097	Dihydropyrimidinase (EC 3.5.2.2), metal/ion binding	Metal/ion binding
EcN1917_1384	-2,096	Uncharacterised protein	Uncharacterised protein
EcN1917_0030	-2,090	Uncharacterised protein	Uncharacterised protein
EcN1917_1613	-2,089	mobile element protein	Uncharacterised protein
EcN1917_4609	-2,087	Uncharacterised protein	Uncharacterised protein
EcN1917_3830	-2,078	Fimbrial protein	Fimbrial protein
EcN1917_0442	-2,067	Putative inner membrane protein	Membrane protein
EcN1917_2824	-2,059	Uncharacterised protein	Uncharacterised protein
EcN1917_4889	-2,039	Uncharacterised protein	Uncharacterised protein

EcN1917_2425	-2,038	Galactose/methyl galactoside ABC transport system 2C ATP-binding protein MglA (EC 3.6.3.17), galactose transport system	Carbohydrate metabolism
EcN1917_0632	-2,032	Hypothetical small protein yjiX /replicative DNA helicase, DNA metabolism	Nucleotide, RNA, DNA metabolism
EcN1917_3522	-2,029	D-glucarate permease, carbohydrate metabolism and transport related genes	Carbohydrate metabolism
EcN1917_3534	-2,017	Galactosamine-6-phosphate isomerase, carbohydrate metabolism	Carbohydrate metabolism

Table S3: Details of the genes upregulated in EcN when incubated with T4 phages after 2 h (log₂foldchange>2, padj<0.05)

Upregulated genes	log₂foldchange	Functional prediction	Functional group
EcN1917_1772	4,9758	Putative transport protein	Membrane protein or transport related genes
EcN1917_3763	3,4163	TsgA protein homolog, involved in transmembrane transport	Membrane protein or transport related genes
EcN1917_4067	2,7274	Putative inner membrane protein	Membrane protein or transport related genes
EcN1917_2418	2,6690	Cytidine deaminase, nucleoside metabolism	Nucleotide, RNA, DNA metabolism
EcN1917_2679	2,6261	Nucleoside permease NupC, nucleotide transport	Nucleotide, RNA, DNA metabolism
EcN1917_3557	2,5741	DEAD-box ATP-dependent RNA helicase CshA, ribosome biogenesis	Transcription or translation

EcN1917_0131	2,5133	PQQ-dependent glucose dehydrogenase (EC 1.1.5.2), pentose phosphate pathway	Carbohydrate metabolism
EcN1917_4569	2,4706	YjcB protein, membrane protein	Membrane protein or transport related genes
EcN1917_1507	2,4084	ABC-type sugar transport system periplasmic binding protein YcjN	Membrane protein or transport related genes
EcN1917_2337	2,4051	Polysaccharide export lipoprotein Wza, LPS synthesis	Membrane protein or transport related genes
EcN1917_4973	2,4040	Thymidine phosphorylase (EC 2.4.2.4), nucleoside metabolism	Nucleotide, RNA, DNA metabolism
EcN1917_1631	2,3324	Bdm protein, positive regulation of bacterial-type flagellum assembly	Flagella protein
EcN1917_4972	2,2810	Deoxyribose-phosphate aldolase (EC 4.1.2.4), involved in deoxyribose phosphate catabolic process	Nucleotide, RNA, DNA metabolism
EcN1917_0626	2,2317	Isochorismate synthase (EC 5.4.4.2), siderophore biosynthesis	Siderophore biosynthesis
EcN1917_0474	2,2233	Nucleoside-specific channel-forming protein Tsx precursor	Nucleotide, RNA, DNA metabolism
EcN1917_3698	2,1533	LSU ribosomal protein L15p (L27Ae)	Transcription or translation
EcN1917_2278	2,1440	Putrescine importer	Membrane protein or transport related genes
EcN1917_3978	2,1340	Outer membrane protein A precursor	Membrane protein or transport related genes
EcN1917_4843	2,1094	LysR family transcriptional regulator	Transcription or translation

EcN1917_1516	2,0761	Outer membrane protein G precursor	Membrane protein or transport related genes
EcN1917_1275	2,0746	Spermidine putrescine ABC transporter permease component PotB	Membrane protein or transport related genes
EcN1917_3579	2,0602	Permease of the drug/metabolite transporter (DMT) superfamily	Membrane protein or transport related genes
EcN1917_0624	2,0518	Enterobactin exporter EntS	Membrane protein or transport related genes
EcN1917_0059	2,0513	Ribosomal large subunit pseudo-uridine synthase A	Transcription or translation
EcN1917_3693	2,0468	SSU ribosomal protein S4p (S9e)	Transcription or translation
EcN1917_3710	2,0467	LSU ribosomal protein L16p (L10e)	Transcription or translation
EcN1917_3657	2,0434	DNA-binding protein Fis	Transcription or translation
EcN1917_0936	2,0347	SSU ribosomal protein S1p	Transcription or translation
EcN1917_3709	2,0342	LSU ribosomal protein L29p (L35e)	Transcription or translation
EcN1917_3697	2,0294	Preprotein translocase <i>secY</i> subunit, secretory pathway	Membrane protein or transport related genes
EcN1917_1625	2,0177	Formate dehydrogenase O beta subunit (EC 1.2.1.2), electron carrier activity	Transcription or translation
EcN1917_3692	2,0050	DNA-directed RNA polymerase alpha subunit (EC 2.7.7.6), DNA templated transcription	Transcription or translation
EcN1917_0821	2,0030	ATP-dependent RNA helicase RhIE, ribosome assembly	Transcription or translation

References:

1. Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. .
2. Edgar, R., Domrachev, M., and Lash, A.E. (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* 30, 207-210.
3. Forstner, K.U., Vogel, J., and Sharma, C.M. (2014). READemption-a tool for the computational analysis of deep-sequencing-based transcriptome data. *Bioinformatics* 30, 3421-3423.
4. Freese, N.H., Norris, D.C., and Loraine, A.E. (2016). Integrated genome browser: visual analytics platform for genomics. *Bioinformatics* 32, 2089-2095.
5. Hoffmann, S., Otto, C., Kurtz, S., Sharma, C.M., Khaitovich, P., Vogel, J., Stadler, P.F., and Hackermuller, J. (2009). Fast mapping of short sequences with mismatches, insertions and deletions using index structures. *PLoS Comput Biol* 5, e1000502.
6. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, 550.
7. Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, 17, 3.