1 **Wild-type *Caenorhabditis elegans* Isolates Exhibit Distinct Gene Expression Profiles in**

2 **Response to Microbial Infection**

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Abstract

The soil-dwelling nematode *Caenorhabditis elegans* serves as a model system to study innate immunity against microbial pathogens. *C. elegans* have been collected from around the world, where they, presumably, adapted to regional microbial ecologies. Here we use survival assays and RNA-sequencing to better understand how two isolates from disparate climates respond to pathogenic bacteria. We found that, relative to N2 (originally isolated in Bristol, UK), CB4856 (isolated in Hawaii), was more susceptible to the Gram-positive pathogen, *Staphylococcus epidermidis*, but equally susceptible to *Staphylococcus aureus* as well as two Gram-negative pathogens, *Providencia rettgeri* and *Pseudomonas aeruginosa*. We performed transcriptome analysis of infected worms and found gene-expression profiles were considerably different in an isolate-specific and pathogen-specific manner. We performed GO term analysis to categorize differential gene expression in response to *S. epidermidis*. In N2, genes that encoded detoxification enzymes and extracellular matrix proteins were significantly enriched, while in CB4856, genes that encoded detoxification enzymes, C-type lectins, and lipid metabolism proteins were enriched, suggesting they have different responses to these bacterial pathogens, despite being the same species. Overall, discerning gene expression signatures in an isolate by pathogen manner can help us to understand the different possibilities for the evolution of immune responses within organisms.

39 **Introduction**

40 Antimicrobial resistance is a looming public health crisis as microbial pathogens evade drugs
41 of last resort. When pathogenic microbes bypass the physical barriers of the host (*e.g.,* an
42 epidermis), an organism must rely on cellular and humoral defenses (*e.g.,* the innate immune
43 system) to protect itself from infections that can compromise organismal fitness. While all
44 organisms will inevitably become infected over their lifespan, genetic variation in individuals will
45 influence the outcomes of infections. Thus, our ability to link genetic variation to infection
46 susceptibility may enable more sophisticated strategies for deploying antibiotics or help us
47 enhance host immunity to treat microbial infection.[1,2]

48 Genetic variation in organisms can create tradeoffs in susceptibility to different pathogens.
49 The presence of non-synonymous single nucleotide polymorphisms (SNPs) in the coding
50 sequence of immune effector genes has been shown to alter the propensity of individuals to
51 become infected and develop disease.[3] For instance, a SNP in the *TLR5* gene introduces a
52 premature stop codon, creating a truncated form of the protein and impairing the ability of TLR5
53 to recognize the flagellin protein in flagellated bacteria.[4] Individuals carrying this SNP are more
54 susceptible to infection by *Legionella pneumophila*, the vector for Legionnaires' disease, but not
55 *Salmonella enterica serotype Typhi,* the microbial vector for typhoid fever.[5,6]

56 Animal models have been critical to our understanding of the innate immune system.
57 However, the reliance on canonical laboratory strains often ignores genetic variation in innate
58 immunity and susceptibility to pathogens.[7] The soil-dwelling nematode *Caenorhabditis elegans*
59 is a genetically tractable model organism to examine host-pathogen interactions.[8,9] *C. elegans*

60 possesses a robust microbial defense system with evolutionarily conserved mechanisms in
61 microbial pathogen detection, immune activation and pathogen killing (*e.g.* the induction of
62 reactive oxygen species (ROS), deployment of antimicrobial and antifungal peptides, and
63 expression of bacterial-binding C-type lectins).[10-14] These evolutionarily conserved responses to
64 infection make *C. elegans* an ideal model to study bacterial pathogenesis.[9]

65 *C. elegans* wild isolates collected from geographically discrete regions are apt to vary in their
66 ability to fight infections either because of a history of different selective pressures or simply
67 genetic drift. Many discoveries regarding *C. elegans* host-microbe interactions have come from
68 studying wild isolates, including identifying virus that infect *C. elegans* and the characterization
69 of the *C. elegans* gut microbiome.[15-17] The genomic diversity of wild isolates has also provided a
70 platform for quantitatively examining the genetic variation underlying complex traits, including
71 behavioral differences in foraging as well as pathogen avoidance and susceptibility.[18-23] However,
72 less attention has been paid to the variation in the transcriptomic responses of wild isolates to
73 multiple, discrete microbial pathogens.

74 In this study, we show that the *C. elegans* Hawaiian wild isolate CB4856 is significantly more
75 susceptible to infection by the Gram-positive pathogen *Staphylococcus epidermidis* than the N2
76 lab-conditioned strain. We used high-throughput RNA sequencing (RNA-seq) to identify
77 differentially expressed genes in staged N2 and CB4856 worms exposed to pathogenic bacteria.
78 The differentially expressed genes identified in pathogen-infected animals provide insight into
79 isolate-specific transcriptomic responses of *C. elegans* to pathogenic bacteria. We believe that

80 this knowledge will provide a foundation for better understanding the role of natural genetic

81 variation in the *C. elegans* innate immune response to microbial pathogens.

82 ## Materials and Methods

83 ### *C. elegans* and Bacterial Strains

84 *C. elegans* N2 and CB4856 wild-type strains were maintained at 20°C on nematode growth
85 medium (NGM) agar plates seeded with *Escherichia coli* OP50.[24] *Staphylococcus epidermidis*
86 (EVL2000, this study), *Staphylococcus epidermidis* (1191; ATCC#700562), *Providencia rettgeri*
87 (Dmel1)[25], *Pseudomonas aeruginosa* (PA-14)[26], and *Staphylococcus aureus* (Newman)[27] were
88 used as pathogens in this study. All bacteria strains were grown at 37°C with shaking in low-salt
89 Luria Bertani (LB) medium (10 g Bacto-tryptone, 5 g yeast extract, 5 g NaCl).

90

91 ### Identification of *S. epidermidis* Strain

92 DNA was extracted from 1mL of fresh culture using a QiaQuick miniprep column (Qiagen) and
93 the 16S ribosomal region was amplified using universal primers 27F (5'-
94 AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3'). PCR cycling
95 conditions were as follows: 1) 92°C for 2 minutes; 2) 35 cycles at 92°C for 15-sec, 55°C for 15-sec,
96 68°C for 75-seconds; and 3) 68°C for 10 minutes. The amplified PCR product was purified, Sanger
97 sequenced, and the NCBI BLAST tool was used to identify the species.

98

99 ### Survival Assays

100 NGM plates were seeded with 40 µL of bacteria (*E. coli*, *S. epidermidis*, *P. rettgeri*, *P.*
101 *aeruginosa*, *or S. aureus*) and incubated overnight at 37°C. Plates were acclimated to room

102 temperature and 30 late-stage L4 worms were added. Each day, living worms were transferred
103 to fresh plates and the number of dead worms was recorded.
104
105 **Pathogen Avoidance Assays**
106 NGM plates were seeded with a 10 µL dot of pathogenic bacteria (*S. epidermidis*, *P. rettgeri*,
107 *S. aureus*, or *P. aeruginosa*) on one half, a 10 µL dot of non-pathogenic *E. coli* OP50 on the other
108 half and incubated overnight at 37°C. 20 late-stage L4 worms were placed on blank plates for 30
109 minutes to remove any external bacteria while pathogen avoidance plates acclimated to room
110 temperature. Worms were placed at the midline and their location on the plate was recorded at
111 1-hour, 2-hour, 4-hour, 8-hour, and 24-hour time points. To avoid ambiguity in the number of
112 animals associated with the specific bacteria, only animals residing within the spot of bacteria
113 were assigned "preference" for either the control (OP50) or pathogen.
114
115 **Exposure of Nematodes to Pathogen**
116 NGM plates were seeded with 250 µL of bacteria incubated overnight at 37°C. Approximately
117 2,000 L4 stage worms grown on *E. coli* OP50 were transferred to NGM plates seeded with bacteria
118 and incubated at 20°C for 24 hours. After 24 hours, the worms were washed with M9 buffer three
119 times to remove any residual bacteria. The worms were resuspended in 100 µL of M9 buffer and
120 mechanically disrupted in liquid nitrogen using a ceramic mortar and pestle. Frozen tissue was
121 transferred to a microcentrifuge tube and 1 mL TRIZOL reagent was added. The tubes were flash

122 frozen in liquid nitrogen and stored at -80°C. Three biological replicates were collected for each
123 worm and pathogen strain.
124
125 **RNA isolation and Sequencing**
126 Frozen worm tissue was thawed, vortexed for 5 seconds and incubated at room temperature
127 for 5 minutes. After the addition of 470 µL chloroform (mixed by inversion and phase separated
128 for 2 minutes at room temperature), the samples were centrifuged at 15,000 RPM at 4°C for 15
129 minutes. The upper aqueous phase containing RNA (approximately 600 µL) was transferred to a
130 new RNase-free Eppendorf tube. Total RNA extraction was carried out using the Monarch RNA
131 Cleanup Kit (New England Biolabs) according to the manufacturer's instructions. Total RNA was
132 quantified using the Qubit (ThermoFisher Scientific) and RNA quality and integrity was assessed
133 using the Agilent Tapestation 2200 (Agilent Technologies). Sequence libraries were prepared
134 using the TruSeq Stranded mRNA Library Prep Kit (Illumina) and sequenced using single-end 1x75
135 bp sequencing on the Illumina NextSeq 550.
136
137 **RNA-seq Analysis and Quantification of Differentially Expressed Genes**
138 The quality of the 75-bp single-end reads generated using the Illumina NextSeq 550 was
139 assessed using FastQC[28] (v.0.11.5). Illumina sequencing adapters and low-quality bases (Phred
140 score <30) were trimmed from reads using Fastp[29] (v.0.19.8). Reads were first aligned to the *E.*
141 *coli*, *S. epidermidis*, *P. rettgeri*, *S. aureus*, or *P. aeruginosa* genomes to evaluate bacterial
142 contamination, then aligned to the *C. elegans* genome assembly (Wormbase WS273 release)

143 using HISAT2[30] (v.2.1.0) with default settings. Aligned reads were mapped to the *C. elegans*
144 annotated genome (Wormbase WS273 release) and transcript abundances were quantified using
145 featureCounts[31] (v.1.6.0). Data normalization and differential gene expression analysis was
146 performed with DESeq2[32] (v.3.1.0) in R (v.3.6.0) using a false discovery rate (FDR)-adjusted P-
147 value <0.05 and fold change ≥2 as cutoffs. Separate design formulas were used to determine
148 differential gene expression for infection (~Infection), Gram-stain (~Gram-stain), and individual
149 pathogens (~Pathogen). Geneset enrichment analysis was performed with WormCat[33] using an
150 FDR-adjusted P-value <0.05 as the significance cutoff.
151
152 **Statistical Analysis**
153 All statistical analysis was carried out using SigmaPlot 14.0 (Systat Software, Inc.). Log rank
154 (Mantel-Cox) analysis was used for pairwise comparison of survival curves and to calculate
155 median survival (LT_{50}). Chi-squared or Fisher's Exact Test was used to assess statistical
156 significance of the pathogen avoidance data. Spearman's correlation was used to determine the
157 correlation coefficient between log_2 fold changes in gene expression. Statistically significant
158 differences are defined in the figure legend and noted in the figure with asterisks.
159
160

161 ## Results

162 **CB4856 and N2 exhibit differences in susceptibility to the pathogen *S. epidermidis***

163 During our studies on susceptibility to microbial pathogens we serendipitously cultured a
164 bacterial contaminant that exhibited differences in a *C. elegans* isolate-specific manner. We
165 identified this bacterium as *Staphylococcus epidermidis* by 16S sequencing (see Materials and
166 Methods) and provided it with the strain designation EVL2000. Survival assays conducted with
167 the EVL2000 *S. epidermidis* strain found CB4856 animals to be significantly more susceptible than
168 N2, with a median survival (LT_{50}) of 11.3 ± 1.6 days, whereas 50% of N2 animals were killed after
169 an average of 18.8 ± 1.5 days (p<0.001) (Figure 1A, Table S1).

170 We then compared N2 and CB4856 susceptibility to other known *C. elegans* pathogens,
171 *Staphylococcus aureus, Providencia rettgeri*, and *Pseudomonas aeruginosa.* In each case, the
172 susceptibility of N2 and CB4856 to these pathogens was not significantly different (Figures 1B-D,
173 Table S1). We also grew animals on non-pathogenic *E. coli* OP50 to evaluate the possibility that
174 the increased susceptibility of *S. epidermidis* was due to a general defect in lifespan. Interestingly,
175 the lifespan of N2 worms was significantly shorter than CB4856, with 50% of animals dying at
176 12.0 ± 0.4 and 16.2 ± 0.6 days for N2 and CB4856 animals, respectively (p<0.001) (Figure 1E, Table
177 S1). While there were differences in lifespan on *E. coli*, our data are consistent with the idea that
178 the increased susceptibility of CB4856 to *S. epidermidis* is not due to a universal decrease in
179 lifespan.

180 Relative to worms fed *E. coli*, infection with the EVL2000 *S. epidermidis* strain actually
181 prolonged the median lifespan of N2 animals (*E. coli* OP50 LT_{50} = 12.0 ± 0.4 days, *S. epidermidis*

182 EVL2000 LT_{50} = 18.8 ± 1.5 days; $p<0.001$). To determine if the differences in susceptibility were
183 unique to the EVL2000 strain, we infected N2 and CB4856 animals with an additional *S.*
184 *epidermidis* strain (1191). CB4856 animals infected with the 1191 strain had a median survival of
185 5.7 ± 1.2 days, whereas 50% of N2 animals were killed after an average of 12.3 ± 0.9 days
186 ($p<0.001$) (Figure 1F, Table S1). Together, our results indicate that CB4856 is more susceptible to
187 *S. epidermidis* infection and that this increase in susceptibility is observed across multiple strains
188 of *S. epidermidis.* Since infection by the *S. epidermidis* EVL2000 strain provided the greatest
189 difference in susceptibility between N2 and CB4856, follow-up experiments used this bacterial
190 strain.
191
192 **Pathogen avoidance of N2 and CB4856 isolates depends on the pathogen and time of**
193 **observation**
194 We performed two-choice pathogen avoidance assays to determine if the difference in
195 susceptibility could be explained in part by differences in animal behavior (Figure 2A). At the 1-
196 hr time point there was a significant difference in *S. epidermidis* avoidance, with 100% of N2 and
197 70% of CB4586 animals on the OP50 lawn (Figure 2B) ($p<0.001$). By 24 hours the difference in
198 pathogen avoidance was no longer significant and nearly 100% of worms from both isolates were
199 found on the OP50 lawn. This suggests that both genotypes perceived *S. epidermidis* as
200 pathogenic and avoided them accordingly.
201 We also examined avoidance behavior in N2 and CB4856 animals exposed to *S. aureus*, *P.*
202 *rettgeri* or *P. aeruginosa* and found differences in the initial avoidance of pathogenic bacteria. At

203 the 1-hr time point avoidance of *S. aureus* was not significantly different between the two
204 isolates, with 80% and 70% of animals observed on the OP50 lawn for N2 and CB4856,
205 respectively (Figure 2C) (p=0.390). Avoidance of *P. rettgeri* at the 1-hr time point was significantly
206 more likely in N2 worms, with 53% of N2 and 19% of CB4856 animals on the OP50 lawn (Figure
207 2D) (p<0.001). The avoidance of *P. aeruginosa* trended in the opposite direction with 46% of N2
208 and 65% of CB4856 animals on the OP50 lawn (Figure 2E) (p=0.051). Neither the N2 nor CB4856
209 genotypes had significant changes in pathogen avoidance between the 1-hr and 24-hr time points
210 for these three pathogens (Figure 2C-E). Natural variation in olfactory preference may partially
211 explain these differences in pathogen avoidance.[34] While the results are interesting and warrant
212 follow-up, they suggest that the avoidance behavior of N2 and CB4856 animals likely has little
213 impact on susceptibility to *S. epidermidis*.
214

215 **Gene expression changes during infection cluster by genotype, rather than pathogen type**

216 To identify gene expression changes due to pathogen we performed RNA sequencing
217 comparing CB4856 and N2 animals fed pathogenic bacteria versus non-pathogenic *E. coli* OP50
218 for 24 hours. *E. coli* OP50 is a standardized food source for laboratory-bred *C. elegans*, and thus
219 represents the nonpathogenic control diet. We analyzed gene expression in N2 and CB4856
220 animals exposed to either *S. epidermidis, S. aureus*, *P. rettgeri* or *P. aeruginosa*.

221 Although both *C. elegans* isolates showed similar susceptibility to three of these pathogens,
222 we hypothesized geographical isolation might have resulted in variations in the immune
223 response, which could be analyzed through transcriptional profiling. Principal component

224 analysis (PCA) of gene expression found that N2 and CB4856 samples exposed to *E. coli*, the
225 Gram-positive pathogen *S. epidermidis* or the pathogenic Gram-negative *P. rettgeri* clustered in
226 an isolate-specific manner (Figure 3A). N2 samples exposed to the virulent Gram-negative *P.*
227 *aeruginosa* were also part of this N2-specific cluster, whereas CB4856 samples exposed to *P.*
228 *aeruginosa* formed their own distinct cluster. Further, both genotypes exposed to the moderately
229 virulent Gram-positive *S. aureus* formed distinct groups that did not cluster with other samples
230 from the same genotype, nor did they cluster with each other (Figure 3A).

231 We also performed PCA of gene expression using samples from a single genotype. For both
232 *C. elegans* isolates *S. epidermidis* and *P. rettgeri* samples again clustered with *E. coli*, whereas. *S.*
233 *aureus* samples formed a separate group along the first principal component axis (PC1). *P.*
234 *aeruginosa* samples also formed individual clusters for both isolates, with separation along the
235 second principal component axis (PC2) in N2 and separation along both the PC1 and PC2 axes in
236 CB4856 (Figures 3B, 3C). Thus, it appears that both nematode genotype and microbial taxonomy
237 contribute to the gene expression differences between N2 and CB4856.

238

239 **Differential Gene Expression is Largely Unique in N2 and CB4856 Animals After Infection with**
240 **Pathogenic Bacteria**

241 We first sought to identify how gene expression changes in these two isolates, as a function
242 of infection. Thus, we identified genes differentially expressed in response to all pathogens (for
243 all analyses, differentially expressed genes (DEGs) were defined as those with an FDR-adjusted P-
244 value <0.05 and fold change ≥2, infected vs. control). We identified 273 and 117 genes that were

245 differentially expressed in N2 and CB4856, respectively, as a function of infection. In either
246 genotype the majority of DEGs were upregulated (N2, 236/273 = 86%; CB4856, 96/117 = 82%)
247 (Table 1, Table 2, Table S2). Interestingly, only six out of the 384 total genes were differentially
248 expressed in both N2 and CB4856 (N2-only, 267/273 = 98%; CB4856-only, 111/117 = 95%) (Figure
249 4A, Table S2).

250 We examined the correlation of gene expression using the $\log_2$ fold changes after infection.
251 Expression levels were weakly, but positively, correlated between N2 and CB4856 ($\rho = 0.097$; $p <$
252 0.001) (Figure 4B). A correlation of baseline gene expression was determined using normalized
253 gene counts from worms exposed to the control bacteria (OP50 *E. coli*). In that context N2 and
254 CB4856 had similar patterns of expression ($\rho = 0.826$), suggesting that gene expression in
255 response to infection diverges from a common baseline. Taken together, these data suggest that
256 while there is some conservation of expression in response to infection, the pattern is relatively
257 weak between these two isolates.

258 We further examined the DEGs identified in both isolates. Of them, only Y65B4BR.1 has any
259 functional annotation relating to infection. Previous data suggests that RNAi knockdown
260 increases susceptibility to infection by *S. aureus.*[35] A single gene, *oac-14*, was identified as
261 differentially expressed in opposing directions, *i.e.*, up in N2 and down in CB4856. RNAi
262 knockdown delays or slows development, suggesting *oac-14* contributes to organismal growth.[36]

263 To take a more holistic approach, we used geneset enrichment analysis to analyze DEGs. The
264 GO terms "Stress response: pathogen", "Transmembrane transport: lipid", and "Metabolism:
265 lipid" were observed to be enriched in N2 (N2-only), whereas genes corresponding to the GO

266 term "Metabolism: lipid" were overrepresented in CB4856 animals (CB4856-only) (Table 3, Table
267 S3). Taken together, these results indicate that although there was little overlap in the
268 transcriptomic response to microbial pathogen in N2 and CB4856 animals, both genotypes
269 exhibit an enrichment of genes involved in lipid metabolism.

270 Finally, to examine the change in transcriptional networks, we cross referenced our DEG lists
271 with a table of known *C. elegans* transcription factors (File S1). We found that in N2, the
272 transcription factors *cky-1, nhr-19, nhr-99, nhr-116, nhr-127, peb-1* and *unc-42* were all
273 upregulated in response to infection by pathogens, whereas in CB4856-infected animals, *ham-1*
274 expression was repressed and *klu-2* expression was induced (Table S2). These results are
275 consistent with the divergence of gene expression profiles between N2 and CB4856, suggesting
276 that, upon infection, these isolates rely on different immune pathways.

277

278 **N2 and CB4856 Transcriptomic Responses Exhibit More Overlap Following Infection with Gram-**
279 **positive than Gram-negative Pathogens**

280 We then asked how gene expression profiles differed when we grouped pathogens by Gram-
281 stain. We identified 3833 and 2565 genes that were differentially expressed in response to Gram-
282 positive bacteria in N2 and CB4856, respectively. In either genotype the majority of DEGs were
283 upregulated (N2, 2964/3833 = 77%; CB4856, 1962/2565 = 76%) (Table 1, Table 2, Table S4). While
284 most genes were unique to a genotype (N2-only, 2661/3833 = 69%; CB4856-only, 1393/2565 =
285 54%), 1172 genes were differentially expressed in both N2 and CB4856 worms (Figure 5A, Table
286 S4). Further, the majority of DEGs identified in both genotypes were expressed in the same

287 direction, while only 1% of genes were differentially expressed in opposing directions, *i.e.*, down
288 in N2 and up in CB4856 (14/1172 = 1%) (Figure 5C, Table S4). These results were reflected in a
289 strong, positive correlation between N2 and CB4856 gene expression levels (ρ = 0.613; $p < 0.001$)
290 (Figure 5A).

291 We again looked for transcription factors that were differentially expressed in both N2 and
292 CB4856 as a function of infection by Gram-positive bacteria. In this list, there were 50
293 transcription factor genes that were significantly different from baseline (Table S4). As observed
294 for the overall gene expression similarity, for each of the transcription factors, we observed a
295 similar pattern for the direction of expression (*e.g.,* genes upregulated in N2 were upregulated
296 in CB4856, *etc.*).

297 In response to Gram-negative bacteria, 628 and 1720 genes were differentially expressed in
298 N2 and CB4856 animals, respectively. Interestingly, in both genotypes a minority of DEGs were
299 upregulated (N2, 254/628 = 40%; CB4856, 456/1720 = 27%) (Table 1, Table 2, Table S5). Like the
300 transcriptomic response when all pathogens were grouped together, most genes were unique to
301 a genotype with a total of 124 genes differentially expressed in both N2 and CB4856 (N2-only,
302 504/628 = 80%; CB4856-only, 1596/1720 = 93%) (Figure 5B, Table S5). Further, the majority of
303 DEGs identified in both genotypes were expressed in opposing directions (82/124 = 66%), leaving
304 only 42 genes that were differentially expressed in both genotypes and in the same direction
305 (Figure 5C, Table S5). Gene expression profiles were again weakly, but positively correlated (ρ =
306 0.107; $p < 0.001$) (Figure 5B). Finally, there were no transcription factors that were coordinately
307 regulated in both isolates in response to Gram-negative bacteria (Table S5).

308 These results highlight the dramatic differences when we compare the transcriptomic
309 response of N2 and CB4856 worms infected with Gram-positive or Gram-negative pathogens.
310 Interestingly, exposure to Gram-positive pathogens resulted in differential gene expression
311 profiles with considerable overlap between the two isolates, suggesting that there are conserved
312 responses to some bacteria. In contrast, the transcriptomic responses of N2 and CB4856 animals
313 to Gram-negative pathogens were relatively divergent, with few differentially expressed genes
314 in common.

315

316 **Overlap in Differentially Expressed Genes between N2 and CB4856 Depends on the Microbial**
317 **Pathogen**

318 Finally, we analyzed gene expression by individual pathogen exposure. This identified 2384
319 (*S. epidermidis*), 8754 (*S. aureus*), 5802 (*P. aeruginosa*), and 1029 (*P. rettgeri*) differentially
320 expressed genes in N2 and 1776 (*S. epidermidis*), 7033 (*S. aureus*), 5171 (*P. aeruginosa*), and 2253
321 (*P. rettgeri*) differentially expressed genes in CB4856 (Table 1, Table 2). In response to *S.*
322 *epidermidis*, upregulated DEGs outnumbered downregulated DEGs at a ratio of 3:1 (N2,
323 1754/2384 = 74%; CB4856, 1296/1776 = 73%), whereas the response to *S. aureus* was split more
324 evenly between upregulated and down regulated genes, although upregulated DEGs still
325 represented a majority (N2, 4901/8754 = 56%; CB4856, 3597/7033 = 51%) (Table 1, Table 2, Table
326 S6, Table S7).

327 Although both belong to the *Staphylococcus* genus, the overlap between N2 and CB4856
328 transcriptomic responses to *S. epidermidis* and *S. aureus* were considerably different. In *S.*

329 *epidermidis*-infected animals the minority of DEGs, 511 in total, were differentially expressed in
330 both N2 and CB4856 (N2-only, 1873/2384 = 79%; CB4856-only, 1265/1776 = 71%) (Figure 6A,
331 Table S6). The correlation of gene expression in response to *S. epidermidis* was ρ = 0.294 ($p<$
332 0.001). In contrast, a majority of differentially expressed genes, 5039 in total, overlapped in N2
333 and CB4856 animals infected with *S. aureus* (N2-only, 3715/8754 = 42%; CB4856-only, 1994/7033
334 = 28%) (Figure 6B, Table S7), with a correlation of ρ = 0.721 ($p<$ 0.001).

335 The majority of DEGs identified in both genotypes following either *S. epidermidis* or *S. aureus*
336 infection were expressed in the same direction (*S. epidermidis*, 410/511 = 80%; *S. aureus*,
337 4928/5039 = 98%) (Figure 6C, Table S6, Table S7). Lastly, we only identified nine transcription
338 factors that were coordinately regulated in *S. epidermidis*-infected animals, whereas 346
339 transcription factors genes were differentially expressed in both genotypes in the same direction
340 after *S. aureus* infection (Table S6, Table S7). Together, these results suggest N2 and CB4856
341 worms exhibit considerable similarity in their transcriptomic response to *S. aureus*, whereas
342 there is much less overlap in their response to *S. epidermidis* infection, which may perhaps
343 explain some of the difference in susceptibility to infection by *S. epidermis*.

344 Exposure to either Gram-negative pathogen resulted in a minority of DEGs being upregulated
345 in both genotypes. In *P. aeruginosa*-infected animals, upregulated genes represented less than
346 one-third of all DEGs in both genotypes (N2, 1804/5802 = 31%; CB4856, 1460/5171 = 28%),
347 whereas, after *P. rettgeri* infection, differential expression analysis found nearly equivalent
348 numbers of upregulated and downregulated genes in N2 and upregulation of less than one-third

349 of all DEGS in CB4856 (N2, 504/1029 =49%; CB4856, 694/2253 =31%) (Table 1, Table 2, Table S8,
350 Table S9).

351 We observed considerable differences in the overlap of gene expression profiles between N2
352 and CB4856 worms infected with *P. aeruginosa* or *P. rettgeri*. In animals infected with *P.*
353 *aeruginosa*, a majority of differentially expressed genes, 3092 in total, overlapped (N2-only,
354 2710/5802 = 47%; CB4856-only, 2079/5171 = 40%) (Figure 7A, Table S8), with a correlation of ρ
355 = 0.539 (p< 0.001). Like infection with *S. epidermidis* or *S. aureus*, the majority of DEGs identified
356 in both genotypes following *P. aeruginosa* infection were expressed in the same direction
357 (2971/3092 = 96%) (Figure 7C, Table S8). In response to *P. rettgeri*, 581 genes were differentially
358 expressed in both genotypes, representing a majority of DEGs in N2 but a minority in CB4856
359 (N2-only, 448/1029 = 44% CB4856-only, 1672/2253 = 74%) (Figure 7B, Table S9). Interestingly,
360 the vast majority of DEGs found in both genotypes following *P. rettgeri* infection were expressed
361 in opposing directions (564/581 = 97%), leaving only 17 genes that were coordinately expressed
362 (Figure 7C, Table S9). Finally, we identified 114 transcription factor genes that were differentially
363 expressed in both genotypes in the same direction after *P. aeruginosa* infection, whereas no
364 transcription factors were coordinately regulated in *P. rettgeri*-infected animals (Table S8, Table
365 S9).

366 Taken together, these results indicate that the N2 and CB4856 transcriptomic responses to *P.*
367 *aeruginosa* have more in common, whereas little overlap in differential gene expression exists
368 when N2 and C4856 are infected with *P. rettgeri*. In fact, of all the gene expression profiles
369 compared, *P. rettgeri* infection was the only one to have a negative correlation (ρ = -0.387; p<

370 0.001). These results are consistent with the responses to infection being driven both by the
371 isolate and the pathogen.
372
373 **Geneset Enrichment Analysis Identifies Unique GO Terms Enriched in N2 and CB4856 Worms**
374 **Exposed to *S. epidermidis***
375 Since N2 and CB4856 exhibited survival differences after *S. epidermidis* infection but not *S.*
376 *aureus* infection, we were interested in identifying biological processes that may be enriched in
377 these animals. We cross-referenced DEGs identified in N2 following *S. epidermidis* and *S. aureus*
378 infection and identified 556 genes that were either exclusively expressed in response to *S.*
379 *epidermidis* or were expressed in response to both pathogens but in opposing directions (*e.g.,*
380 upregulated in *S. epidermidis* and downregulated in *S. aureus*). The same analysis performed for
381 DEGs in CB4856 animals identified 749 genes. Only 73 of these genes were differentially
382 expressed in both N2 and CB4856 (N2-only, 483/556 = 87%; CB4856-only, 676/749 = 90%) (Table
383 S10).
384 We performed geneset enrichment analysis on genes that were exclusive to N2 (N2-only),
385 genes that were exclusive to CB4856 (CB4856-only), and genes that were differentially expressed
386 in both N2 and CB4856 animals (N2 & CB4856). Biological functions pertaining to the extracellular
387 matrix, stress response, and metabolism were enriched in N2 and CB4856 worms following *S.*
388 *epidermidis* infection (Table 4, Table S11). The Gene Ontology (GO) terms “Extracellular material:
389 collagen” and “Extracellular material: cuticlin” were enriched in N2-exclusive DEGs, whereas
390 genes corresponding to the GO terms “Stress response: C-type Lectin” and “Metabolism: lipid”

391 were overrepresented in DEGs exclusive to CB4856. Genes in the GO category "Stress response:
392 detoxification" were overrepresented in both isolates, even if the individual genes were not
393 necessarily the same. Genes that were differentially expressed in both isolates were enriched for
394 a single GO term, "Stress response: pathogen". A separate group of genes that was expressed
395 exclusively in CB4856 animals was also enriched for this GO term. Notably, we did not find
396 significant enrichment of the GO term "Stress response: pathogen" in DEGs exclusive to N2
397 animals. Thus, when only considering genes that are differentially expressed in an *S. epidermidis*-
398 specific manner, our results indicate a common enrichment of biological processes related to
399 stress response and innate immunity in N2 and CB4856 animals. At the same time, we also
400 observe an N2-specific enrichment of extracellular matrix genes and a CB4856-specific
401 enrichment of lipid metabolism genes, suggesting a degree of divergence in the transcriptomic
402 response of these isolates to *S. epidermidis* infection.
403
404 **Comparing DEGs between N2 and CB4856 fed OP50 versus *S. epidermidis***
405 The genomes of N2 and CB4856 animals differ at approximately one polymorphism per kb
406 and gene expression between these two isolates, including the expression of innate immune
407 genes, is known to vary across similar developmental stages, even in the absence of infection.[37-
408 39] Since principal component analysis found that genotype is the greatest predictor of isolate-
409 specific differences in gene expression following *S. epidermidis* infection (Figure 3), we performed
410 an additional gene expression analysis to identify genes that were differentially expressed

411 between N2 and CB4856 animals fed either the non-pathogenic *E. coli* OP50 or the pathogenic *S.*
412 *epidermidis*.

413 Using N2 expression as a baseline, we found 3384 and 1892 genes to be differentially
414 expressed in CB4856 animals fed *E. coli* OP50 and *S. epidermidis*, respectively. Upregulated genes
415 outnumbered those that were downregulated at a ratio of 2:1, regardless of whether the worms
416 were fed OP50 or *S. epidermidis* (OP50 Upregulated, 2121/3384 = 63%; OP50 Downregulated,
417 1263/3384 =37%) (*S. epidermidis* Upregulated, 1245/1892 = 66%; *S. epidermidis* Downregulated,
418 647/1892 =34%) (Table S12, Table S13).

419 Next, we cross-referenced genes that were differentially expressed between N2 and CB4856
420 animals in the OP50 and *S. epidermidis* conditions and classified them into four categories based
421 on changes in gene expression, or lack thereof. Relative to N2, a total of 2280 genes were
422 differentially expressed in CB4856 animals fed OP50 but were no longer differentially expressed
423 when worms were fed *S. epidermidis*. This suggests that the final level of expression for these
424 gene was similar in N2 and CB4856 animals following infection. A total of 1072 genes were
425 differentially expressed between N2 and CB4856 animals fed OP50 and continued to be
426 differentially expressed in the same direction in worms fed *S. epidermidis*, suggesting that they
427 are likely uninvolved in the transcriptional response to the pathogen. A third group of genes, 788
428 in total, were exclusively differentially expressed between N2 and CB4856 animals fed *S.*
429 *epidermidis*. Finally, we identified 32 genes that were differentially expressed between N2 and
430 CB4856 animals fed either OP50 or *S. epidermidis* but in opposing directions, *i.e.*, up in CB4856
431 fed OP50 and down in CB4856 fed *S. epidermidis* (Table S14).

432 The isolate- and pathogen-specific expression pattern of genes from these latter two
433 categories prompted us to perform geneset enrichment analysis to identify biological processes
434 that may be differentially enriched in N2 and CB4856 animals following *S. epidermidis* infection.
435 The 788 genes that were exclusively differentially expressed in CB4856 animals fed *S. epidermidis*
436 were enriched for GO terms pertaining to immunity (Stress response: C-type lectin), cell signaling
437 and transduction (Signaling: phosphatase; Signaling: tyrosine kinase), reproduction (Major sperm
438 protein), posttranslational modification (Protein modification: glycoprotein), and cytoskeletal
439 movement (Cytoskeleton: microtubule). From the 32 genes that were differentially expressed in
440 opposing directions in CB4856 animals fed either OP50 or *S. epidermidis* geneset enrichment
441 analysis identified GO terms corresponding to immunity (Stress response: pathogen) and the
442 extracellular matrix (Extracellular material: collagen) (Table 5) (Table S15). Thus, by considering
443 differences in gene expression between N2 and CB4856 animals fed non-pathogenic *E. coli* OP50,
444 we have identified additional sets of genes that are enriched for GO terms pertaining to, among
445 other categories, stress response, innate immunity, and the extracellular matrix.
446 Finally, to better understand the transcriptional regulators that might be significantly
447 different upon infection by *S. epidermidis,* we looked for transcription factors that were
448 differentially expressed only upon infection. There were 22 protein coding genes (1 pseudogene),
449 that fit these criteria. Interestingly, 13 of the 22 were members of the nuclear hormone receptor
450 (*nhr*) family. There are 266 *nhr* genes in our list of 1077 transcription factors. The incidence of
451 *nhr* genes in our DEG list was significantly enriched (P = 0.007, Fisher's exact test, odds ratio 4.4).
452

453 ## Discussion

454 We conducted survival, behavioral and transcriptional assays using the *C. elegans* strains N2
455 and CB4856 to identify differences in pathogen susceptibility between two wild-type isolates.
456 CB4856 was significantly more susceptible than N2 to infection by the Gram-positive pathogen
457 *S. epidermidis*. CB4856 and N2 were equally susceptible to a related species, *S. aureus,* and two
458 non-*Staphylococcus* pathogens, *P. aeruginosa* and *P. rettgeri.* We confirmed the CB4856
459 susceptibility using a second isolate of *S. epidermidis*.

460 What might underlie the differences in susceptibility? Initially we hypothesized that, perhaps,
461 the CB4856 strain lacked the ability to detect *S. epidermidis* as pathogenic. However, CB4856
462 worms avoided *S. epidermidis,* when given *E. coli* as an alternative, suggesting they were averse
463 to the bacteria. Further, we found robust changes in gene expression in response to the *S.*
464 *epidermis* infection. Unexpectedly, we found that, despite *P. rettgeri* being equally pathogenic to
465 both isolates, CB4856 animals preferred *P. rettgeri* to *E. coli,* while N2 did not. This observation
466 was also highlighted by the fact that changes in gene expression were negatively correlated in
467 these two isolates in response to *P. rettgeri.*

468

469 **Isolates exhibit distinct transcriptomic responses to infection**

470 The pathogens used in this study are diverse both in terms of their genome size and known
471 virulence mechanisms.[40-43] Principal component analysis of the sequencing dataset found that
472 gene expression changes during infection clustered largely by genotype, rather than pathogen,
473 even in instances where susceptibility was equivalent. At a glance, this suggests that the

474 strategies these strains have adopted for dealing with microbial infection have diverged since
475 they were separated from one another.

476 When we looked for genes that were differentially expressed in response to infection, *i.e.,*
477 was identified in response to all pathogens, there were hundreds, compared to the lists for
478 individual pathogen (thousands). Further, we found only six DEGs in common between N2 and
479 CB4856, with those genes largely uncharacterized functionally. Overall, these results suggest that
480 N2 and CB4856 respond to infection by altering gene transcription, but the core infection
481 response represents only a small fraction of the total genes regulated, and that the core
482 transcriptional response in each background has diverged. It also suggests there are still a
483 number of genes involved in immunity that need to be characterized.

484 By categorizing the genes that were differentially expressed in response to all pathogens, we
485 found an overrepresentation of genes involved in lipid metabolism in both N2- and CB4856-
486 infected animals. Lipid metabolism has been shown to be required for proper innate immune
487 system activation and pathogen defense.[44] These genes could play similar roles in both isolates
488 and thus, while they may reflect a divergence in specific gene activation, they could represent a
489 similarity in how N2 and CB4856 animals utilize lipid metabolism enzymes to facilitate the innate
490 immune response.

491

492 **Gram-stain level analysis suggests an absence of conserved master regulatory pathways**

493 In many organisms, the Toll signaling pathway is activated by infection with Gram-positive
494 bacteria and/or fungi, while the IMD pathway is activated in response to Gram-negative bacteria.

495 Thus, we examined the results grouping pathogens by Gram-stain, and then compared between
496 isolates. Our results found that the majority of DEGs were strain-specific, consistent with a
497 divergence of the responses to pathogens, but that, generally, there was a stronger conservation
498 of responses to Gram-positive bacteria, compared to Gram-negative. Our results are consistent
499 with the evidence that Toll and IMD signaling is diminished or absent in *C. elegans* as master
500 regulators of immunity and suggests each isolate deals with Gram-negative and Gram-positive
501 pathogens differently.

502 We examined the list of DEGs for transcription factors that could be contributing to the
503 differential expression profiles in each of the isolates. There were hundreds of transcription
504 factors regulated by infection with Gram-positive pathogens, with 50 overlapping between the
505 N2 and CB4856 isolates. In contrast, for Gram-negative pathogens, N2 had only nine differentially
506 expressed transcription factors, while CB4856 had 97, with none in common.

507 One possible explanation for the large overlap in N2 and CB4856 transcriptomic responses to
508 Gram-positive bacteria is the identity of the microbial pathogens. *S. aureus* and *S. epidermidis*
509 are both members of the *Staphylococcus* genus, share a number of virulence factors and invoke
510 overlapping host immune responses following infection.[41,45,46] Thus, the greater proportion of
511 overlapping DEGs in N2 and CB4856 animals infected with Gram-positive bacteria may reflect a
512 common response to *Staphylococcus* pathogens. Similar results for infection of N2 animals by
513 *Enterococcus faecalis* and *Enterococcus faecium* suggest that, within species of pathogens, there
514 are core transcriptional similarities.[47]

515

516 **Individual pathogens induce different responses in *C. elegans* isolates**

517 Since N2 and CB4856 worms exhibited similar susceptibility to infection by *P. aeruginosa*, *S.*
518 *aureus* or *P. rettgeri*, we posited that RNA sequencing would reveal considerable overlap in gene
519 expression between the two isolates. Indeed, after infecting N2 and CB4856 worms with either
520 *P. aeruginosa* or *S. aureus* we identified thousands of differentially expressed genes, a majority
521 of which were coordinately regulated in both isolates, supporting the idea that infection by either
522 of these pathogens invokes similar kinds of transcriptomic responses in N2 and CB4856 animals.
523 However, animals infected with *P. rettgeri* exhibited very little overlap in gene expression with
524 fewer than 20 genes differentially expressed in both isolates and expressed in the same direction.

525 It is possible that N2 and CB4856 worms have adopted two very different immune responses
526 to *P. rettgeri* infection, with neither providing a perceivable survival advantage. But why do we
527 not observe a similar lack of overlap in gene expression after infection with the Gram-negative
528 pathogen, *P. aeruginosa*, which also showed similar lethality in N2 and CB4856 animals? Analysis
529 of gene expression at regular intervals throughout an infection has demonstrated that the host
530 immune response is a dynamic process which needs to be initiated and sustained to fight an
531 infection.[48,49] At 24 hours post-infection, the timepoint at which RNA was collected for gene
532 expression analysis, animals infected with *P. aeruginosa* were much closer to dying than animals
533 infected with *P. rettgeri* (N2 *P. aeruginosa* LT_{50} = 3.8 ± 0.3 days, CB4856 *P. aeruginosa* LT_{50} = 3.5
534 ± 0.3 days; N2 *P. rettgeri* LT_{50} = 7.8 ± 0.7 days, CB4856 *P. rettgeri* LT_{50} = 7.2 ± 0.6 days). Perhaps
535 animals exposed to *P. aeruginosa* or *P. rettgeri* are at two different stages in combatting their

536 respective infections and the overlap in gene expression between N2 and CB4856 animals grows
537 larger as animals progress from earlier to later stages of infection.
538
539 **Changes in gene expression by infection with *S. epidermidis***
540 We found differences in survival in response to *S. epidermidis.* For each isolate we narrowed
541 down the list of differentially expressed genes to those that were expressed in a *S. epidermidis*-
542 specific manner and performed geneset enrichment analysis on sets of genes that were either
543 exclusive to N2, exclusive to CB4856, or expressed in both N2 and CB4856 animals. We observed
544 an enrichment of genes encoding extracellular matrix (ECM) proteins, such as collagens and
545 cuticlins, exclusively in N2 animals. Cuticular collagens are an integral component of the *C.*
546 *elegans* epidermal barrier and have established roles in the host epidermal immune response to
547 pathogens.[50-52] Further, ECM proteins are likely involved in promoting longevity as changes in
548 collagen gene expression have been used as *in vivo* biomarkers in *C. elegans* aging research.[53]
549 Together, our data reveal isolate-specific changes in ECM gene expression after *S. epidermidis*
550 exposure, suggesting a role for collagen genes in the increased resistance of N2 worms to *S.*
551 *epidermidis* infection. In DEGs exclusive to *S. epidermidis*-infected N2 animals as well as DEGs
552 exclusive to *S. epidermidis*-infected CB4856 animals, our analysis detected an enrichment of
553 genes encoding detoxification enzymes. These enzymes are primarily responsible for
554 metabolizing xenobiotic substances and have demonstrated roles in the *C. elegans* immune
555 response.[54,55] In both N2 and CB4856 animals, the DEGs were either members of the Cytochrome
556 P450 (CYP) family or possessed UDP glucuronosyltransferase (UGT) activity. It is possible that

557 these genes play similar roles in both isolates and thus may reflect a divergence in how N2 and
558 CB4856 animals utilize detoxification enzymes to counteract the microbial infection.

559 Finally, we took advantage of the ability to look for genes with expression differences
560 between N2 and CB4856 on control bacteria. This allowed us to sort differentially expressed
561 genes in the *S. epidermidis* condition into those that started out different, but ended up being
562 similar, and those that changed as a function of infection to end up at different levels of
563 expression. *A priori* we might consider the former group as being less likely to underlie the
564 differences in susceptibility. In those analyses, approximately 90% of the genes were upregulated
565 in response to infection.

566 When we examined the transcription factors that were differentially expressed, we found an
567 overrepresentation of nuclear hormone receptors. What is more, of the 13 *nhr* genes, seven were
568 upregulated, while six were downregulated during infection. Nuclear hormone receptors are
569 used in many contexts to systemically coordinate signaling. In *C. elegans* multiple *nhr* genes have
570 been linked to innate immunity, including *nhr-8, nhr-23, nhr-25, nhr-45, nhr-49, nhr-57, nhr-80,*
571 and *nhr-112.*[56-63] Thus, this is an interesting family of genes to potentially contribute to
572 differences in organismal response and/or susceptibility *in vivo.*

573

574 **Conclusions**

575 In populations, genetic and genomic diversity drive phenotypes. Immune signaling is no
576 exception, and yet, our understanding of *C. elegans* immunity has largely been driven by the
577 analysis of the canonical laboratory strain, N2. We found that the N2 strain and CB4856 wild

578 isolate were significantly different when we measured the susceptibility to *S. epidermidis.* It is
579 possible that the relative abundance of *S. epidermidis* or related pathogens in their respective
580 ecological niches resulted in the shift in immune capacity between the N2 and CB4856 lines.
581 Unfortunately, without sufficient metagenomic sampling of the environment, this would be
582 difficult to address. However, the robust differences observed suggests that isolating the animals
583 and transferring them to the laboratory environment has fixed genetic variation that underlies
584 pathogen-specific susceptibility.

585 Perhaps more surprisingly, we found significantly divergent transcriptomic responses
586 between these isolates, even when pathogen susceptibility was equivalent. These differences
587 could come from protein coding variants in the molecules that detect pathogens or the
588 transcription factors that coordinate immune defenses, non-coding variants in regulatory
589 regions, or, more likely, a combination of the two. By narrowing down the regions of the genome
590 that are important for the observed differences, we will be able to better understand how genetic
591 variation contributes to different immune outcomes.

592

593 ## Declarations

594 **Ethics approval and consent to participate**

595 Not Applicable

596 **Consent for publication**

597 Not Applicable

598 **Availability of data and materials**

599 The raw sequence files used for differential gene expression analysis are available in FASTQ
600 format in the NCBI BioProject repository (BioProject Accession number: PRJNA770277;
601 https://www.ncbi.nlm.nih.gov/sra/PRJNA770277).

602 **Competing interests**

603 "The authors declare that they have no competing interests"

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610 manuscript.

611 **Authors' contributions**

612 PL and BDA designed and conceived the project; PL, MC and BDA acquired data; PL and BDA
613 analyzed and interpreted data and wrote the manuscript. All authors have approved the
614 manuscript.

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618

619 **Figures**

620

621

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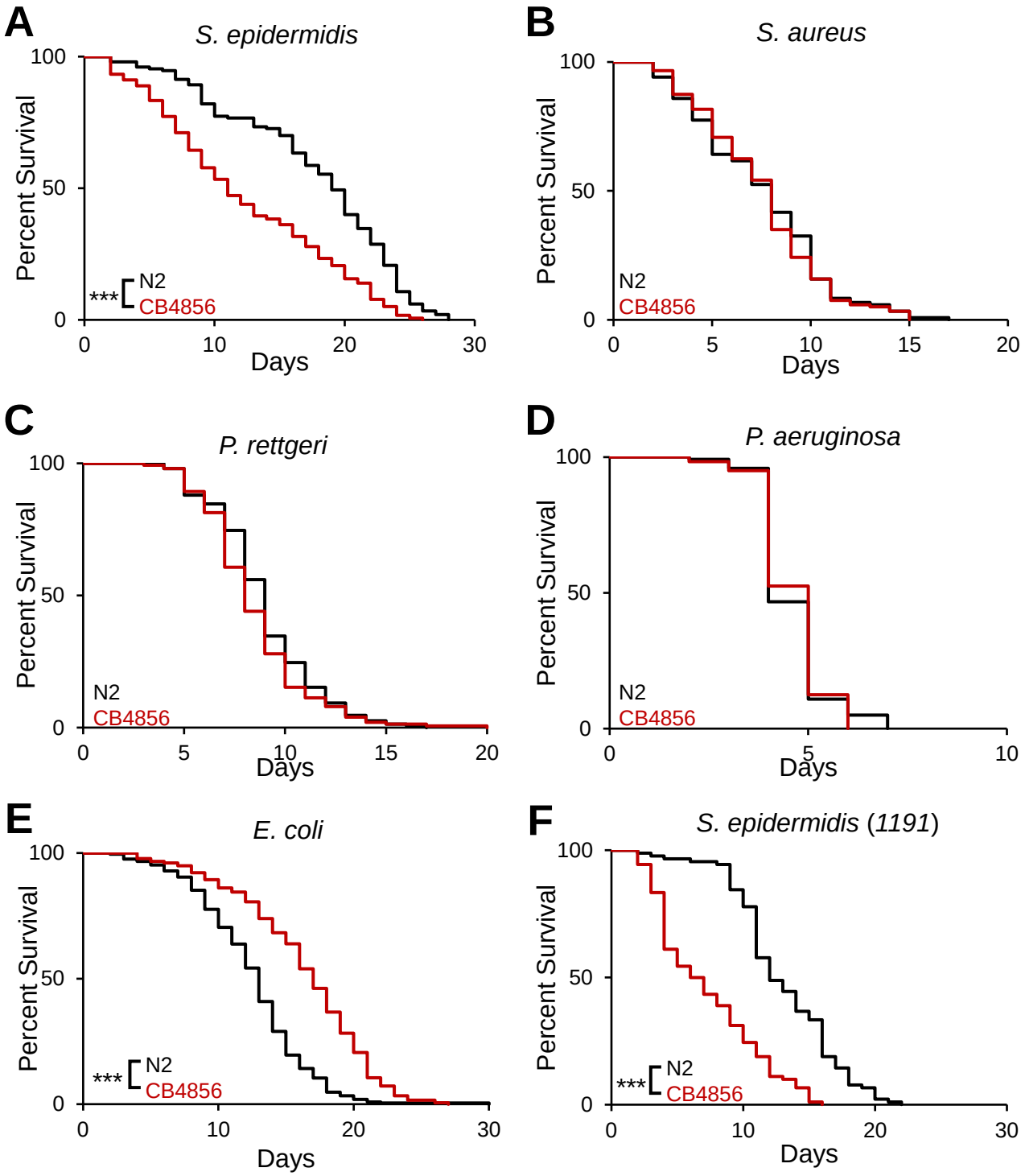


Figure 1: CB4856 is more susceptible to *S. epidermidis,* but not other bacterial pathogens, relative to N2.

(A-E) Survival of the *C. elegans* Hawaiian wild isolate, CB4856, versus N2 on nematode growth medium (NGM) with a lawn of *S. epidermidis* (A), *S. aureus* (B), *P. rettgeri* (C), *P. aeruginosa* (D), or *E. coli* (E). We tested a second *S. epidermidis* strain (1191) to confirm the differences in genotypes (F). In each experiment, 30 worms were placed on the bacterial lawn and transferred every day while scored for survival. Survival curves represent 3-7 independent experiments. For each pathogen, statistical analysis by Mantel-Cox (CB486 vs N2). ***, $p<0.001$.

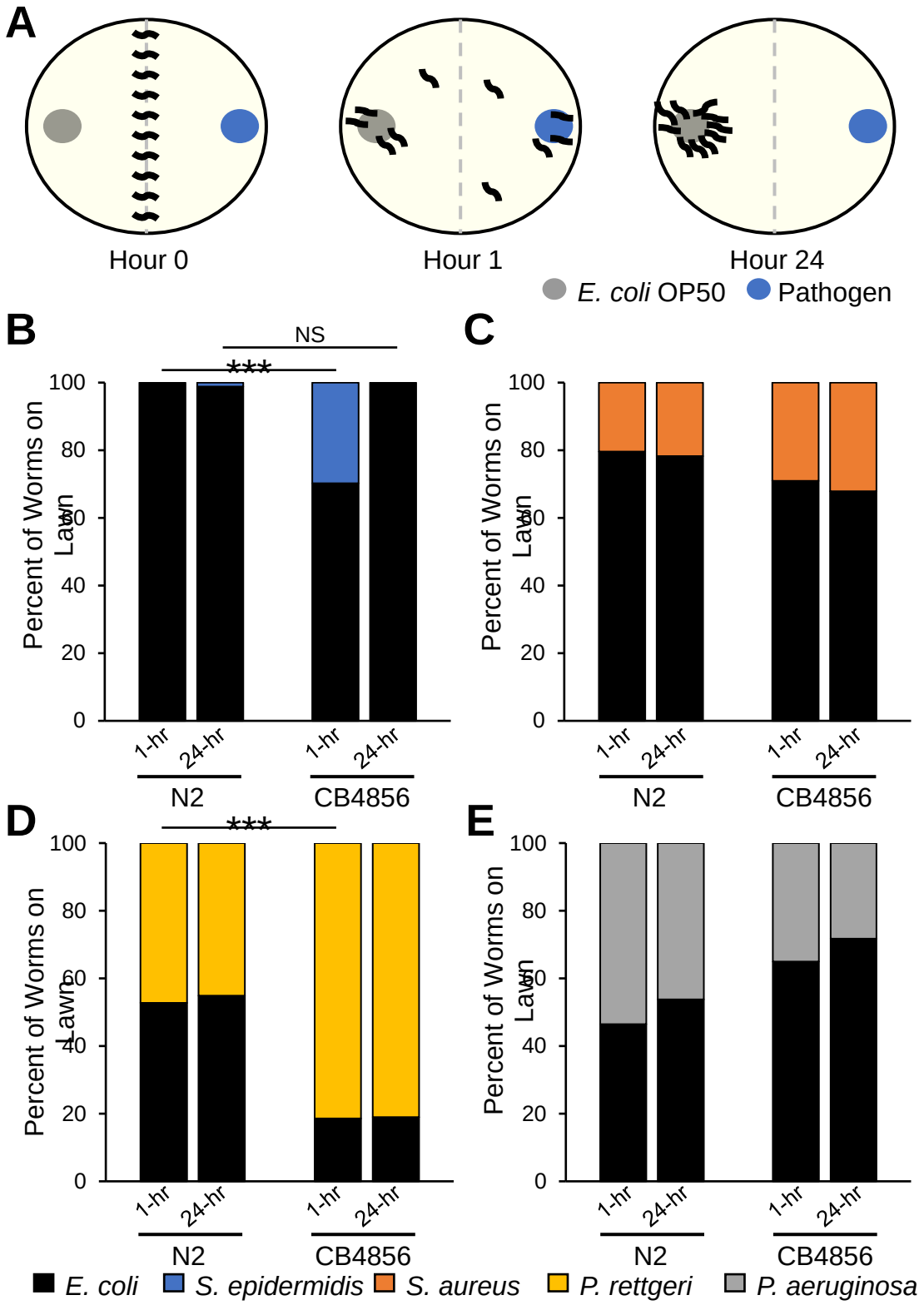


Figure 2: Pathogen avoidance of N2 and CB4856 isolates depends on the pathogen and time of observation.

(A) Two-choice pathogen avoidance assay. L4 worms were washed and then placed at the midline of test plates containing two bacterial lawns, OP50 or pathogen. At 1-hr and 24-hr timepoints the percentage of worms on each lawn was calculated. (B-E) Percentage of N2 and CB4856 isolates on a bacterial lawn when given a choice of *E. coli* OP50 or. *S. epidermidis* (B), *S. aureus* (C), *P. rettgeri* (D) or *P. aeruginosa* (E). In each experiment, 20 worms were placed on NGM plates and the number of worms on the bacterial lawn was recorded at regular intervals. Data represent 5 independent experiments. ***, $p<0.001$; NS, $p>0.05$.

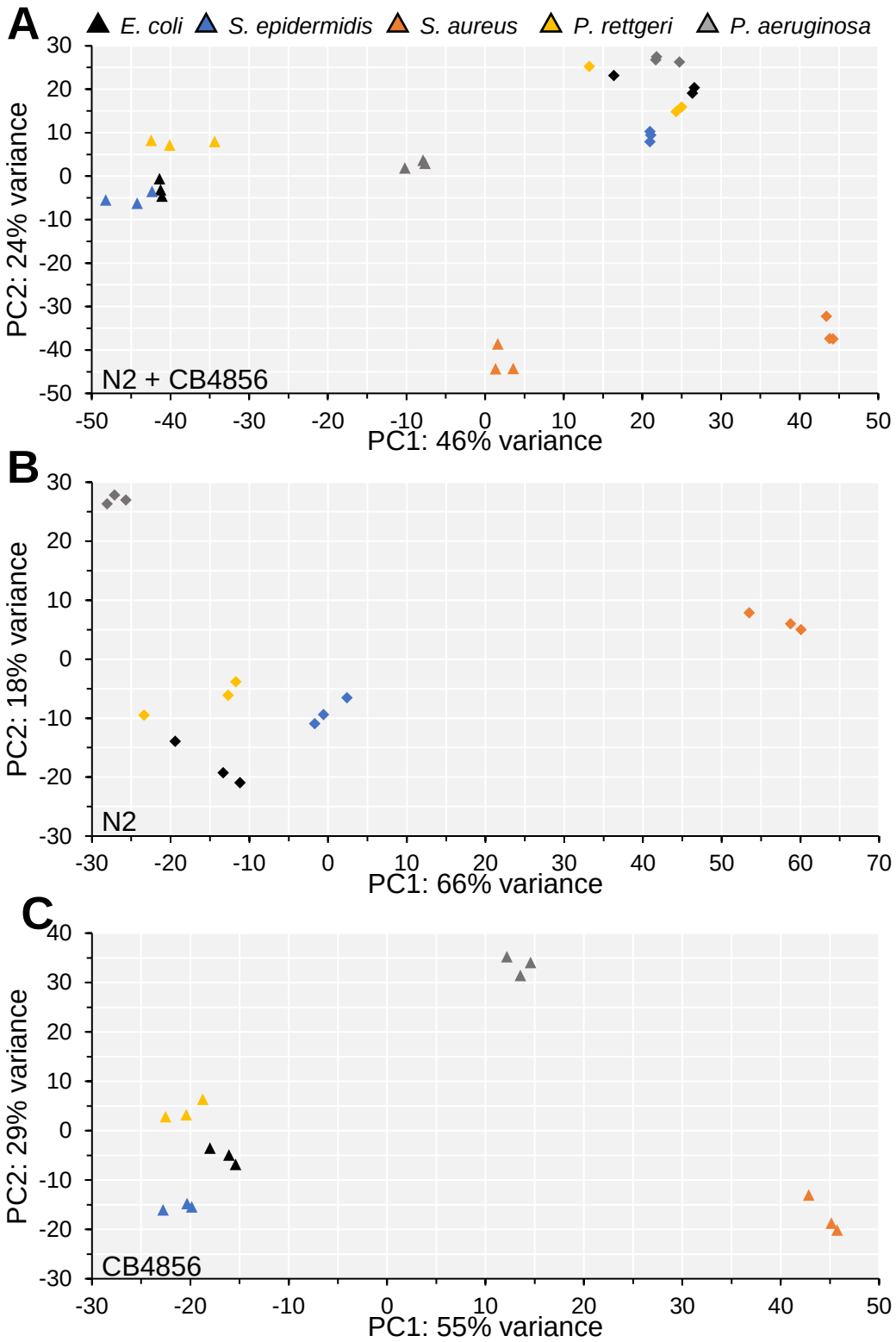


Figure 3: Principal component analysis clusters samples based on genotype, rather than pathogen exposure.

(A-C) Principal component plots of expression data from the Hawaiian wild isolate, CB4856 (triangles) and N2 (diamonds) samples (A), only N2 samples (B), and only CB4856 samples (C).

Table 1: Differentially expressed genes (DEGs) in N2 animals following 24-hour exposure to microbial pathogens.

Condition	[1]Total DEGs	Total Upregulated	Total Downregulated	[2]Total Genes Expressed
All Pathogens	273	236	37	23938
Gram Positive	3833	2964	869	22934
Gram Negative	628	254	374	21646
Staphylococcus epidermidis	2384	1754	630	21124
Staphylococcus aureus	8754	4901	3853	21433
Pseudomonas aeruginosa	5802	1804	3998	20304
Providencia rettgeri	1029	504	525	20295
Escherichia coli	-	-	-	19961

[1]Genes with a false discovery rate (FDR)-adjusted p-value of <0.05 and log2 fold change >1
[2]Total number of genes with >0 counts

Table 2: Differentially expressed genes (DEGs) in CB4856 animals following 24-hour exposure to microbial pathogens.

Condition	[1]Total DEGs	Total Upregulated	Total Downregulated	[2]Total Genes Expressed
All Pathogens	117	96	21	24333
Gram Positive	2565	1962	603	23297
Gram Negative	1720	456	1264	21847
Staphylococcus epidermidis	1776	1296	480	21252
Staphylococcus aureus	7033	3597	3436	21675
Pseudomonas aeruginosa	5171	1460	3711	20963
Providencia rettgeri	2253	694	1559	19731
Escherichia coli	-	-	-	20288

[1]Genes with a false discovery rate (FDR)-adjusted p-value of <0.05 and log2 fold change >1
[2]Total number of genes with >0 counts

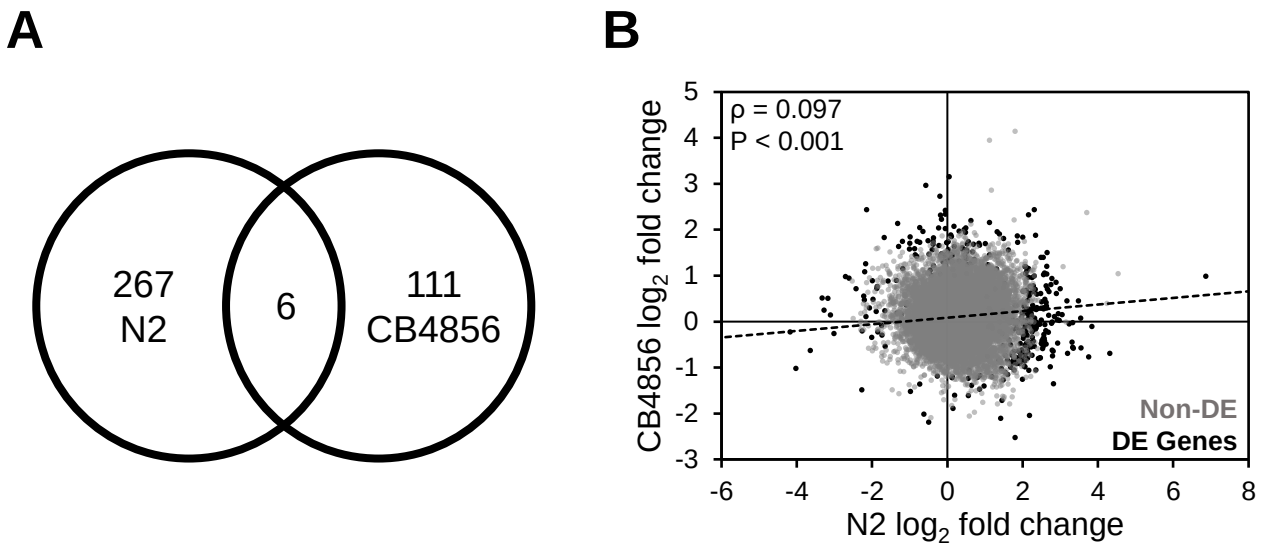
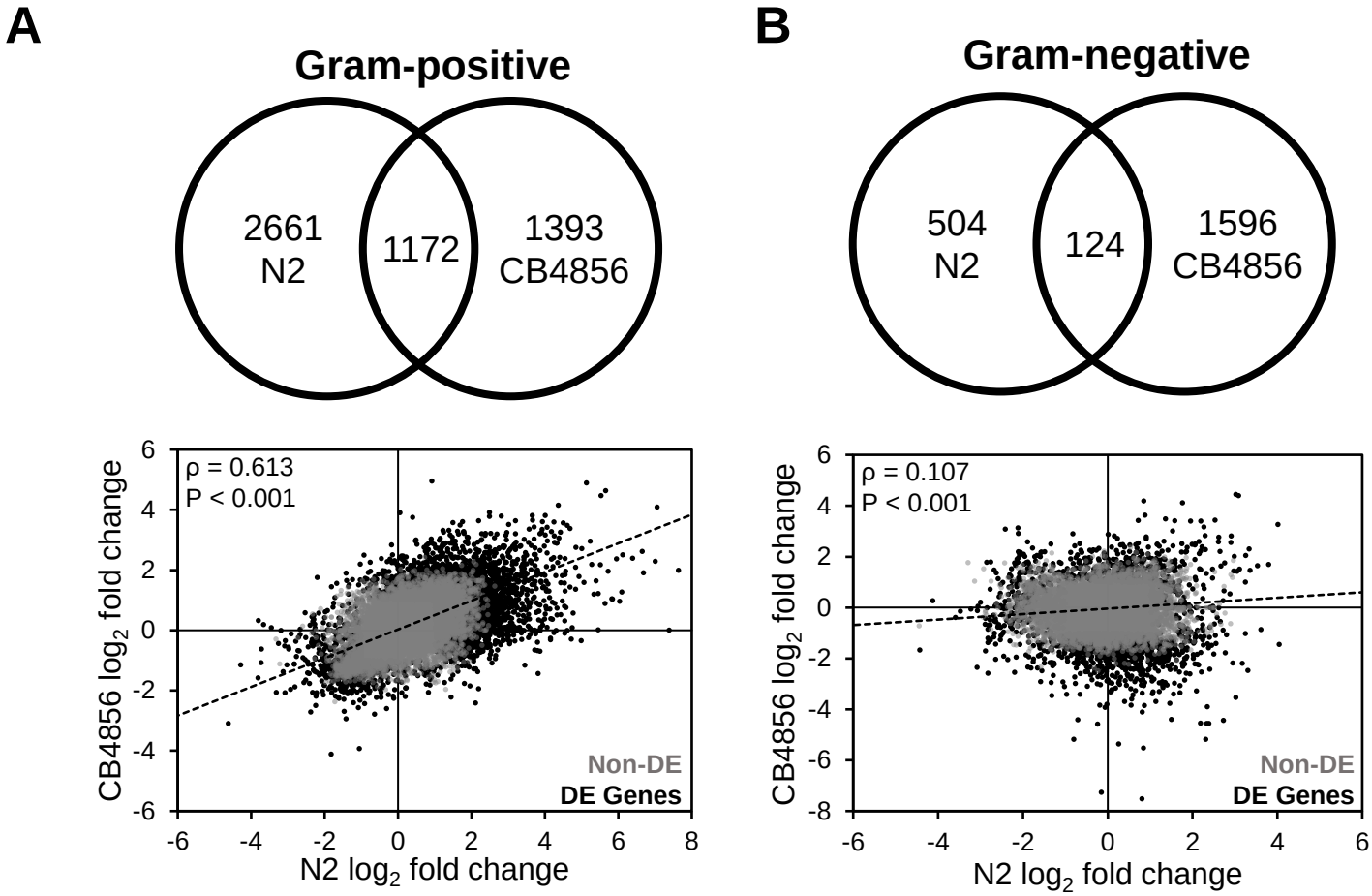


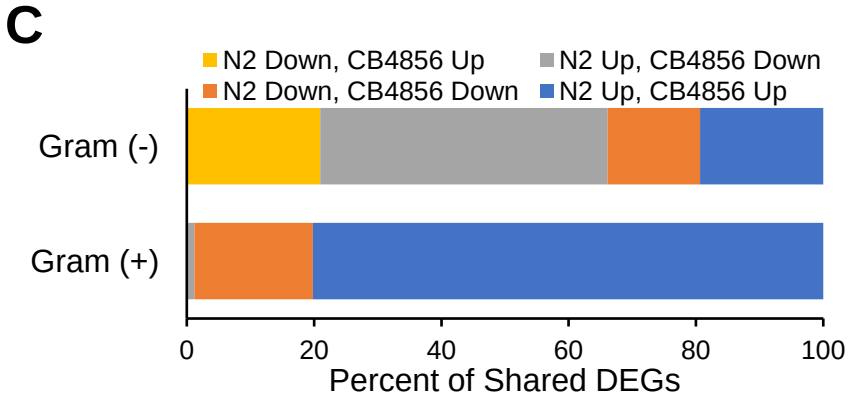
Figure 4: Expression differences in N2 and CB4856 animals after infection with pathogenic bacteria.
(A) Intersection of differentially expressed genes and (B) correlation between $\log_2$ fold changes in N2 and CB4856 animals infected with pathogenic bacteria. Spearman's rho value and P-values are presented in the upper left corner. Gray (Non-DE) and black dots (DE Genes) represent expressed and differentially expressed genes, respectively.

Table 3: Enriched Gene Ontology Terms after Infection with Pathogenic Bacteria

	Gene Ontology Term	Number of Genes	Percent[1]	FDR-adjusted p-value[2]
N2-only	Stress response: pathogen	13	7.3	9.4×10^{-7}
	Transmembrane transport: lipid	4	26.7	1.1×10^{-3}
	Metabolism: lipid	16	3.0	1.7×10^{-3}
CB4856-only	Metabolism: lipid	10	1.9	1.7×10^{-3}

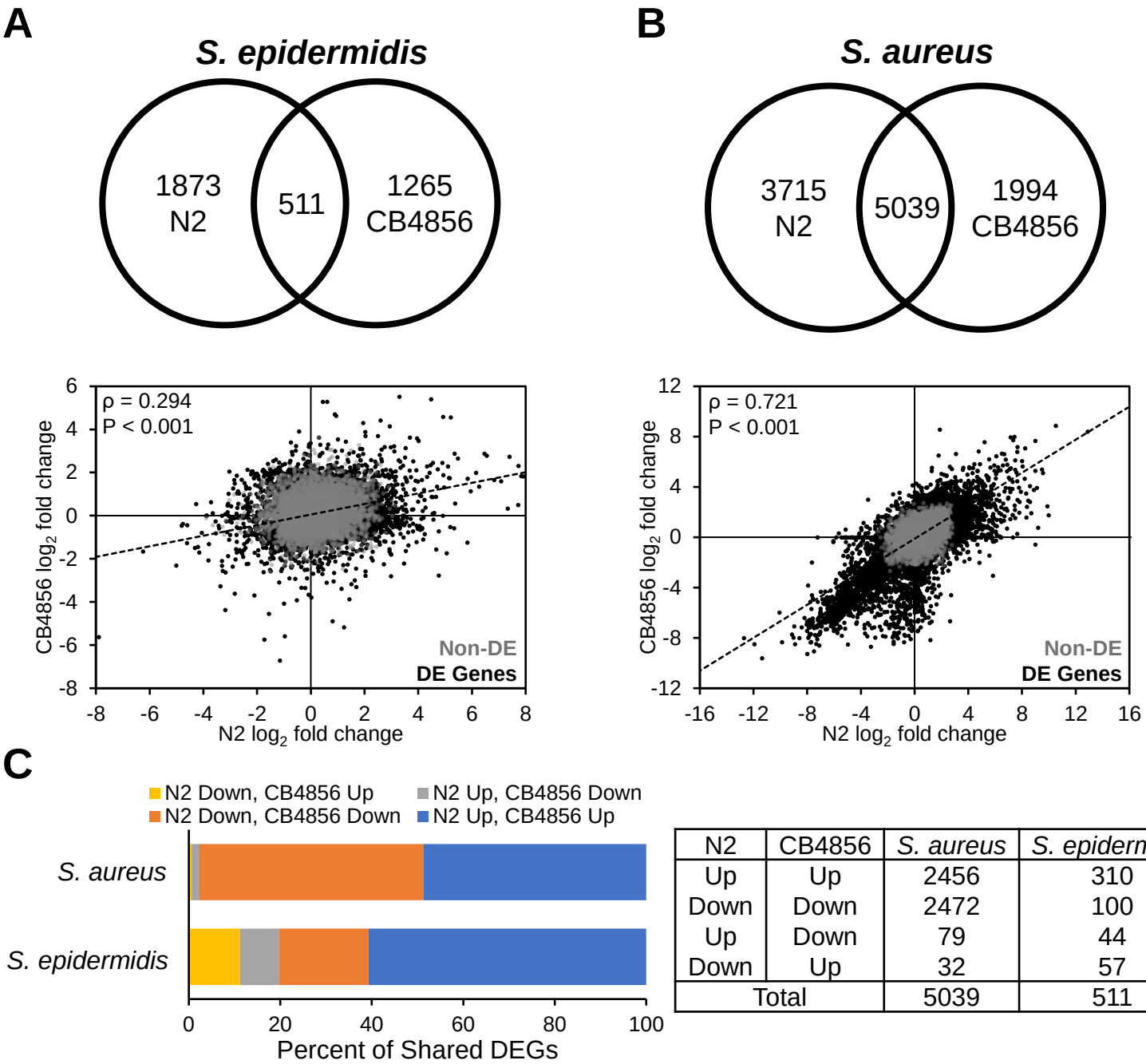
[1]Percentage of genes annotated with the indicated GO term that are differentially expressed
[2]FDR-adjusted *p*-value is a measure of enrichment (Fisher exact test; Bonferroni correction) of the GO term among differentially expressed genes





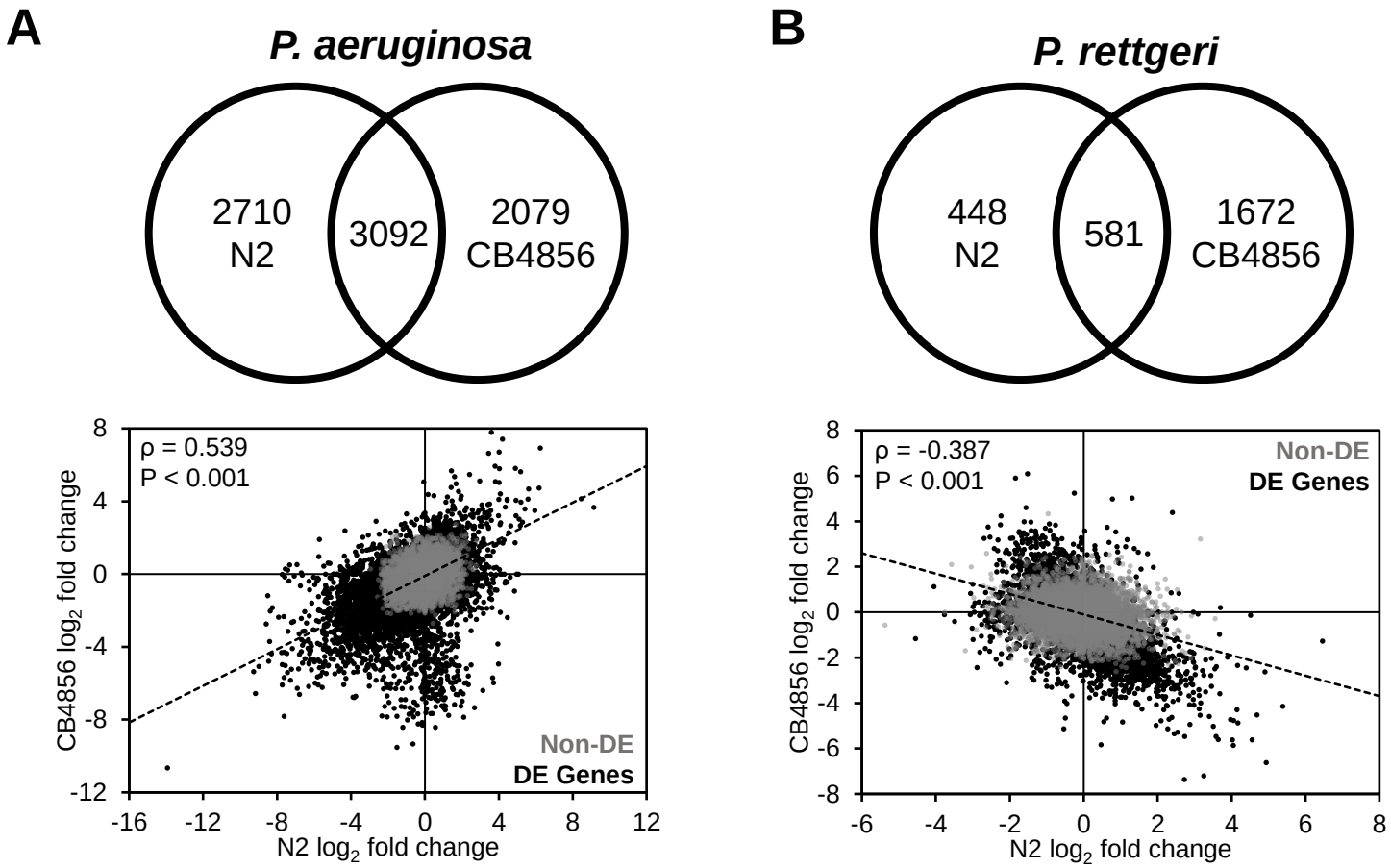
N2	CB4856	Gram (-)	Gram (+)
Up	Up	24	940
Down	Down	18	218
Up	Down	56	12
Down	Up	26	2
Total		124	1172

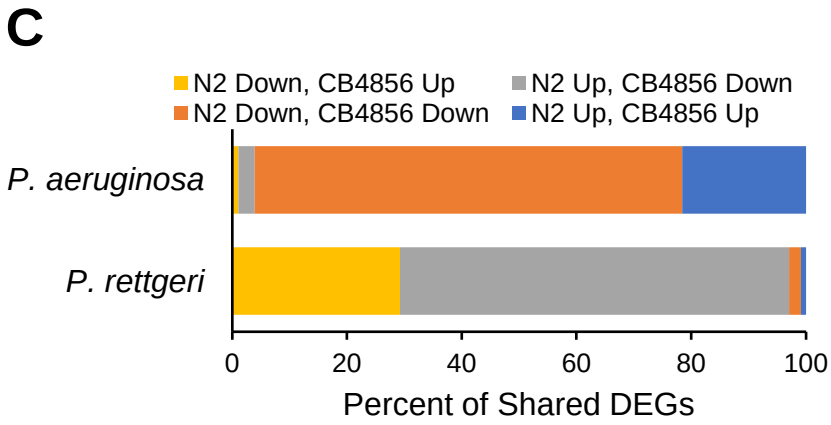
Figure 5: Expression differences in N2 and CB4856 animals infected with Gram-positive or Gram-negative pathogens.
(A) Intersection of differentially expressed genes (top) and correlation between $\log_2$ fold changes (bottom) in N2 and CB4856 animals infected with Gram-positive pathogens (*S. epidermidis, S. aureus*) or (B) Gram-negative pathogens (*P. rettgeri*, *P. aeruginosa*). Spearman's rho value and P-values are presented in the upper left corner. Gray (Non-DE) and black dots (DE Genes) represent expressed and differentially expressed genes, respectively. (C) The distribution of up- and down-regulated genes that are differentially expressed in both N2 and CB4856 animals infected with either Gram-negative or Gram-positive pathogens.



N2	CB4856	*S. aureus*	*S. epidermidis*
Up	Up	2456	310
Down	Down	2472	100
Up	Down	79	44
Down	Up	32	57
Total		5039	511

Figure 6: Expression differences in N2 and CB4856 animals infected with the gram-positive pathogens, *S. epidermidis* or *S. aureus*.
(A) Intersection of differentially expressed genes (top) and correlation between $\log_2$ fold changes (bottom) in N2 and CB4856 animals infected with either *S. epidermidis* or (B) *S. aureus*. Spearman's rho value and P-values are presented in the upper left corner. Gray (Non-DE) and black dots (DE Genes) represent expressed and differentially expressed genes, respectively. (C) The distribution of up- and down-regulated genes that are differentially expressed in both N2 and CB4856 animals infected with either *S. aureus* or *S. epidermidis*.





N2	CB4856	*P. aeruginosa*	*P. rettgeri*
Up	Up	667	5
Down	Down	2304	12
Up	Down	87	394
Down	Up	34	170
Total		3092	581

Figure 7: Expression differences in N2 and CB4856 animals infected with the gram-negative pathogens, *P. rettgeri* or *P. aeruginosa*.
(A) Intersection of differentially expressed genes (top) and correlation between $\log_2$ fold changes (bottom) in N2 and CB4856 animals infected with either *P. rettgeri* or (B) *P. aeruginosa*. Spearman's rho value and P-values are presented in the upper left corner. Gray (Non-DE) and black dots (DE Genes) represent expressed and differentially expressed genes, respectively. (C) The distribution of up- and down-regulated genes that are differentially expressed in both N2 and CB4856 animals infected with either *P. rettgeri* or *P. aeruginosa*.

Table 4: Enriched Gene Ontology Terms in N2 and CB4856 Worms Exposed to *S. epidermidis*

	Gene Ontology Term	Number of Genes	Percent[1]	FDR-adjusted p-value[2]
N2-only	Extracellular material: collagen	55	29.7	4.5×10^{-44}
	Extracellular material: cuticlin	7	20.0	1.5×10^{-4}
	Stress response: detoxification	13	8.6	2.8×10^{-4}
CB485-only	Stress response: pathogen	22	12.4	4.6×10^{-8}
	Stress Response: C-type Lectin	26	10.1	7.8×10^{-8}
	Stress response: detoxification	20	13.2	1.0×10^{-7}
	Metabolism: lipid	30	5.7	5.2×10^{-4}
N2 & CB4856	Stress response: pathogen	7	4.0	1.4×10^{-5}

[1]Percentage of genes annotated with the indicated GO term that are differentially expressed
[2]FDR-adjusted *p*-value is a measure of enrichment (Fisher exact test; Bonferroni Correction) of the GO term among differentially expressed genes

Table 5: Enriched Gene Ontology Terms when Comparing N2 and CB4856 Worms fed OP50 versus *S. epidermidis*

	Gene Ontology Term	Number of Genes	Percent[1]	FDR-adjusted p-value[2]
Genes expressed exclusively in worms fed *S. epidermidis*	Signaling: phosphatase	33	16.8	7.3×10^{-14}
	Cytoskeleton: microtubule	24	18.8	6.3×10^{-11}
	Major sperm protein	12	38.7	3.3×10^{-8}
	Signaling: tyrosine kinase	12	16.0	1.4×10^{-4}
	Protein modification: carbohydrate	15	11.8	2.4×10^{-4}
	Stress response: C-type Lectin	20	7.8	2.1×10^{-3}
Genes expressed in opposing directions in worms fed *E. coli* OP50 or *S. epidermidis*	Extracellular material: collagen	7	3.8	4.5×10^{-8}
	Stress response: pathogen	4	2.3	6.8×10^{-4}

[1]Percentage of genes annotated with the indicated GO term that are differentially expressed
[2]FDR-adjusted *p*-value is a measure of enrichment (Fisher exact test; Bonferroni Correction) of the GO term among differentially expressed genes