

Supplementary material

Title *Caenorhabditis elegans* employs innate and learned aversion in response to bacterial toxic metabolites tambjamine and violacein

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Running title: Nematode avoidance of bacterial toxic metabolites

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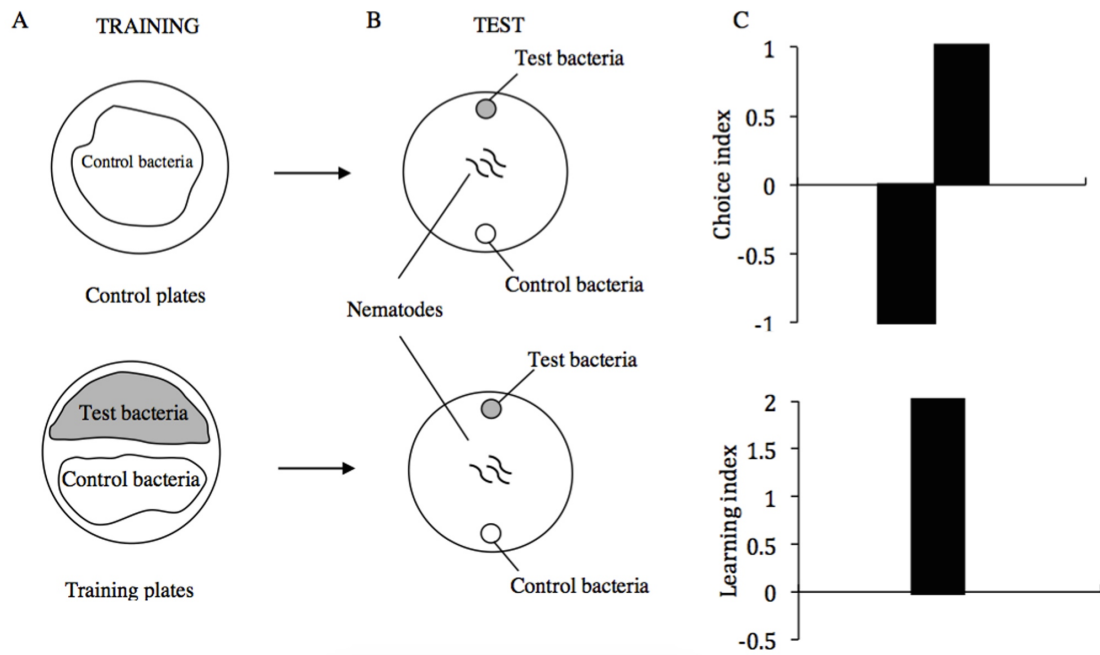


Figure S1. Schematic representation of the protocol for the food choice assay. (A) training plates, and (B) test plates. (C) Data from the food choice assay plotted as choice index or Learning index. A choice index of -1.0 represents complete preference for the control bacterium, an index of 1.0 represents complete preference for the test bacterium, and an index of zero represents an equal distribution. A positive learning index indicates a learned avoidance of toxic bacteria with the value 2 as maximum learning index. Adapted from (Zhang et al., 2005).

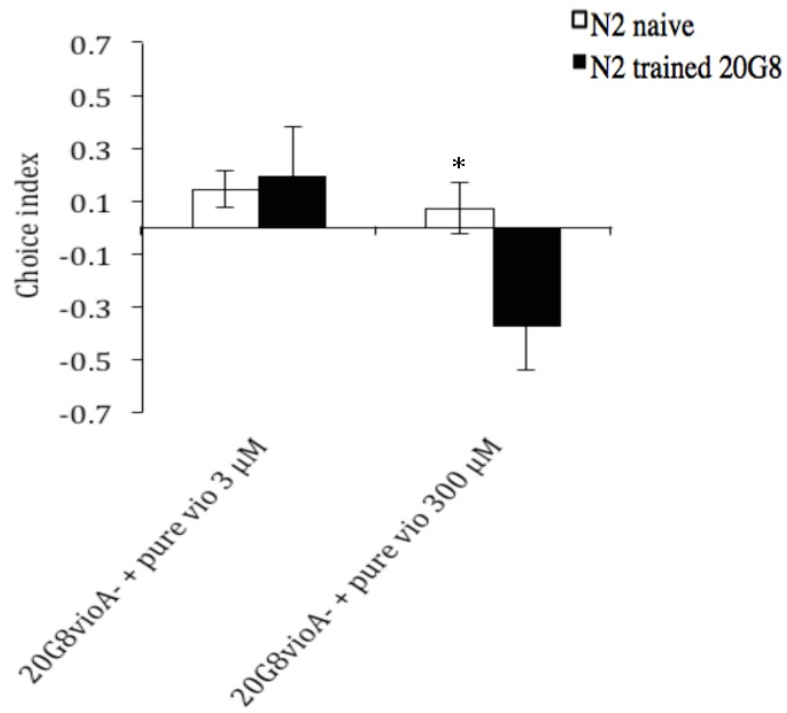


Figure S2. *C. elegans* assessed for aversive olfactory learning behavior in the food choice assay tested using 20G8vioA- and 20G8vioA- supplemented with pure violacein. Naïve (white bars) and trained (black bars). Nematode preference was tested against three and 300 μ M of pure violacein. Each data point represents means \pm the standard error of three replicates. * denotes $0.001 < p < 0.05$.

Table S1. Bacterial and nematode strains and vectors used in this study.

Strain/Vector	Relevant characteristic or genotype	Source or reference
<u>Bacterial strains</u>		
<i>E. coli</i> EPI300- T1 ^R	F- <i>mcrA</i> Δ(<i>mrrhsdRMSmcrBC</i>) φ80 <i>dlacZ</i> ΔM15Δ <i>lacX</i> 74 <i>recA1 endA1 araD139</i> Δ(<i>ara, leu</i>) 7697 <i>galU galK</i> λ- <i>rpsL nupG trfA tonA dhfr</i>	Epicentre
<i>E. coli</i> AA11	Fosmid AA11 cloned in <i>EPI300-T1^R</i> ; Cm ^r	(Burke, et al. 2007)
<i>E. coli</i> AA11 <i>tamG</i> ⁻	Fosmid AA11 mutated in <i>tamG</i> gene and cloned in <i>EPI300-T1^R</i> ; Cm ^r , Tetr	(Ballestriero, et al. 2014)
<i>E. coli</i> 20G8	Fosmid 20G8 cloned in <i>EPI300-T1^R</i> ; Cm ^r	(Penesyanyan, et al. 2012)
<i>E. coli</i> 20G8 <i>vioA</i> ⁻	Fosmid 20G8 mutated in <i>vioA</i> gene and cloned in <i>EPI300-T1^R</i> ; Cm ^r , Kan ^r	(Ballestriero, et al. 2014)
<i>E. coli</i> 20G8 <i>vioC</i> ⁻	Fosmid 20G8 mutated in <i>vioC</i> gene and cloned in <i>EPI300-T1^R</i> ; Cm ^r , Kan ^r	(Ballestriero, et al. 2014)
<i>E. coli</i> OP50	Uracil auxotroph	(Brenner 1974)
<u>Nematode strains</u>		
N2 Bristol	<i>C. elegans</i> wild type isolate	CGC ^b
PR811	<i>osm-6(p811)</i> V. <i>osm-6</i> mutants are defective in the ability to avoid high osmolarity. <i>osm-6</i> is required for proper sensory cilium structure. In <i>osm-6</i> mutants most of the ciliated neurons have severely shortened cilia that are not exposed to the environment. <i>osm-6</i> mutant shows reduced avoidance responses to repellents and fail to stain amphids with FITC.	CGC ^b
GR1321	<i>tph-1(mg280) cam-1(vs166)</i> II. Mutation in <i>tph-1</i> , which encodes the enzyme tryptophan hydroxylase that catalyses the rate-limiting first step in serotonin biosynthesis. Some phenotypic defects originally attributed to <i>mg280</i> in this strain are likely due to <i>vs166</i> , a deletion in the <i>cam-1</i> gene.	CGC ^b
MT15434	<i>tph-1(mg280)</i> II. Backcrossed strain carrying <i>mg280</i> allele without the <i>cam-1</i> mutation.	(Flavell, et al. 2013) ^c
<u>Vectors</u>		
pCC1FOS ^a	Fosmid backbone for genomic library; Cm ^r	Epicentre

^a Copy number inducible by arabinose. ^b *Caenorhabditis* Genetics Center. ^c Strain provided by Cori Bargmann, Rockefeller University New York

References

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