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REVIEW

www.wormgenes.org , a new gene centered database.

Danielle et Jean Thierry-Mieg[1,2,3], Michel Potdevin[2], Mark Sienkiewicz[1], Vahan Simonyan[1], Tadasu Shin-i[3], Yuji Kohara[3]

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Wormgenes is a new resource for C.elegans offering a detailed summary about each gene and a powerful query system.

P> P>

The interface is very simple: you ask a question, you get a list of genes. If you open a gene, the information will come as a single little illustrated story, with paragraphs about function, expression, interactions and so on, and the selected biblio at the bottom. From there, you can jump to a second page, where we annotated the mRNA variants and proteins. You can query across the database for proteins sharing a motif or belonging to the same family. The database can of course be queried by name, but also by content, using natural language. For example you may ask for 'mitotic spindle orientation', 'expressed in nerve' 'protruding vulva and essential', 'axon guid', 'stress response', 'growth factor and transduct' or 'membrane protein in endoplasmic reticulum'. The system looks everywhere for the simultaneous presence of all these (possibly truncated) words and returns a list of genes. If you find a gene missing from the list, please send us a note, so that we can improve our annotation.

It is also possible to use a blast interface, in which case you will get the best matches to your query in worm, human, and soon arabidopsis and drosophila, the 4 species for which so far we offer a similar integrated annotation of all the genes we reconstruct from alignment on the genome of the entire mRNA and EST dataset in Genbank. In the worm, we are limited now to the genes with complete mRNAs from Genbank.

P> P>

The data are derived in part from a selection of CGC and Wormbase, in part from our own annotations, in part from direct contributions. Wormgenes (also called AceView at NCBI) offers direct links to Yuji Kohara's NextDB in situ hybridization, to Marc Vidal's WorfDB, to Tony Hyman's RNAi sites and to various other places including Wormbase. The bibliography leads to Leon Avery's site and to Medline. The site is updated quarterly in synchrony with the worm sections of the NCBI Refseq and Locuslink resources.

P> P>

We would be very pleased to receive your comments and suggestions on the interface, but mostly to integrate under your name any addition to the data that you might like to contribute.

P> P>

Acknowledgments: A database is only as good as the data it presents, and we are grateful to the whole worm community for their experimental results, to the CGC, Wormbase and the other sites for data gathering, and to all our friends who helped over the years in the development of acedb.

Updated protocols for Generating *C. elegans* Gene Deletion Mutants

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We have recently released a substantially updated and improved version of our protocols for generating gene knockouts in *C. elegans*. The document can be downloaded from the "protocols" section of our Web site:

http://info.med.yale.edu/mbb/koelle/

Our methods are adapted from those originally developed at NemaPharm and in the Plasterk, Barstead, and Moerman labs. Briefly, mutagenized *C. elegans* are cultured in 96-well microtiter dishes. A mutant "library" representing 920,000 mutagenized genomes is thus generated. A portion of each culture is frozen alive and the remainder of each culture is used to prepare genomic DNA. Any gene of interest will suffer deletions of 100-1000 bp at a frequency of ~1/200,000 mutagenized genomes. Using PCR, a genomic DNA sample containing such a deletion can be detected, allowing the corresponding frozen microtiter culture to be thawed. Thus live animals carrying a deletion mutation in the gene of interest can be recovered.

Our methods require an initial investment of about 2 weeks of part-time work to pilot the techniques followed by ~3 weeks of full-time work (if two individuals work together) to construct a frozen mutant "library". The library can be stored indefinitely and can be screened at least 200 times. Once the library is constructed one can isolate a live mutant in a gene of interest in only 2-3 weeks of part-time work. Using these methods we have so far succeeded in obtaining one to three mutant alleles for almost every gene we have worked on.

The newly-released version of our protocols includes technical improvements both in library construction and in methods for PCR-detection of deletions. We have also included a new outline and flowchart of the procedures, making them easier to understand. Our new methods are substantially more successful than those we have previously released. In the past we failed to isolate a deletion in about 1/3 of genes we attempted to knock out. With the new methods we are virtually always successful and can expect to obtain multiple alleles per gene. We believe this technology is now successful enough that it is worth the investment of time and effort for any small *C. elegans* laboratory.

Modelling of *C. elegans* pharyngeal neural network

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This work describes a dynamical model of the neural circuitry and muscles for the pharynx of *C. elegans*. Our goal is to build a model of the pharyngeal neural network dynamics with respect to the generation of signals (action potentials) for controlling the feeding. We also include in the model the processing of feedback from the sensory endings in the muscles. A dynamical system of two variables is used to mimic the myogenic rhythm of the pharyngeal muscles.

A set of simulations is described in detail where some connections are tuned to mimic apropriate behaviour. We also try to use a genetic algorithm to tune parameters for the system, where a population of pharynx models is evolved through random mutation and crossover.

Our model is limited to the information about the pharynx dynamics that we could gather from published literature, and we were successfull in building and simulating a simple model. The simulations mimic some some behaviours observed in the laboratory with respect to feeding and it's control by the pharyngeal neural circuitry. In the text, we give a detailed discription of the pharynx aspects and behaviour that we'd like to model, and discribe what we can model using only the data collected.

Download the report here: http://marcelo69.vila.bol.com.br/Celegans

The genetic map of *Caenorhabditis briggsae*

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The *Caenorhabditis briggsae* genome sequence has facilitated experiments related to the evolution of gene function and their networks. Towards classical genetic approach to study *C. briggsae* genes, we have started building linkage maps using markers such as Unc (Uncoordinated, Aka Movement impaired *mip*), Dpy (Dumpy, Aka Chubby *Cby*), Rol (Roller, Aka Rotator *Rot*), Con (Constipated), Sma (Small), Bli (Blister), Lin (Lineage defective), Daf (Dauer formation) as well as GFP expressing integrated arrays. Revision of the gene nomenclature for *C. briggsae* and other nematodes is under discussion. The phenotypic markers have been isolated following EMS mutagenesis of the wild-type AF-16 strain. The GFP markers contain *C. elegans* genomic sequences *myo-2* (pPD118.33, Fire lab vector), *daf-4* and *egl-17* (*egl-17::GFP*, Burdine et al., 1998). So far, mapping studies have revealed six independent linkage groups (5 autosome and one X-linked). To correlate the genetic and physical maps, we are cloning the *C. briggsae* loci of known *C. elegans* genes by transgene rescue and/or allele sequencing (e.g., *lin-11*, *unc-4* and *daf-4*).

So far EMS screens have identified more than 250 mutants displaying Dpy, Unc, Rol, Bli, Sma, Con, Lin and Daf phenotypes, of which we have mapped 23 Dpy, 20 Unc, 2 Rol, 2 Sma, one Con, 8 Lin and 3 Daf to various linkage groups. This was followed by 2- and 3-factor mappings to determine precise linkage and gene order (see Figure 1). We are continuing the mapping project with the objective of achieving high density and resolution.

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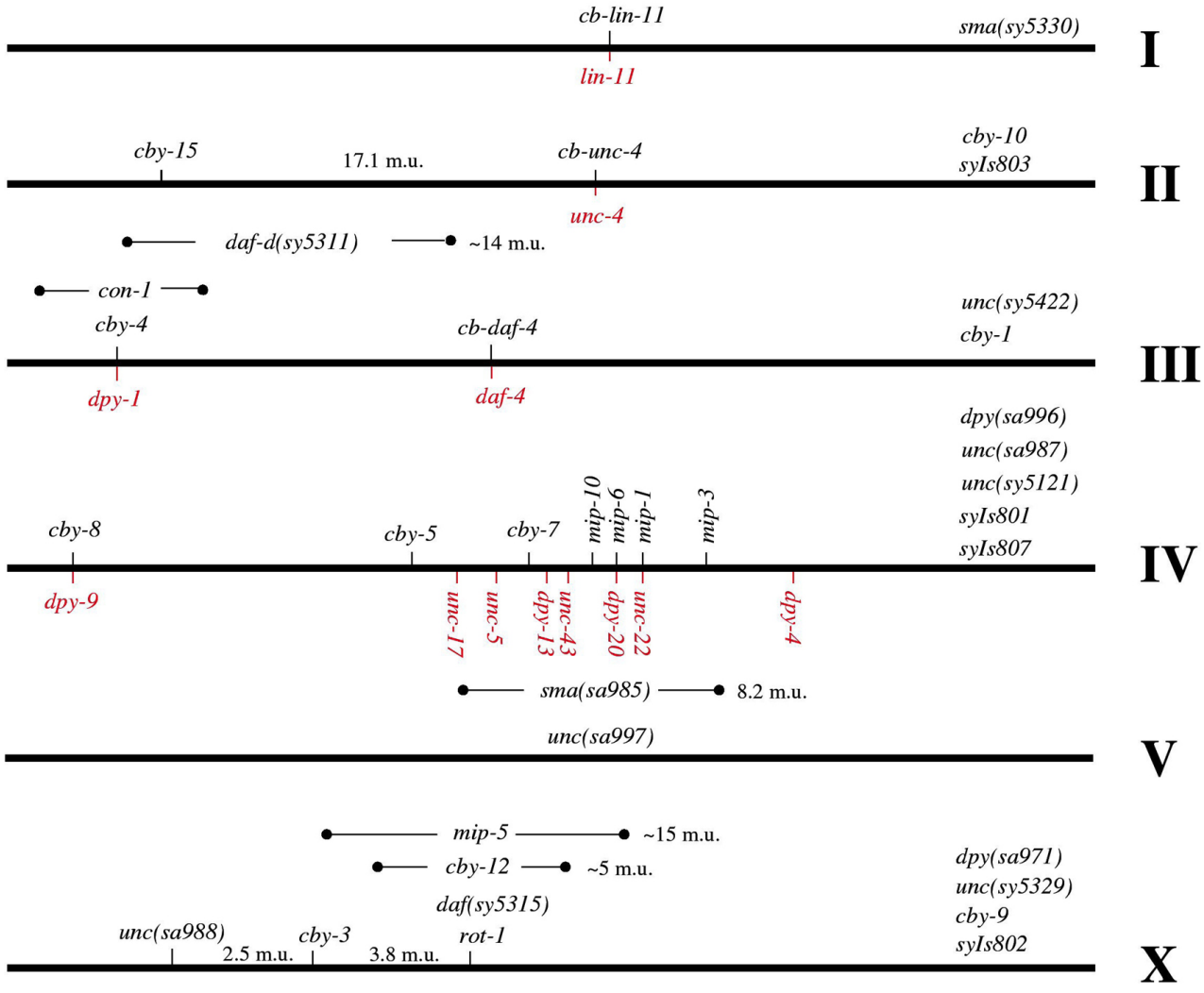

Figure 1. *C. briggsae* linkage map. For correlation *C. elegans* genes have been placed below the lines.

Baseline data for worms grown in *C. elegans* Maintenance Medium

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Growth of *C. elegans* in axenic medium was proposed prior to development of the worm as a genetic system. Dougherty saw the utility of *C. elegans* as a genetic system and was interested in axenic cultivation in order to apply genetics to the study of worm metabolism. Development of a chemically defined axenic medium for *C. elegans* cultivation ultimately proved a long road and in the interim the worm has become a genetic model system. Most of our community grows animals on NGM and because of the large base of knowledge of animals on NGM there is good cause to continue to use NGM.

However, there are a number of reasons to consider use of an alternative medium for worm growth and as a result a number exist. Unlike some media the chemically defined, axenic *C. elegans* Maintenance Medium[1] (CeMM) can be used either in a liquid or solid state in petris or any of a number of standard cell culture containers. Solid state use provides the same general benefits as NGM although it is harder to get animals to mate and animals tend to burrow. Liquid phase use provides the ability to cultivate large numbers of animals and to automate culturing and experimentation. Because CeMM is chemically defined one has the advantage of altering the chemical composition if desired and its use removes the caveats associated use of undefined medium or *E. coli*.

To assist in wide spread use of CeMM we have collected data on the life-cycle of *C. elegans* in CeMM, just as baseline data was collected for grown on NGM early in the development of *C. elegans* research. Animals grown in or on CeMM grow and reproduce for at least 8 weeks regardless of temperature (15°->25°C) and without medium exchange. Similar to NGM grown animals, animals grown in CeMM undergo four moults and display temperature sensitivity of growth rate, brood size, and life-span. At 15°C animals reach a length of 1 mm in about 9.5 days, begin laying eggs on day 9, lay a total of 107 ± 58 eggs and cease laying eggs on day 19. At 15°C animals have mean and maximum life spans of 41 and 112 days. At 20°C animals reach a length of 1 mm in about 7 days, begin laying eggs on day 7, lay a total of 81 ± 31 eggs and cease laying eggs on day 14. At 20°C animals have mean and maximum life spans of 30 and 93 days. At 25°C animals reach a length of 1 mm in about 5 days, begin laying eggs on day 5, lay a total of 42 ± 18 eggs and cease laying eggs on day 9. At 25°C animals have mean and maximum life spans of 17 and 37 days. These data combined with the results of analyses of lipid and protein stores and microarray analysis of CeMM grown versus NGM grown animals suggest that animals have different metabolisms depending upon the media type. Thus, those wishing to use CeMM should be aware that, as with all media, there are caveats associated with use of CeMM and potential pitfalls of applying what is known of the animals on NGM to animals in CeMM. Pre-made 2x CeMM can be obtained from Fisher, please contact the Conley Lab for further details.

This research was funded by NASA Fundamental Biology and the NASA Astrobiology Institute.

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Exposure To Sodium Azide Reduces Lifespan In C. elegans

Christen M. Kyre, *Jill Y. MacAlpine*, *Geneva A. Stork*, *Jason N. Crawford*, *Colleen D. Root*, *Jacqueline M. White*, *Melissa S. White*, *Glenn E. White*

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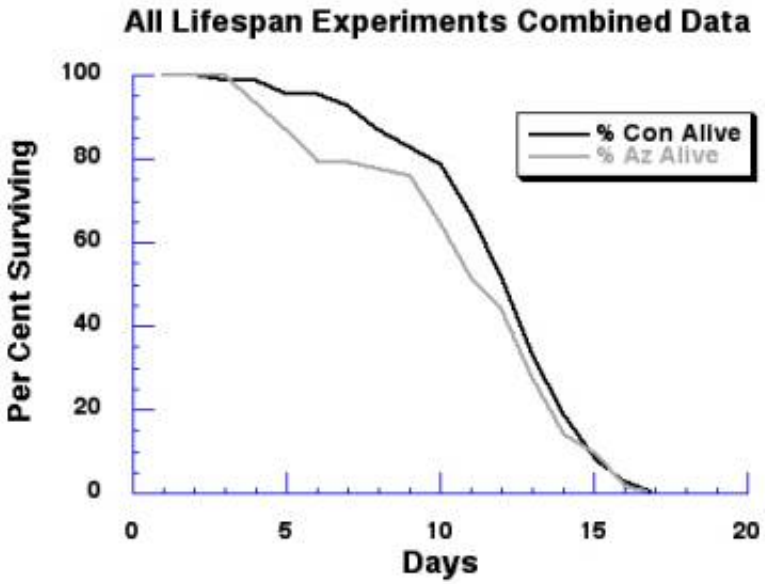
Previous work in our lab demonstrated that exposing C. elegans to the metabolic inhibitor sodium azide induces thermotolerance and the expression of the stress proteins hsp70 and hsp16 (Massie et al., 2003). We have continued our efforts to characterize further the physiological response of worms to sodium azide exposure. We sought to determine what effect, if any, sodium azide exposure had on the lifespan of the animal as well as on its reproductive viability since azide continues to be the anesthetic of choice for C. elegans researchers. In four separate double-blinded experiments consisting of 57 control and 50 experimental worms total (L1 and L2), a one hour exposure to sodium azide resulted in animals consistently showing a 7.1% decrease in lifespan. In our lab, the average age at death of control worms was 11.2±0.5 days, as compared to 10.4±1 days for azide treated worms (see Figure I). A two-tailed Z score demonstrated a confidence interval of $p<0.001$. We are also interested in determining whether azide exposure had an effect on the reproductive viability of the animal. For the reproductive viability study, we were concerned that the manual transfer of L1s and L2s may be quite traumatic for those animals and could be affecting their reproductive viability. As such, we decided to use L3 and L4 animals. Three separate experiments using L3 and L4 experimental worms (14 control and 15 azide exposed), failed to demonstrate a consistent effect on brood size or the number of days that the animals laid eggs. Our results demonstrate that the use of sodium azide as an anesthetic is not without immediate physiological consequences for the animal in terms of stress protein induction and a shortening of its lifespan.



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An attempt to slow aging in *C. elegans*. 30. No positive effect of kudesan

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The purpose of this study was to investigate the effect of kudesan (a water-soluble medicine, containing 30.0 mg of ubiquinone and 4.5 mg of α-tocopherol in 1 mL) in water solutions on the nematode life span. In this experiment kudesan was used in following dilutions: 1000, 100, 10, 1.0 and 0.1 mg/L. Three adult animals (3 - 5 days old) were kept in microtitre wells containing 0.5 ml of liquid medium (with *E. coli* and without kudesan) during 4 hours, then they were discarded and newborn larvae were transferred in next wells (without kudesan in medium) every day (one worm in one well) beginning from third day. Then, beginning from 3rd day, these worms were transferred every day in next wells containing medium with kudesan in any concentration. This investigation was carried out in temperature +21°C and in the darkness. The obtained results are presented in the following table.

Concentration of kudesan (mg/L)	n	Longevity (days)	
		Mean±S.E.	Maximal
Control	12	11.08±1.29	17
100 000	12	11.08±1.56	19
10 000	12	4.75±1.27	18
1 000	12	12.75±2.00	25
100	12	10.33±1.37	20
10	12	8.08±1.20	17
1	12	13.50±1.47	21
0.1	12	13.75±1.32	23

Conclusion: If kudesan solution was applied to *C. elegans*, it was not able to increase their mean longevity significantly in comparison with control.

Acknowledgment: The authors wish to express their thanks to CGC for providing *C. elegans* (Bristol, N2) and *E. coli* OP50.

An attempt to slow aging in *C. elegans*. 31. No positive effect of glycerol

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The purpose of this study was to investigate the effect of glycerol in water solutions on the nematode life span. In this experiment glycerol was used in following dilutions: 100 g/L, 10 g/L, 1 g/L, 100 mg/L, 10 mg/L, 1 mg/L and 0.1 mg/L. Three adult animals (3-5 days old) were kept in microtitre wells containing 0.5 ml of liquid medium (with *E. coli* and without glycerol) during 4 hours, then they were discarded and newborn larvae were transferred in next wells (without glycerol in medium) every day (one worm in one well) beginning from third day. Then, beginning from 3rd day, these worms were transferred every day in next wells containing medium with glycerol in any concentration. This investigation was carried out in temperature +21°C and in the darkness. The obtained results are presented in the following table.

Concentration of glycerol	n	Longevity (days)	
		Mean±S.E.	Maximal
Control	12	18.08±1.60	26
100 g/L	12	16.00±1.93	21
10 g/L	12	16.08±1.44	24
1 g/L	12	15.67±1.85	27
100 mg/L	12	16.83±1.66	26
10 mg/L	12	16.00±2.12	26
1 mg/L	12	10.75±1.47	21
0.1 mg/L	12	13.00±1.56	24

Conclusion: If glycerol solution was applied to *C. elegans*, it was not able to increase their mean longevity significantly in comparison with control.

Acknowledgment: The authors wish to express their thanks to CGC for providing *C. elegans* (Bristol, N2) and *E. coli* OP50.

An attempt to slow aging in *C. elegans*. 32. No positive effect of hydrogen peroxide

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The purpose of this study was to investigate the effect of hydrogen peroxide in water solutions on the nematode life span. In this experiment hydrogen peroxide was used in following dilutions: 100 mg/L, 10 mg/L, 1 mg/L, 0.1 mg/L, 0.01 mg/L and 0.001 mg/L. Three adult animals (3-5 days old) were kept in microtitre wells containing 0.5 ml of liquid medium (with *E. coli* and without hydrogen peroxide) during 4 hours, then they were discarded and newborn larvae were transferred in next wells (without hydrogen peroxide in medium) every day (one worm in one well) beginning from third day. Then, beginning from 3rd day, these worms were transferred every day in next wells containing medium with hydrogen peroxide in any concentration. This investigation was carried out in temperature +21°C and in the darkness. The obtained results are presented in the following table.

Concentration of hydrogen peroxide	n	Longevity (days)	
		Mean±S.E.	Maximal
Control	12	12.42±1.57	18
100 mg/L	12	11.92±1.72	20
10 mg/L	12	10.33±1.39	17
1 mg/L	12	9.17±1.48	20
0.1 mg/L	12	7.42±0.87	16
0.01 mg/L	12	8.92±1.34	19
0.001 mg/L	12	10.33±1.45	19

Conclusion: If hydrogen peroxide solution was applied to *C. elegans*, it was not able to increase their mean longevity significantly in comparison with control.

Acknowledgment: The authors wish to express their thanks to CGC for providing *C. elegans* (Bristol, N2) and *E. coli* OP50.

Author index

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An attempt to slow aging in *C. elegans*. 28. A positive effect of acetylsalicylic acid

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The purpose of this study was to investigate the effect of acetylsalicylic acid in water solutions on the nematode life span. In this experiment acetylsalicylic acid was used in following dilutions: 1000, 100, 10, 1.0 and 0.1 mg/L. Three adult animals (3 - 5 days old) were kept in microtitre wells containing 0.5 ml of liquid medium (with *E. coli* and without acetylsalicylic acid) during 4 hours, then they were discarded and newborn larvae were transferred in next wells (without acetylsalicylic acid in medium) every day (one worm in one well) beginning from third day. Then, beginning from 3rd day, these worms were transferred every day in next wells containing medium with acetylsalicylic acid in any concentration. This investigation was carried out in temperature +21°C and in the darkness. The obtained results are presented in the following table.

Concentration of acetylsalicylic acid (mg/L)	n	Longevity (days)	
		Mean±S.E.	Maximal
Control	36	9.14±0.50	15
100	36	11.36±0.78	21
10	36	11.78±0.77	21
1	36	13.14±0.74	21

Conclusion: If acetylsalicylic acid solution was applied to *C. elegans* in concentration of 100; 10 and 1 mg/L, it was able to increase significantly (P>0.01; >0.02 and >0.001) their mean longevity in comparison with control to 24.29; 28.88 and 43.76 percent.

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An attempt to slow aging in *C. elegans*. 29. No positive effect of sodium salicylate

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The purpose of this study was to investigate the effect of sodium salicylate in water solutions on the nematode life span. In this experiment sodium salicylate was used in following dilutions: 1000, 100, 10, 1.0 and 0.1 mg/L. Three adult animals (3 - 5 days old) were kept in microtitre wells containing 0.5 ml of liquid medium (with *E. coli* and without sodium salicylate) during 4 hours, then they were discarded and newborn larvae were transferred in next wells (without sodium salicylate in medium) every day (one worm in one well) beginning from third day. Then, beginning from 3rd day, these worms were transferred every day in next wells containing medium with sodium salicylate in any concentration. This investigation was carried out in temperature +21°C and in the darkness. The obtained results are presented in the following table.

Concentration of sodium salicylate (mg/L)	n	Longevity (days)	
		Mean±S.E.	Maximal
Control	12	10.67±1.82	22
10 000	12	toxic	
1 000	12	11.08±0.65	16
100	12	10.75±1.40	22
10	12	10.08±0.82	16
1	12	10.75±1.15	19
0.1	12	9.33±0.54	12

Conclusion: If sodium salicylate solution was applied to *C. elegans*, it was not able to increase their mean longevity in comparison with control.

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