NUTRITIONAL ALTERATION OF LIFE SPAN IN THE NEMATODE *CAENORHABDITIS ELEGANS*

C. De Cuyper and **J. R. Vanfleteran***
Laboratory of Animal Morphology
and Systematics
State University of Ghent
Ledeganckstraat 35
B-9000 Ghent (Belgium)

Abstract

The longevity of the free-living nematode *Caenorhabditis elegans* was studied under two different nutritional regimes, one axenic and the other monoxenic. Axenic nematodes showed typical sigmoidal survival curves with exceptionally long tailing. Monoxenic worms died off much faster and the maximum life-span in bacterial culture was generally three to four times shorter than that obtained in axenic culture. When nematodes were transferred from axenic to monoxenic culture and *vice versa* at near adulthood the survival patterns observed were reminiscent of the final medium. These results are in agreement with the hypothesis that worms may die off prematurely in bacterial culture by toxins given off by the bacteria.

Introduction

Free-living nematodes, among which *C. elegans* are gaining popularity as model systems for studying aging (1-3). They are small, can easily be grown in either bacterial (*Escherichia coli*) or axenic culture and have short life spans. In addition they offer the advantage of a system which is accessible to combined genetic and biochemical analysis (4, 5).

It has become clear that culture conditions considerably influence the actual life span of *C. elegans*. Thus the median life span, i.e. the time by which 50% of the nematode population cultured at 20-21 °C on a bacterial diet has died, is about 12-16 days (6-8) and in axenic conditions 17-22 days (6, 8), exceptionally 59 (9) days. The rapid decline of live nematodes observed in bacterial culture even with daily transfer to fresh medium led Hansen (10) and Croll and his associates (6) to believe that the intestinal bacterial flora somehow might cause their premature death. If so, serious restraints would be imposed to the use of the monoxenic regime for studying the process of aging in *C. Elegans*.

Alternatively it might be argued that the time needed for nematodes to mature in axenic culture is delayed and that there is some intrinsic relationship of life span to maturation time. Worms grown in bacterial culture would then live shorter as a consequence of their ability to complete their life cycle in considerably less time.

*To whom all correspondence should be addressed

The present study is an attempt to further investigate the influence of the nutritional regime on nematode aging by interchanging the diet when the worms reach maturity and examining the effects on reproduction and life span.

Results

The effects of temperature and nutritional regime on some biological parameters of *C. elegans* are shown in Table 1. For comparison the average fecundity of *C. elegans* on a bacterial diet may be taken at 200-280 at 21 °C (7, 11). Both the increased generation time and the few progeny produced in axenic culture may reflect partial starvation. CbMMS-Hb allows faster maturation than CbMMS-Hm but this appears to have no effect on life span. However, when the worms were unable to mature, the median life span increased to as much as 88 days. It is interesting to note that the median life span observed in axenic culture is not significantly shorter at 25 °C as compared to 21 °C. This is in contrast to comparable results in monoxenic culture (7).

The survival curves observed with axenic and monoxenic nematodes are strikingly different (Fig. 1). Axenic worms show sigmoidal survival curves with a small but consistent number of long term survivors. These are absent in the monoxenic regime. We then resolved to examine whether this could be due to some toxic effect produced by the bacteria. Nematode larvae were cultured in a suspension of *E. coli* cells (5.10^8/ml) until they reached the late fourth juvenile or early adult stages. Then they were axenized and grown further in axenic medium. Corollarly larvae were raised in axenic medium and transferred to *E. coli*. Fecundity and life span data are presented in Table 2. The survival curves are shown in Fig. 2, A and B. From these data it is clear that the shape of the curves is largely determined by the final culture medium. The shorter median life span observed in some of these experiments is not relevant largely due to stressing factors related to the washing in antibiotics and adaptation to another medium. Careful examination of the survival curves reveals a decline of live nematodes which coincides with the event of transfer.

Table 1. Effects of Temperature and Nutritional Regime on Generation Time, Life Span and Fecundity of *C. Elegans*

Culture medium	*Temp.* °C	*Generation time* (days)	*Median life span* (days)	*Average number* of progeny[a]	*Number of nematodes* in each trial
CbMMS-Hb[b]	25	4.5- 5	25	—	100
	21	6- 6.5	19.5	36 ± 2	100
	16	9.5-10	24	—	100
CbMMS-Hm[b]	25	7.5- 8	18	—	20
	21	7 - 9	19	28 ± 4[d]	140
	16	nm[c]	88	—	100
E. coli	21	3 - 3.5	11.5	—	25

[a]Mean values ± S.E.

[b]CbMMS-Hb = *Caenorhabditis briggsae* Maintenance Medium (GIBCO Bio-Cult, Grand Island, N.Y.) supplemented with 50 μg/ml sterol mixture and 1 mg/ml hemoglobin; CbMMS-Hm = *Caenorhabditis brigssae* Maintenance Medium supplemented with 50 μg/ml sterol mixture and 200 αg-ml hemin chloride.

[c]nm = non-maturing.

[d]Mean ± S.E. of 25 animals only. Figures have been rounded to whole numbers.

Table 2. Effect of Alteration of the Nutritional Regime on Generation Time, Life Span and Fecundity of *C. Elegans*

Culture medium	Generation time (days)	Median life span (days)	Number of progeny[a]	Reproductive period (days)	Age at transfer
E. coli → CbMMS-Hb	3-3.5	19	19 ± 2	12	young adult
E. coli → CbMMS-Hm	3-3.5	9.5	17 ± 2	12	L_4
E. coli → CbMMS-Hm	3-3.5	9.5	10 ± 1	12	young adult
CbMMS-Hb → *E. coli*	8.5-9	13.7	81 ± 28	8	young adult
CbMMS-Hm → *E. coli*	7.5-8	12.5	23 ± 6	7	young adult

[a]The values cited are mean ± S.E. The figures have been rounded to whole numbers. Each condition represents 20-25 worms. Temperature was 21 °C.

Discussion

Our results are in agreement with the hypothesis proposed by Hansen (10) and Croll and his associates (6) that the shorter nematode longevity observed in bacterial culture is caused by toxins given off by the bacteria. Nematodes which are fed on *E. coli* until they reach maturity and then are axenized and transferred to axenic culture medium age like worms raised in axenic medium. They show the typical sigmoidal survival curve, the long tail of which is due to a small but consistent number of long term survivors. Corollarly axenic nematodes which are transferred to a bacterial diet show the rapid decline in numbers typical for monoxenic culture conditions. The production of many more progeny is a minor factor which may explain the rapid death in monoxenic culture. At this point it is interesting to compare life span data from two trials (Table 2) in which worms were transferred from axenic medium to bacterial culture. These are very similar but in one experiment the average production of eggs was 81 whereas in the second no more than 23 eggs were laid. Further support for this may be derived from the experiments of Mitchell and Santelli (12) who observed nearly identical median life spans when nematodes were grown on *E. coli* in the presence and absence of sufficient amounts of 5-fluorodeoxyuridine so as to prevent reproduction.

In conclusion we believe that the study of aging in bacterial culture is complicated by toxic effects given off by the bacteria. It is also true, however, that this effect can be controlled to some extent by dietary restriction. Monoxenically grown nematodes may also show survival curves that are sigmoidal rather than parabolic but long term survivors comparable to those found in axenic culture are absent (8).

It is not clear why aging nematodes but not larvae nor young adults suffer from the putative toxins. There may be a gradual accumulation of toxins in the nematode tissues reaching detrimental levels later on in life. On the other hand the decreased ability to withstand physiological stress is a general characteristic of aging organisms.

From our present investigation it is not possible to determine whether there is some intrinsic relationship of maturation time to life span, i.e. whether the lengthening or shortening of the juvenile phase has an equal or inverse effect on the adult life span. Some of the data presented in Table 1 would suggest that life span is rather independent of lengthening or shortening of the larval phase. On the other hand there is some indirect evidence that maturation by itself triggers the onset of the aging process somehow. Thus we

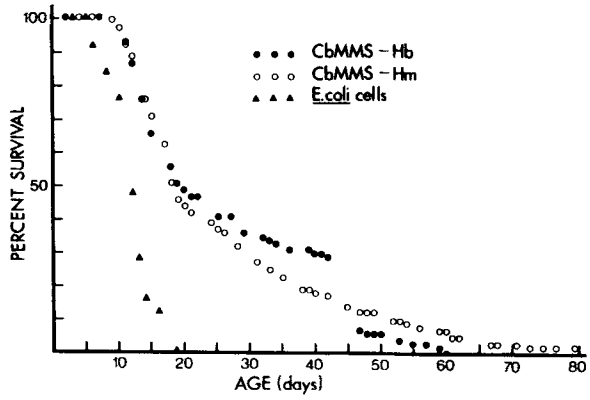

Figure 1. Life span of *C. elegans* in axenic and in bacterial culture. Newly hatched larvae were transferred to each of two axenic media (CbMMS-Hb and CbMMS-Hm) and to *E. coli* cells suspended in S medium (15) at 5.10^8 cells/ml and kept in these media throughout their lifetime. Temperature was 21 °C. Each condition represents 100 worms except for the monoxenic culture which contained 25 worms.

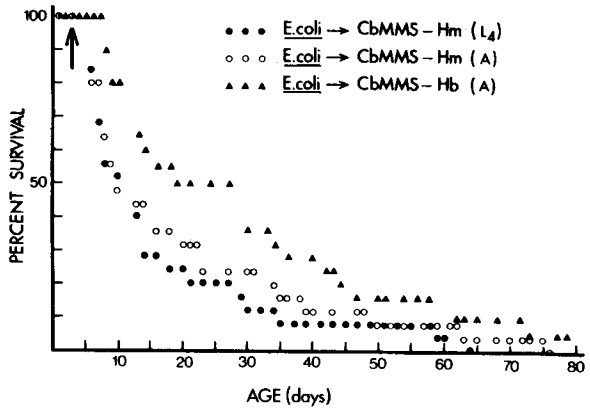

Figure 2A. Life span of *C. elegans* after transfer from monoxenic to axenic culture. Newly hatched larvae were raised in monoxenic medium (5.10^8 *E. coli* cells/ml) until they just began to mature. Then they were axenized and transferred to axenic culture medium (arrow). Each trial represents 20-25 worms. Temperature was 21 °C. L_4 = fourth stage larvae; A = adults.

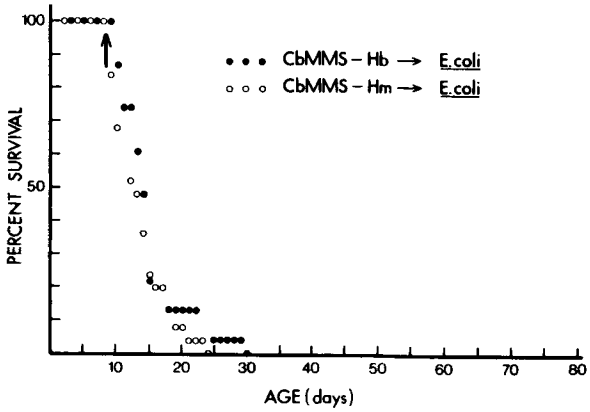

Figure 2B. Life span of *C. elegans* after transfer from axenic to monoxenic culture. Newly hatched larvae were raised in axenic medium until maturation. Then they were washed and transferred to monoxenic medium (arrow). Each condition represents 20-25 nematodes. Temperature was 21 °C.

have consistently observed exceptionally high survival rates, even at room temperature, when nematode larvae were grown in axenic media that were unable to support maturation. These larvae behave like adult aging nematodes in becoming very sluggish and loosing transparency (accumulation of age pigment?).

Owing to the shape of the survival curves a decrease from 60% to 40% survival currently correlates with a considerable shift on the time axis. This is a serious restraint to the usefulness of the 50% survival value for characterizing longevity. Thus we have generally found that replicate experiments performed simultaneously produced fairly reproducible results. However, when longevity was investigated later on in identical conditions but using other egg masses and a new batch of medium, the shape of the survival curves substantially remained unchanged whereas the time at which 50% survival was observed sometimes varied considerably. Differences in physiological condition of the first stage larvae may be partly responsible for this event. In addition we have noticed that batches of axenic culture medium may exhibit variable growth promoting potency. Improved axenic media may enhance reproducibility. In the meantime it will be necessary to compare all experimental trials to simultaneously run control series so as to provide the internal consistency which is required for quantitative comparisons.

Experimental Procedures

Culture Media

Caenorhabditis elegans strain N_2 (originally obtained from Dr. H. F. Epstein, Departments of Neurology and Biochemistry, Baylor College of Medicine, Houston, Texas) was used in all experiments. Stock cultures were maintained axenically on a medium consisting of 3% dry yeast extract, 3% soy peptone and 0.5 mg/ml hemoglobin. The axenic media used throughout the experiments were chemically more defined. One medium designated CbMMS-Hb was composed of CbMM (*Caenorhabditis briggsae* Maintenance Medium, available from GIBCO Bio-Cult, Grand Island, New York) to which was added 50 µg/ml sterol mixture and 1 mg/ml hemoglobin. In the second medium called CbMMS-Hm hemoglobin was replaced by 200 µg/ml hemin chloride. These media have been described in detail elsewhere (13, 14). Monoxenic culture was performed on *E. coli* cells (strain 3000-38) grown in tryptone broth (1% bacto tryptone, 0.5% bacto yeast extract and 0.5% sodium chloride) and suspended in S medium (15) at about 5.10^8 cells/ml. All experiments were performed at 21 °C unless otherwise stated.

Axenization

Worms grown in bacterial medium were transferred with a pasteur pipette through a series of small petri dishes containing M9 buffer (4) to which were added 10,000 U/ml penicillin and 10 mg/ml streptomycin so as to remove the bulk of the bacterial cells. The worms were then suspended for 2 hours in M9 buffer containing 1,000 U/ml penicillin and 1 mg/ml streptomycin and finally transferred to the axenic culture medium to which were added 200 U/ml penicillin and 0.2 mg/ml streptomycin. Sterility was checked by adding a drop from each test culture onto nutrient agar.

Generation Time, Fecundity and Longevity

Egg masses are typically formed by dense nematode populations grown in axenic culture. These were harvested, washed and allowed to hatch in M9 buffer. The newly hatched larvae were then inoculated one in each culture tube containing 0.30 ml of culture medium and generation time was determined as the time elapsed from inoculation to hatching of the first progeny. The adults were then transferred daily to fresh medium so as to prevent confusion with their growing offspring. All progeny were counted to determine the mean fecundity in a given medium. Unlike worms grown on a bacterial diet, axenic nematodes were no longer transferred to fresh medium after reproduction had stopped. Median longevity (survival, life span) was determined as the time in days by which 50% of the inoculated worms were dead. Death was determined by visual inspection under a dissecting microscope. Worms which did not move at all after swirling the culture tube by hand were defined as dead. Premature death may occur from endotokia matricida (progeny hatching in the uterus and feeding on the tissues of the maternal organism) in both axenic and monoxenic culture though it is much more frequent in axenic culture. Worms which became endotokic were not used to plot survivorship.

Acknowledgments

J. R. Vanfleteren is sponsored by the Belgian National Fund for Scientific Research (research associate career).

References

1. Gershon, D.: Studies on aging in nematodes. I. The nematode as a model organism for aging research. Exptl. Gerontol., 5:7-12, 1970.

2. Zuckerman, B. M. and Himmelhoch, S.: Nematodes as models to study aging, in Nematodes as Biological Models. Vol. 2, edited by Zuckerman, B. M., New York, Academic Press, 1980, pp. 4-28.

3. Rothstein, M.: Effects of aging on enzymes, in Nematodes as Biological Models. Vol. 2, edited by Zuckerman, B. M., New York, Academic Press, 1980, pp. 29-46.

4. Brenner, S.: The genetics of *Caenorhabditis elegans.* Genetics, 77:71-94, 1974.

5. Nematodes as Biological Models. Vol. 1, edited by Zuckerman, B. M., New York, Academic Press, 1980.

6. Croll, N. A., Smith, J. M. and Zuckerman, B. M.: The aging process of the nematode *Caenorhabditis elegans* in bacterial and axenic culture. Exper. Aging Res., 3:175-189, 1977.

7. Klass, M.: Aging in the nematode *Caenorhabditis elegans:* major biological and environmental factors influencing life span. Mech. Ageing and Devel., 6:413-419, 1977.

8. Mitchell, D. H., Stiles, J. W., Santelli, J. and Sanadi, D. R.: Synchronous growth and aging of *Caenorhabditis elegans* in the presence of fluorodeoxyuridine. J. Gerontol., 34:28-36, 1979.

9. Tilby, M. J. and Moses, V.: Nematode ageing. Automatic maintenance of age synchrony without inhibitors. Exptl. Gerontol., 10: 213-223, 1975.

10. Hansen, E., Buecher, E. J. and Yarwood, E. A.: Development and maturation of *Caenorhabditis briggsae* in response to growth factor. Nematologica, 10:623-630, 1964.

11. Byerly, L., Cassada, R. C. and Russell, R. L.: The life cycle of the nematode *Caenorhabditis elegans* I. Wild type growth and reproduction. Develop. Biol., 51:23-33, 1976.

12. Mitchell, D. H. and Santelli, J.: Fluorodeoxyuridine as a reproductive inhibitor in *Caenorhabditis elegans:* applications to aging research. Personal communication.

13. Vanfleteren, J. R.: Axenic culture of free-living, plant-parasitic, and insect-parasitic nematodes. Ann. Rev. Phytopathol., 16: 131-157, 1978.

14. Vanfleteren, J. R.: Nematodes as nutritional models, in Nematodes as Biological Models. Vol. 2, edited by Zuckerman, B. M., New York, Academic press, 1980, pp. 47-79.

15. Sulston, J. E. and Brenner, S.: The DNA of *Caenorhabditis elegans.* Genetics, 77: 95-104, 1974.