Journal of Nutrition & Intermediary Metabolism 13 (2018) 20–25

Contents lists available at ScienceDirect

Journal of Nutrition & Intermediary Metabolism

journal homepage: www.elsevier.com/locate/jnim

Rapid induction of vitamin B12 deficiency in *Caenorhabditis elegans* cultured in axenic medium

Surafel M. Tegegne[a,*], Markandeya Jois[a], Matthew R. Flavel[a], Damien L. Callahan[b], Devin Benheim[a]

[a] School of Life Sciences, La Trobe University, Crn Plenty Road and Kingsbury Drive, Bundoora, VIC, 3083, Australia
[b] School of Life and Environmental Sciences, Deakin University, 221 Burwood Highway, Burwood, VIC, 3125, Australia

HIGHLIGHTS

- *C. elegans* grown in defined axenic medium without added B12 exhibit signs of deficiency in one generation.
- Worms grown in deficient media had retarded growth, reduced fertility, reduced quiescence and shortened lifespan.
- *C. elegans* cultured in a defined axenic medium is a suitable and rapid model for B12 deficiency study.

ARTICLE INFO

Keywords:
Axenic
C. elegans
Deficiency
Fertility
Growth
Lifespan
Quiescence
Vitamin B12

ABSTRACT

Animal models of B_{12} deficiency have proven to be difficult due to storage of substantial amounts in the liver and the length of time required to maintain animals on a B_{12} deficient diet to induce deficiency. *Caenorhabditis elegans (C. elegans)*, due to its short lifespan, has recently emerged as an alternate model to investigate vitamin B12 (B12) deficiency. However, when *C. elegans* are maintained on bacterial diet, five generations of B12 deficient diet is required before the worms show signs of deficiency. Here we show that *C. elegans* grown in chemically defined axenic medium without added B12 exhibit signs of deficiency within one generation. Worms grown in deficient media had lower cobalt concentration, retarded growth, reduced fertility, increased motility, reduced quiescence and a shortened lifespan. In conclusion, *C. elegans* cultured in a defined axenic medium is a suitable and rapid model for studies on B12 deficiency.

1. Introduction

Research on vitamin B12 (B12) deficiency has long been constrained by lack of a suitable model organism due to the fact that animals store B12 in their liver for long term use [1], which prolongs the process of generating B12 deficient models. The commonly used model animals such as rodents require several months on a B_{12} deficient diet to deplete their body reserve before symptoms of deficiency become apparent.

The free-living soil nematode *Caenorhabditis elegans* is a microscopic (1 mm body length at its adult stage) and free-living organism with about 1000 cells. It needs only 3 days to complete its life cycle (L1 to adult) and its lifespan is 3–4 weeks. It has been well established that the nematode is an important model to study the functions of disease-related genes underpinning a number of major advances in the field of developmental biology and neurobiology. Watson, Olin-Sandoval [2] also reported that B12 deficient diets in *C. elegans* transcriptionally activate a B12-independent propionate breakdown shunt. Hence, these are believed to be important characteristics which back up the organism's strong potential to serve as a suitable model to study B12 deficiency.

Bito, Matsunaga [3] managed to induce B12 deficiency in *C. elegans* after feeding B12-deficient *E. coli* (0.2 μg/g wet weight) for five successive generations. According to their report, signs of B12 deficiency including reduced growth and fertility were not observed until the third generation. Here, we report that, in chemically defined axenic media without added B12, *C elegans* show signs of B12 deficiency including reduced growth, fertility and lifespan within one generation.

* Corresponding author.
E-mail address: S.Tegegne@latrobe.edu.au (S.M. Tegegne).

https://doi.org/10.1016/j.jnim.2018.08.001
Received 3 July 2018; Received in revised form 6 August 2018; Accepted 6 August 2018
Available online 07 August 2018
2352-3859/ © 2018 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

2. Methods

2.1. Maintenance of C. elegans

Wild-type *C. elegans* strain (N2) and a dietary sensor strain (*Pacdh-1*::*GFP*) were acquired from *C. elegans* Genetics Center (CGC) and University of Massachusetts respectively and maintained on NGM agar medium with a lawn of *E. coli* OP50 bacteria as food. Prior to conducting all the experiments in CeHR medium, the worms were allowed to adapt to the axenic environment for three generations before use.

Stock solutions of salts, vitamins and growth factors, amino acids, nucleic acids, buffers were prepared separately with deionised water from Milli-Q system before assembling them with 20% UHT skim milk. All components of the media were filtered with 0.22 μm filter and stored in light protective containers. A mix of antibiotics (Nalidixic acid, 250 μg/mL; Streptomycin, 250 μg/mL and Tetracycline, 100 μg/mL) were also added after assembly to keep the medium sterile and axenic [4]. The pH was adjusted to 6.5 prior to culturing the worms. The media preparation steps were done as reported by Samuel, Sinclair [5]. Accordingly, the final concentrations of the ingredient in CeHR were as follows: Cyanocobalamine (2.77 μM), Biotin (15.35 μM), Folinate (Ca μM) (7.33 μM), Folic acid (16.99 μM), Niacinamide (61.41 μM), Pantetheine (6.76 μM), Pantothenate (Ca μM) (31.47 μM), Pyridoxal 5′-phosphate (15.18 μM), Pyridoxamine.2HCl (15.55 μM), Pyridoxine.HCl (36.48 μM), Niacine (60.92 μM), Riboflavine 5-PO4 (15.68 μM), Thiamine.HCl (22.24 μM), DL-6,8-Thioctic Acid (18.18 μM), Glucose (72.97 μM), HEPES Na salt (20 μM) and Cholestrol (12.93 μM), Adenosine 2'- & 3′-PO4 (1002.3 μM), Cytidine 5′-PO4 (1002.18 μM), Guanosine 5' - PO4 (999.45 μM), Uridine 5′-PO4 (999.73 μM), Thymine (999.05 μM), KH2PO4 (9 mM), Choline di-acid citrate (1.998 mM), i-Inositol (2.4 mM), Hemin Chloride (20 μM), MgCl2.6H2O (2016.62 μM), Sodium Citrate (986.06 μM), Potassium Citrate.H2O (1510.48 μM), CuCl2.2H2O (41.06 μM), MnCl2.4H2O (101.06 μM), ZnCl2 (73.37 μM), Fe(NH4)2(SO4)2.6H2O (153 μM), CaCl2.2H2O (136.04 μM), *N*-Acetylglucosamine (67.81 μM), DL-Alanine (168.35 μM) and *p*-Aminobenzoic Acid (54.69 μM). Additionally, 20 mL essential of Amino Acid mix (GIBCO 11130–051) and 10 mL of non-essential Amino Acid mix (GIBCO 11130–050) were added in 1L CeHR at the final media assembly stage.

2.2. B12 in individual worms

The B12 content of individual worms was determined by measuring their Cobalt (Co) concentration, using Inductively coupled plasma mass spectrometry (ICP-MS) as reported by Ganio, James [6]. Co concentration of ten independent biological replicates of individual *C. elegans* were analysed from those cultured in the absence of B12 and from the control group. Synchronous L1 *C. elegans* were cultured in the two groups until they reached mature adult age. They were then removed from the axenic media and transferred into 1.5 mL polypropylene test tubes containing 200 μL S-basal ($5.85\,g\,L^{-1}$ NaCl; $1\,g\,L^{-1}\,KH_2PO_4$; $6\,g\,L^{-1}\,K_2HPO_4$). The test tubes were gently inverted to clean the remnants of the culturing media attached with the bodies the worms. Samples were then washed three times with 200 μL S-basal, followed by three washes in 200 μL ultra-pure water to remove any remaining bacteria. To avoid sample loss, a stereomicroscope was used to monitor during aspirations between washes. Samples were then flash frozen in liquid nitrogen and lyophilized overnight. After lyophilisation, samples were digested in 20 μL of 65% HNO_3 for 12 h at room temperature and diluted 1:10 to a final volume of 200 μL using 1% HNO_3. To correct for any contamination that may occur during the digestion process, ten blanks were prepared parallel to each. Finally, Cobalt concentration along with other metals was quantified using NexION 350X ICP-MS (Perkin Elmer).

2.3. Growth, fertility and lifespan

Change in body length, the number of offspring produced by each adult worm and survival data were collected for each worm in both treatment and control group. Growth rate data was obtained from images collected every other day by measuring changes in length. Additionally, fertility assay was conducted by counting the number of progenies excluding unhatched eggs. Moreover, lifespan assay was also carried out by counting the number of dead animals every other day. In this study, instead of using Floxuridine (FuDR) to stop reproduction of worms, they were transferred to new plates with fresh media until they finished laying eggs. When the worms ceased laying eggs, they were left on the same plate and monitored for lifespan until all the worms died. Collecting lifespan data was started once most of the worms in each treatment became adults. For convenience in transferring and data collection, single worms were kept in individual wells of 24 or 48 well plates.

2.4. Acyl-CoA dehydrogenase expression

Acyl-CoA dehydrogenase *acdh-1* expression was determined by measuring the fluorescence of Pacdh-1:GFP strain grown and maintained in CeHR media with and without added B12. Adult worms were placed on 2% agar pads on mounting slides and covered with cover slides. Then the worms were immobilised by quick heat shock on a flame for 3–5 s to take fluorescence images using EVOS cell imaging system. Analysis of fluorescence images were done using the open access image and video analysis software imageJ where fluorescence intensity was measured based on image thresholding.

2.5. Measurement of quiescence and motility

MBF WormLab software version 4.1 was used for video analysis. Motility of worms exposed to different B12 treatments were analysed. Parameters selected from the video analysis were track length (μm) and speed (μm/s) (See Fig. 1). Track length is the length of forward motion plus the length of reverse motion from mid-point in a total number of tracks. Whereas, speed represents the track length in every second.

2.6. Data collection and analysis

EVOS FL Auto Cell Imaging System was used to follow up growth, fertility and survival of worms. Growth and development of the worms were measured using Image J open access image analysis software. Image J open access image analysis software was also used for setting the scale of images and videos using based on scale bars. Video

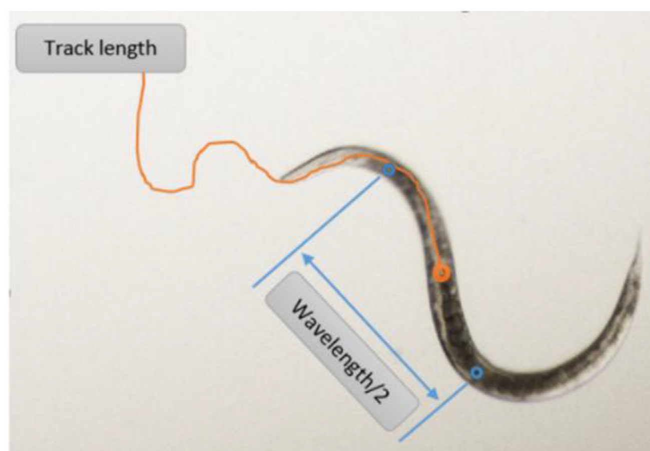

Fig. 1. Illustration of wavelength and track length measurements made by MBF-WormLab video analysis software.

Download English Version:

https://daneshyari.com/en/article/8589125

Download Persian Version:

https://daneshyari.com/article/8589125

Daneshyari.com