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**Note**

# D-Allose, a Stereoisomer of D-Glucose, Extends the Lifespan of *Caenorhabditis elegans* via Sirtuin and Insulin Signaling

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**Abstract: D-Allose (D-All), C-3 epimer of D-glucose, is a rare sugar known to suppress reactive oxygen species generation and prevent hypertension. We previously reported that D-allulose, a structural isomer of D-All, prolongs the lifespan of the nematode *Caenorhabditis elegans*. Thus, D-All was predicted to affect longevity. In this study, we provide the first empirical evidence that D-All extends the lifespan of *C. elegans*. Lifespan assays revealed that a lifespan extension was induced by 28 mM D-All. In particular, a lifespan extension of 23.8 % was achieved ($p < 0.0001$). We further revealed that the effects of D-All on lifespan were dependent on the insulin gene *daf-16* and the longevity gene *sir-2.1*, indicating a distinct mechanism from those of other hexoses, such as D-allulose, with previously reported antiaging effects.**

**Key words: anti-aging, *daf-16*, *sir-2.1*, *Caenorhabditis elegans*, D-allose, lifespan**

D-Allose (D-All), a structural isomer of D-allulose, is a rare sugar that is present in a limited quantity in nature.[1)2)] D-All has been detected at low levels in human cord blood[3)] and in Indian seaweed.[4)] However, it is challenging to study allose owing to its low abundance. D-All could be produced by the Izumoring strategy, which is a systematic method for the production of all monosaccharide isomers using microbial enzymes.[1)] Mass production methods developed by Izumori have provided insight into biological properties. The safety of D-All has been established in rats[5)] and in clinical studies, and its utilizable energy value is approximately zero.[6)] Studies have demonstrated various functions of D-All, such as anti-tumor activity,[7)] anti-hypertension effects,[8)] and brain protection from ischemic injury,[9)] and these functions are mainly due to the suppression of reactive oxygen species (ROS) generation by competing for D-glucose utilization.[10)11)] Based on the beneficial health effects, D-All is expected to be a potent anti-metabolic syndrome drug and a pharmaceutical precursor.

*Caenorhabditis elegans* is a powerful animal model for lifespan assays and studies of the mechanisms underlying aging owing to its short lifespan and well-defined genetic pathways.[12)] Moreover, most longevity genes and signaling pathways are evolutionarily conserved from nematodes to mammals.[13)] A Forkhead box O transcription factor, the primary downstream target of the insulin/IGF-1 signaling pathway, is encoded by *daf-16* in *C. elegans*. It modulates lifespan, metabolism, dauer formation, and stress resistance.[14)] *Sir-2.1* encodes an $NAD^+$-dependent histone deacetylase (Sirtuin) that regulates lifespan by modulating the transcription of genes involved in the stress response.[15)]

We have reported that a low dose of D-allulose, a structural isomer of D-All, prolongs the lifespan of nematodes.[16)] The anti-aging effect of D-allulose may be attributed to its ability to mimic the structure of metabolizable D-sugars, thereby modulating carbohydrate metabolism to extend the life span. Therefore, we hypothesized that D-All is also a functional sugar with an effect on longevity, similar to D-allulose. However, the impact of D-All on longevity has not been demonstrated.

We determined the concentration of D-All that did not influence nematode growth and demonstrated its antiaging activity. In addition, to determine the mechanisms underlying the D-All-induced lifespan extension, we evaluated mutant strains deficient for well-established longevity pathway-related genes, *daf-16,* and *sir-2.1*.

## EXPERIMENTAL

D-All was prepared enzymatically at the International Institute for Rare Sugar Research and Education, Kagawa

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Abbreviations: AMP, Adenosine 5′-monophosphate; NAD, nicotinamide adenine dinucleotide; NGM, nematode growth medium; ROS, reactive oxygen species.

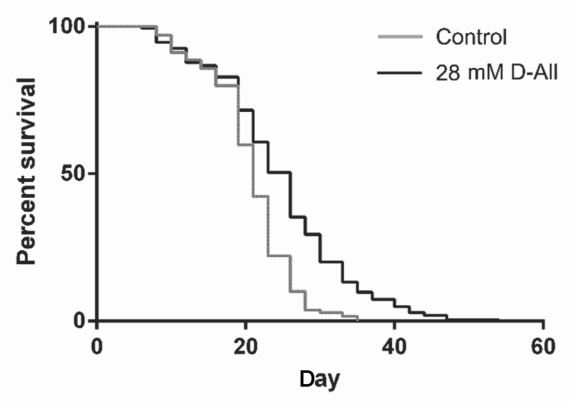


**Fig. 1.** Survival curves of wild type *C. elegans* N2 cultured with D-All.

**Table 1.** Summary and statistical analysis of *C. elegans* lifespan assays.

| Strain | Sugar | Mean lifespan ± SE (days) | Extension (%) | Number of worms | *p*-value (*vs*. None) |
|---|---|---|---|---|---|
| N2 | None | 20.6±0.6 | | 239 | |
| | D-All | 25.5±0.7 | +23.8 | 212 | < 0.0001 |
| *daf-16*(*mgDf50*) | None | 16.8±0.5 | | 101 | |
| | D-All | 17.5±0.4 | +4.2 | 103 | 0.3424, NS |
| *daf-16* (mu86) | None | 14.2±0.6 | | 101 | |
| | D-All | 13.9±0.9 | -2.1 | 100 | 0.3626, NS |
| *sir-2.1*(*ok434*) | None | 19.9±0.8 | | 108 | |
| | D-All | 19.0±0.4 | -4.5 | 104 | 0.7138, NS |

The *p*-value (*vs*. control) was calculated by the log-rank test. NS, not significant.

University. The purity of D-All was higher than 98 %. *C. elegans* N2 (wild-type), *daf-16* two allelic mutant (mgDf50 and mu86), and, *sir-2.1* mutant (ok434) strains were obtained from the *Caenorhabditis* Genetic Center (CGC) at the University of Minnesota. *Escherichia coli* OP50 was used as a food source for the nematodes.

*C. elegans* was maintained at 20 °C on nematode growth medium (NGM)[17] with *E. coli* OP50. As a departure from previous studies, peptone-free NGM medium was used. Eggs of *C. elegans* were collected by treating egg-bearing adults with alkaline hypochlorite solution and were shaken in S basal medium at 20 °C for 20–24 h to prepare synchronized first-stage larvae (L1).[17]

Lifespan assays were performed by conventional methods.[16] One hundred synchronized animals (10 animals/dish) were placed in 2-mL S liquid medium dishes containing D-All with *E. coli* OP50. Control worms were incubated in medium without sugars. 5-Fluoro-2′-deoxyuridine was added (40 μM, final concentration) to prevent progeny growth. In each assay, 10 dishes (10 animals/dish) were used. The dishes were incubated without shaking at 20 °C. The numbers of live and dead animals were counted every other day under a microscope based on their movement, and the survivors were transferred to the new medium. The survival curves were determined using the Kaplan–Meier

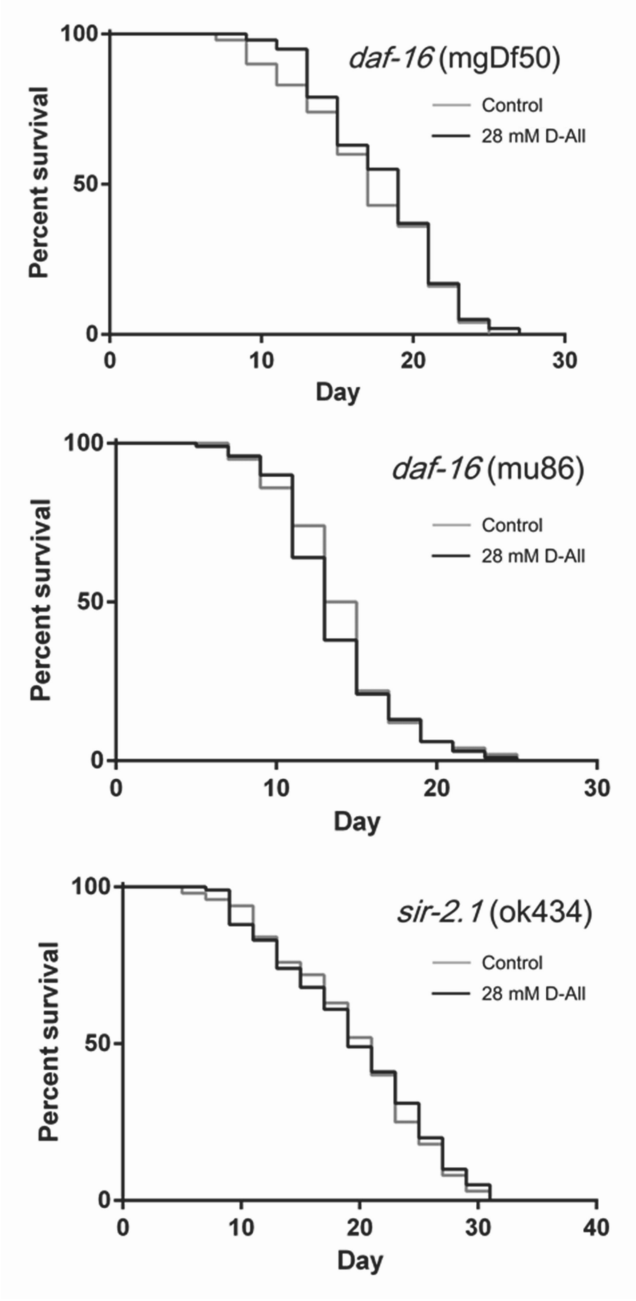


**Fig. 2.** Survival curves of *C. elegans* longevity gene mutants cultured with D-All.

method, and survival differences were tested for significance using the *log-rank* test. Data are expressed as means ± standard error (SE).

D-All was used at a concentration of 28 mM (0.5 %) for the lifespan assay, based on a previous study;[16] this was also in the range of concentrations added to feed in the mixed feed administration method for rodents.[5] Furthermore, we previously showed that 168 mM (3 %) D-All has a weak effect on nematode growth,[18] and our preliminary data showed that 0–56 mM (0–1 %) D-All does not affect nematode growth (data not shown); accordingly, 28 mM is sufficiently low concerning growth inhibition.

We found that the mean lifespan was significantly extended (23.8 %) in the presence of 28 mM D-All as compared to the control ($p < 0.0001$) (Fig. 1, Table 1). Under almost

identical conditions on an OP50 diet for nematodes, the lifetime extension rates of D-allulose were 7.0 and 12.0 % at a sugar concentration of 25 and 10 mM, respectively. Therefore, we can infer that D-All may exert stronger anti-aging activity than D-allulose.

Several molecules involved in longevity, such as DAF-16 and SIR-2.1, have been identified in *C. elegans*.[15)16)] To determine whether the extension of lifespan by D-All is mediated by pathways involving these molecules, we used *daf-16* and *sir-2.1* mutants for lifespan assays using D-All. The lifespans of both *daf-16* and *sir-2.1* mutants did not increase in the presence of D-All (Fig. 2, Table 1). These results indicated that the D-All-induced lifespan extension was dependent on both DAF-16 and SIR-2.1.

D-Allulose and D-glucosamine, that are D-glucose analog as same as D-All, have been reported to extend the lifespan of nematodes not via sirtuin and insulin-dependent signaling pathways but nutrient signaling. Similar to D-glucosamine,[19)] D-allulose enters cells via glucose transporters and inhibits glycolysis, inducing the metabolism of fat and mitochondrial respiration via AMP-activated protein kinase (AMPK). Increased breathing can induce the temporary upregulation of ROS, leading to increased anti-oxidative enzyme activity and survival rates.[16)20)] Orally administrated D-All also reduces body weight in rodents,[5)] which might affect carbohydrate or lipid metabolism. However, the mechanism underlying the antiaging or antimetabolic effect of D-All is not precise, and the role of the nutrient sensor AMPK should be evaluated in the future. Taken together, the anti-aging effect of D-All may be similar to those of D-allulose and D-glucosamine, with differences in the underlying mechanisms and precise outcomes.

In conclusion, we revealed that D-All extends the nematode lifespan and that this effect is dependent on the insulin gene *daf-16* and the longevity gene *sir-2.1*. Further studies are needed to confirm whether D-All extends the lifespan under other experimental conditions, for practical antiaging applications.

## CONFLICTS OF INTEREST

Tomoya Shintani is an employee of Matsutani Chemical Industry Co., Ltd.

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