Talk

The study of the effect of mannose in GALE Caenorhabditis elegans mutants using as food source Escherichia Coli mutants defective in mannose metabolism pathway

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ABSTRACT

Type III galactosemia is a rare disease characterized by mutations in the GALE gene that encodes the enzyme UDP-galactose 4-epimerase. The deficit of this enzyme gives rise to various physical and mental problems in humans (Walter et al. 1999). Several studies in gale-1 mutants of C. elegans worms have shown a positive effect of mannose (Brokate-Llanos et al. 2014), on the longevity and development of these mutants.

These worms have as their main diet E. coli (OP50), so that the positive effects of mannose can be influenced by the transformation these bacteria can produce through metabolic transformation of mannose. Trying to eliminate this possible influence of OP50 mannose metabolism on these experiments, UV radiation inactivated E.coli has been used as worm food in the presence of different concentrations of mannose. This approach presents, among others, the problem that by inactivating bacteria by UV radiation we are eliminating their replication, but not their complete metabolism,demanding other experimental approaches as using E. coli strains unable to metabolize mannose to feed the worms.

Therefore, the objective of this work is to study the effects of mannose in C. elegans, fed with OP50 mutants defective in different steps of mannose metabolism to assess the direct effects of mannose on C. elegans GALE mutants.

- Construction of OP50 isogenic strains defective in different steps of mannose metabolism by transformation with mutant alleles obtained from E. coli (K12) mutant strains. OP50 was transformed by homologous recombination with fragments containing deletions in ΔmanA (gene for the enzyme of the sugars metabolism mannose derived pathway), ΔmanB (gene for the enzyme of the lipopolysaccharide synthesis mannose derived pathway) and ΔmanX (gene for one of the mannose transporters within the cell). These deleted genes were replaced by a kanamycin resistance gene.
- Preparation of eggs of C. elegans and incubation in plates with different concentrations of mannose (0%, 1%, 2%, 3%).The E. coli used are the different mutant strains obtained and a control (wild type OP50).

OP50 mutants for the genes indicated above were correctly obtained. They were tested with the GALE worms, and we observed that ΔmanA and ΔmanX show a great improvement in GALE mutants growth and development with respect to the wt OP50 control. Nevertheless, we did not observe these changes with ΔmanB. It seems that LPS pathway is probably important for the mannose asimilation.

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