Investigating the effect of lactic acid bacteria on the prevention of Alzheimer's disease

Justin Ng (3S2) , Ezeck Chong (3S2), Yeo Rong Quan (3S2)

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that is common among the elderly throughout the world (Alzheimer's Association, n.d.; National Institute of Aging, n.d.). It is mainly characterised by dementia, a disease associated with the loss of memory and other cognitive abilities that eventually incapacitates the patient. The elderly who suffers from Alzheimer's disease will experience short-term memory loss, followed by the decrement in physical or cognitive function. Globally, approximately 44 million people suffer from Alzheimer's related dementia, with more than 4 million and 5.3 million of such case in India and the United States, respectively. By the year 2050, it is estimated that there will be approximately 13.5 million new cases of people suffering from Alzheimer's disease (cited by Mallikarjuna, Kukkarasapalli & Yellamma, 2016).

Alzheimer's disease is caused by brain cell death in the hippocampus cerebral cortex region of the brain which regulates thought processes and memory. The nerve tissue in the brain of Alzheimer's disease patients have excessive buildup of amyloid beta plaques and tau protein tangles. The former are clumps of protein which interfere with cell-to-cell communication and causes brain cell death. The latter are threads of tau proteins twisted into abnormal tangles which interfere with the nutrient transport system (Hu, Wang, & Jin, 2016). This increases oxidative stress and results in the generation of reactive oxygen species that can damage macromolecules such as proteins, lipids and nucleic acids (Mallikarjuna et al., 2016).

Hitherto, there is no known treatment to cure Alzheimer's disease. Only drugs such as galantamine (Razadyne), donepezil (Aricept) and rivastigmine (Exelon) decrease the rate of development of the disease, but cannot fully cure it (Web M.D., 2015). Lactic acid bacteria, considered as GRAS (Generally Recognized as Safe) (Bernardeau, Vernoux, Henri-Dubernet, & Guéguen, 2008) are beneficial microorganisms used in preventing diarrhoea, lactose intolerance, treating ulcer and infectious diseases prevention. In addition, it also helps in restoring the composition of gut microbiome and have useful functions to host through immune neuromodulation (Masood, Qadir, Shirazi, & Khan, 2010 ; Hemarajata & Versalovic, 2012). In rats, lactic acid bacteria have been proven to be able to aid the learning and memory, and reduce anxiety and depression (Akbari et al., 2016). Mallikarjuna, et al.

(2016) reported that *Lactobacillus plantarum* MTCC1325 improved the memory in D-galactose Wistar rats by delaying neurodegeneration. A study done on Alzheimer's disease patients who had probiotics milk which contained *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum,* and *Lactobacillus fermentum* showed that patients had a notable increase in the Mini-metal state examination (MMSE) score, showing that the probiotics positively affected cognitive function in Alzheimer's disease patients (Akbari et al., 2016). It was also discovered that lactic acid bacteria were able to manufacture acetylcholine neurotransmitter, which is a chemical released by nerve cells to send signals to other cells. Lactic acid bacteria were able to revive the nervous system of an elderly suffering from Alzheimer's disease (Girvin & Stevenson, 1954).

Past studies have used lactic acid bacteria on rats and patients with Alzheimer's disease, but there have not been any studies of the effect of lactic acid bacteria on *Caenorhabditis elegans* as model representations for Alzheimer's disease. *Caenorhabditis elegans*, presented as a model organism by Sydney Brenner in 1963, is a free-living and a non-parasitic nematode (Goldstein, 2016). It is 1 mm in length and is transparent. It has a life span of 2 to 3 weeks (Brenner, 1974). *C. elegans* has become a great model representation on the investigation of the molecular and cellular features of human diseases (Baumeister & Ge, 2002). The completion of the *C. elegans* genome sequence in 1998 showed that an estimated 38% of worm genes have a human ortholog, such as the amyloid precursor protein (APP) and tau (Shaye & Greenwald, 2011). Approximately 42% of human disease genes that have an ortholog in the genome of *C. elegans* (Poulin, Nandakumar & Ahringer, 2004). This includes those genes which are related to Alzheimer's disease, juvenile Parkinson's disease, spinal muscular atrophy, hereditary non-polyposis colon cancer, and several others age-related diseases and disorders (Kenyon, 2005). Using *C. elegans*, a simple invertebrate, as a model representation of human diseases, allows for easier differentiation of complex molecular pathways into their component parts, therefore giving a meaningful understanding into the pathogenesis of a complex disease phenotype (Markaki & Tavernarakis, 2010). Hence, *C. elegans* has many advantages as a model representation for the study of Alzheimer's disease and other neurodegenerative diseases.

The strain of model organism used in this study, *C. elegans* CL4176, is a temperature-sensitive mutant that shows increasing human beta-amyloid plaques in the muscle when the temperature increases from 16°C to 25°C, thus resulting in paralysis of the

nematode. In this study, the effect of different species of lactic acid bacteria on reducing paralysis of *C. elegans* CL4176, is studied.

Objectives

The objectives of this study are to investigate the ability of lactic acid bacteria in reducing paralysis of *C. elegans* CL4176 after the temperature upshift to 25°C; and to determine if pretreatment of *C. elegans* with lactic acid bacteria before the temperature upshift can result in a greater reduction in percentage of paralysed *C. elegans*.

Hypotheses

Different species of lactic acid bacteria can reduce paralysis of *C. elegans* CL4176 to different extent. *C. elegans* fed with lactic acid bacteria before the temperature upshift (pretreatment) will result in a greater reduction in percentage paralysed *C. elegans* than those that are not pretreated with lactic acid bacteria.

Experimental Procedures

Growth of bacteria

Escherichia coli OP50 was inoculated and grown in 10 ml of LB broth overnight at 30°C in a shaking incubator. Lactic acid bacteria were inoculated in 10 ml of MRS broth and also grown at 30°C overnight. The absorbance of all bacterial cultures was adjusted to 0.8 at 600 nm.

Preparation of NGM medium for *C. elegans*

The composition of NGM was as follows: 0.9 g NaCl, 7.5 g agar, 0.75 g bacto peptone in 300 ml water. After autoclaving, 0.3 ml cholesterol (5 mg/ml), 0.3 ml $MgSO_4$ (1 M), 0.3 ml $CaCl_2$ (1 M), 7.5 ml potassium phosphate buffer pH 6.0 (1 M) were added.

Pretreatment of *C. elegans* with lactic acid bacteria

0.05 ml of lactic acid bacteria broth culture was added to the NGM plate and grown overnight at 30°C. In the setup without pretreatment, 0.05 ml of *E. coli* OP50 was added instead.

A block of agar containing *C. elegans* was placed at the centre of each NGM plate. The plates were incubated at 16°C for 2 days. Five replicates were prepared.

Temperature upshift and induction of paralysis

0.05 ml of lactic acid bacteria broth culture was added to the NGM plate and grown overnight

at 30°C. In the control setup, 0.05 ml of *E. coli* OP50 was added instead.

A block of agar containing *C. elegans* from the previous NGM agar plate was cut and placed at the centre of a fresh NGM plate. The plate was incubated at 25°C to induce paralysis in the worms. To confirm the paralysis, each worm was gently touched with a platinum worm pick, and paralysis was considered if it moved its head only or did not move at all. Paralysed nematodes were counted 1-2 days after induction of paralysis at 25°C. The percentage of paralysed worms in each setup was determined. Table 1.1 shows the summary of treatments.

Table 1.1 Summary of treatments

Experiment setup	Pretreatment at 16°C	Treatment at 25°C
Test with pretreatment	Lactic acid bacteria	Lactic acid bacteria
Test without pretreatment	None (*E. coli* OP50)	Lactic acid bacteria
Control without pretreatment	None (*E. coli* OP50)	None (*E. coli* OP50)

Data analysis

The Mann-Whitney test was carried out to find out if there were significant differences in the percentage of paralysed worms between *C. elegans* fed with *E.coli* OP50 and lactic acid bacteria at 25°C and between the *C. elegans* fed with lactic acid bacteria in the pre-treated and non-pretreated sets (at 16°C)

Results and Discussion

Lactobacillus gasseri

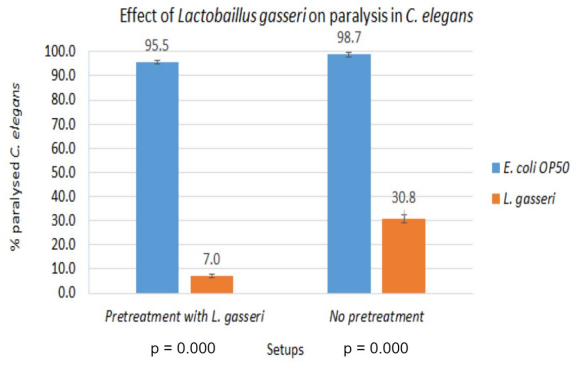

Fig. 1.1

Lactobacillus plantarum

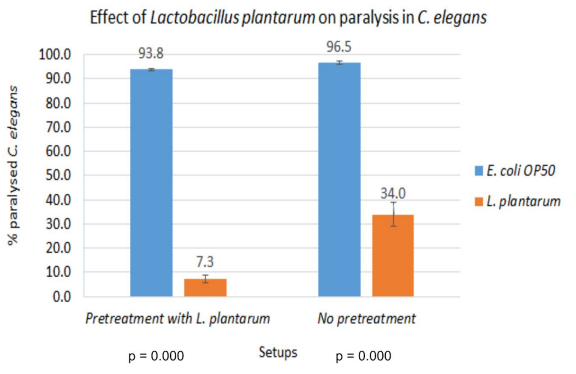

Fig. 1.2

Fig. 1.1 Effect of *Lactobacillus gasseri* on paralysis in *C. elegans*

C. elegans that were fed with *Lactobacillus gasseri* resulted in a significant reduction in paralysis than those that were not fed with *E. coli* OP50 in both the pre-treated and non-pretreated sets. Pretreatment of *C. elegans* with *Lactobacillus gasseri* at 16°C before being fed with OP50 at 25°C had no effect in reducing paralysis of *C. elegans*. Pretreatment of *C. elegans* with *Lactobacillus gasseri* at 16°C before being fed with *Lactobacillus gasseri* at 25°C had a significant effect in reducing paralysis of *C. elegans*. (Mann-Whitney U test p value of 0.000.)

Fig. 1.2 Effect of *Lactobacillus plantarum* on paralysis in *C. elegans*

C. elegans that were fed with *Lactobacillus plantarum* resulted in a significant reduction in paralysis than those that were not fed with *E. coli* OP50 in both the pre-treated and non-pretreated sets. Pretreatment of *C. elegans* with *Lactobacillus plantarum* at 16°C before being fed with OP50 at 25°C had no effect in reducing paralysis of *C. elegans*. Pretreatment of *C. elegans* with *Lactobacillus plantarum* at 16°C before being fed with *Lactobacillus plantarum* at 25°C had a significant effect in reducing paralysis of *C. elegans*. (Mann-Whitney U test p value of 0.000.)

Lactococcus lactis

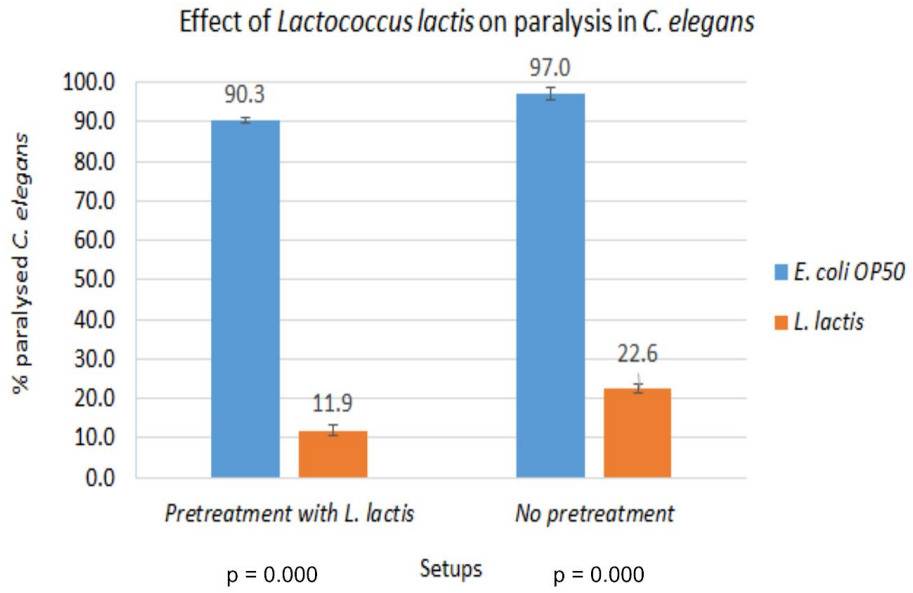

Fig. 1.3 Effect of *Lactococcus lactis* on paralysis in *C. elegans*

C. elegans that were fed with *Lactococcus lactis* resulted in a significant reduction in paralysis than those that were not fed with *E. coli* OP50 in both the pre-treated and non-pretreated sets. Pretreatment of *C. elegans* with *Lactococcus lactis* 16°C before being fed with OP50 at 25°C had no effect in reducing paralysis of *C. elegans*. Pretreatment of *C. elegans* with *Lactococcus lactis* at 16°C before being fed with *Lactococcus lactis* at 25°C had a significant effect in reducing paralysis of *C. elegans*. (Mann-Whitney U test p value of 0.000.)

Lactobacillus casei

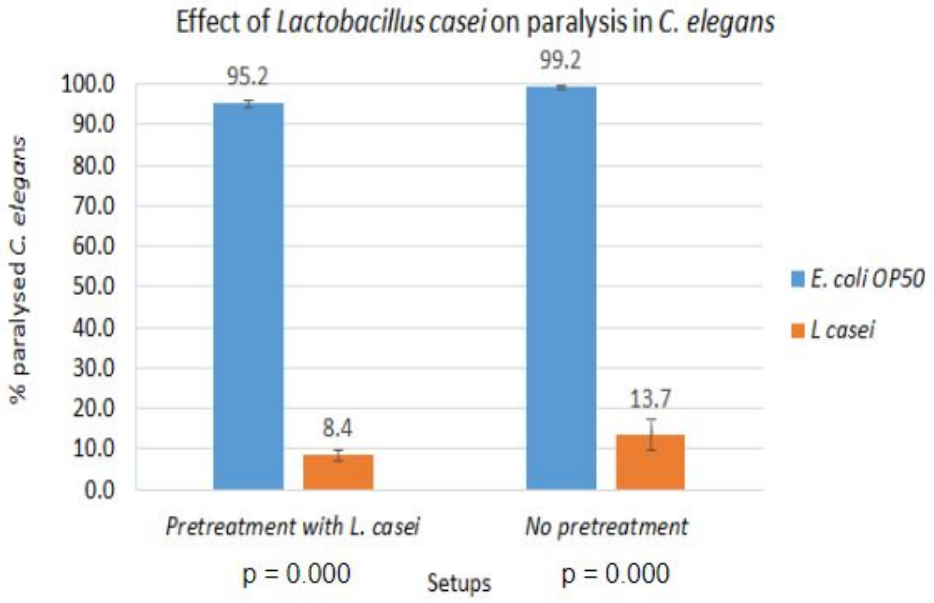

Fig. 1.4 Effect of *Lactobacillus casei* on paralysis in *C. elegans*

C. elegans that were fed with *Lactobacillus casei* resulted in a significant reduction in paralysis than those that were not fed with *E. coli* OP50 in both the pre-treated and non-pretreated sets. Pretreatment of *C. elegans* with *Lactobacillus casei* 116°C before being fed with OP50 at 25°C had no effect in reducing paralysis of *C. elegans*. Pretreatment of *C. elegans* with *Lactobacillus casei* at 16°C before being fed with *Lactobacillus casei* at 25°C had no significant effect in reducing paralysis of *C.elegans*. (Mann-Whitney U test p value of 0.590.)

Table 1.2: Comparison of results for *C. elegans* pre-treated and not pre-treated with lactic acid bacteria before induction of paralysis

Lactic Acid Bacteria	% reduction in paralysis	
	Pretreatment *C. elegans* fed with lactic acid bacteria at 16°C	**No Pretreatment *C. elegans* fed with E. coli at 16°C**
Lactobacillus gasseri	**92.7**	68.8
Lactobacillus plantarum	**92.2**	64.8
Lactococcus lactis	**86.8**	76.7
Lactobacillus casei	**91.2**	86.2

For all four strains of bacteria, *C.elegans* that were fed with lactic acid bacteria at 16°C had a greater reduction in percentage paralysed than those that were not. The Mann-Whitney U-test showed that feeding *C.elegans* with *Lactobacillus gasseri Lactobacillus plantarum* and *Lactococcus lactis* at 16°C reduced paralysis of *C.elegans* significantly below that of *C.elegans* fed with *E.coli* OP50 at 16°C .

Three main conclusions can be derived from this study. Firstly, *C.elegans* fed with lactic acid bacteria in the pretreatment resulted in a greater percentage of reduction in paralysis than those fed with *E.coli* OP50 at 16°C. This suggests the importance of continued intake of probiotics to prevent the onset of Alzheimer's disease in humans. Secondly, *C.elegans* fed with lactic acid bacteria at 25°C during induction of paralysis did not reduce the paralysis of *C.elegans*.

Mallikarjuna and Yellamma (2017) mentioned in their research paper that *Lactobacillus plantarum* MTCC1325 has the ability to produce the neurotransmitter acetylcholine, which is significantly decreased in patients with Alzheimer's disease. Additionally, the potential antioxidant nature of *L.plantarum* MTCC1325 may reduce oxidative stress associated with the development of Alzheimer's disease. This is also supported by the gut-brain communication which suggests that bacteria in the gastrointestinal tract may communicate with the brain and nervous system and stimulate the production of acetylcholine in the hippocampus and cerebral cortex of the brain.

Other researchers such as Akbari et al. (2016) demonstrated that 12 weeks consumption of 200 ml/day probiotic milk containing *Lactobacillus acidophilus*, *Lactobacillus casei, Bifidobacterium bifidum*, and *Lactobacillus fermentum* in 30 AD patients with Alzheimer's disease had favourable effects. Alexander, Marfil, and Li (2014) proposed to use *C.elegans* to study Alzheimer's disease as its neuronal connectivity have been established and it can model learning and memory impairments seen during Alzheimer's disease.

Conclusion

C. elegans fed with lactic acid bacteria in pretreatment resulted in a greater percentage of reduction in paralysis than those fed with *E.coli* OP50 in pretreatment. *C. elegans* fed with lactic acid bacteria at 25°C (during induction of paralysis) is necessary to reduce paralysis. Pretreatment of *C. elegans* fed with lactic acid bacteria at 16°C (prior to induction of paralysis) then with *E.coli* OP50 at 25°C (during induction of paralysis) did not reduce paralysis.

The limitation of our project is the difficulty in standardizing the age and the number of worms for each lactic acid bacteria set-up.

Overall, all the lactic acid bacteria were effective in reducing paralysis of *C. elegans*. Thus, we could use supplements containing probiotics as complementary treatment for patients suffering from Alzheimer's Disease and reduce the dosage of existing drugs such as galantamine (Razadyne), donepezil (Aricept), rivastigmine (Exelon).

For future work, two different species of lactic acid bacteria can be mixed to check whether there is synergistic effects in reducing paralysis to a greater extent in *C. elegans*. We could also study the minimum dosage of lactic acid bacteria required to trigger an effective reaction.

References

Akbari, E., Asemi, Z., Kakhaki, R.D., Bahmani, F., Kouchaki, E., Tamtaji, O.R., ……, Salami, M. (2016). Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: A randomized, double-blind and controlled trial. Frontiers in Aging Neuroscience, 8, 256. doi:10.3389/fnagi.2016.00256.

Alexander, A. G., Marfil, V., & Li, C. (2014, September 05). Use of *Caenorhabditis elegans* as a model to study Alzheimer's disease and other neurodegenerative diseases. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25250042

Alzheimer's Association. (n.d.). What is Alzheimer's? Retrieved from https://www.alz.org/alzheimers_disease_what_is_alzheimers.asp

Baumeister, R. & Ge, L. (2002). The worm in us – *Caenorhabditis elegans* as a model of human disease. Trends in Biotechnology, 20, 147-148. Retrieved from http://www.cell.com/trends/biotechnology/fulltext/S0167-7799(01)01925-4

Bernardeau, M., Vernoux, J., Henri-Dubernet, S. & Guéguen, M. (2008). Safety assessment of dairy microorganisms: The *Lactobacillus* genus. International Journal of Food Microbiology, 126, 278-285. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17889388

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

Girvin G.T., & Stevenson, J.W. (1954). Cell free choline acetylase from *Lactobacillus plantarum.* Canadian Journal of Biochemistry and Physiology, 32(2), 131-146. doi:10.1139/o54-015

Goldstein, B. (2016). Sydney Brenner on the genetics of *Caenorhabditis elegans*. Genetics, 204, 1-2. Retrieved from http://www.genetics.org/content/204/1/1

Hemarajata, P. & Versalovic, J. (2012). Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therapeutic Advances in Gastroenterology, 6, 39-51. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3539293/

Hu, X., Wang, T., & Jin, F. (2016). Alzheimer's disease and gut microbiota. Science China Life Science, 59, 1006-1023. Retrieved March 13, 2018, from https://www.ncbi.nlm.nih.gov/pubmed/27566465

Kenyon, C. (2005). The Plasticity of Aging: Insights from Long-Lived Mutants. Cell, 120, 449-460. Retrieved from http://www.cell.com/cell/fulltext/S0092-8674(05)00110-8?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867405001108%3Fshowall%3Dtrue

Mallikarjuna, N., N. and Yellamma, K. (2017). Anti-Alzheimer Properties of Probiotic, Lactobacillus plantarum MTCC 1325 in Alzheimer's Disease induced Albino Rats. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5620801/

Mallikarjuna, N., Praveen, K., & Yellamma, K. (2016). Role of *Lactobacillus plantarum* MTCC1325 in membrane-bound transport ATPases system in Alzheimer's disease-induced rat brain. Bioimpacts*, 6(4)*, 203-209. doi:10.15171/bi.2016.27

Markaki, M. & Tavernarakis, N. (2010). Modeling human diseases in *Caenorhabditis elegans*. Biotechnology Journal, 5, 1261-1276. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/biot.201000183/abstract;jsessionid=00592AC90A616BBD9CD25079C1DEF209.f04t03

Masood, M., Qadir, M., Shirazi, J., & Khan, I. (2010). Beneficial effects of lactic acid bacteria on human beings. Critical Reviews in Microbiology, 37, 91-98. Retrieved from https://www.tandfonline.com/doi/abs/10.3109/1040841X.2010.536522?journalCode=imby20

National Institute on Aging. (n.d.). What Is Alzheimer's Disease? Retrieved March 9, 2018 from https://www.nia.nih.gov/health/what-alzheimers-disease

Poulin, G., Nandakumar, R. & Ahringer, J. (2004). Genome-wide RNAi screens in *Caenorhabditis elegans*: impact on cancer research. Oncogene, 23, 8340-8345. Retrieved from https://www.nature.com/articles/1208010

Shaye, D.D., & Greenwald, I. (2011). OrthoList: a compilation of *Caenorhabditis elegans* genes with human orthologs. PLoS ONE, 6(5), e20085. doi:10.1371/journal/pone.0020085

Web, M.D. (n.d.). Treatments for Alzheimer's disease. Retrieved March 13, 2018 from https://www.webmd.com/alzheimers/guide/treatment-overview#1

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