The Effect of Gut Microbiome Modifications on Chemosensory Deficiencies in *C. elegans* Models of Alzheimer's Disease

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Abstract

Alzheimer's Disease (AD), the most common form of dementia, is a progressive neurodegenerative disease affecting about 10% of all people over the age of 65. There are no cures for AD, and treatment options are limited. Current evidence suggests a potential correlation between gut microbiome dysbiosis and AD development and progression. This study utilized a transgenic *Caenorhabditis elegans* model of AD to test whether gut microbiome supplementation with individual bacterial strains could hold potential therapeutic power for treatment of AD. Behavioral assays were used to determine the potential amelioration of chemosensation deficiencies in the AD model, and it was found that gut microbiome supplementation with all six tested bacterial strains improved chemosensation in the transgenic AD strain of *C. elegans*. These results provide further evidence for the potential use of microbiome supplementation as a treatment option for AD.

Acknowledgements

This project was made possible due to the guidance and support of many people. I would like to thank Dr. Jagan Srinivasan for his guidance as advisor to this project. I would also like to thank Caroline Muirhead, Elizabeth DiLoreto, and the rest of the Srinivasan Lab for their continued support and assistance throughout this project.

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Background

Alzheimer's Disease

Prevalence of Alzheimer's Disease in the United States

Alzheimer's Disease (AD) is a progressive neurological disorder characterized by destruction of memory and other important mental functions (Centers for Disease Control and Prevention [CDC], 2020). AD is a specific classification of dementia, which itself is not a specific disease, but a grouping of conditions which are characterized by impairment of neurological functions. AD is the most common type of dementia, making up around 60-80% of all dementia cases in the United States (Alzheimer's Association, n.d.-c).

The largest risk factor of AD is age, and the disease is most common in people over the age of 65 (CDC, 2020). As of 2023, 6.7 million Americans over the age of 65 (equivalent to 1 in 9 people within the age group) were living with the disease (Alzheimer's Association, 2024). The risk of AD also continues to increase with age after the age of 65. Of all AD cases, 73% are in individuals aged 75 and over (Alzheimer's Association, 2024). *Figure 1* shows the breakdown of the number and ages of people aged 65 and older who were living with AD.

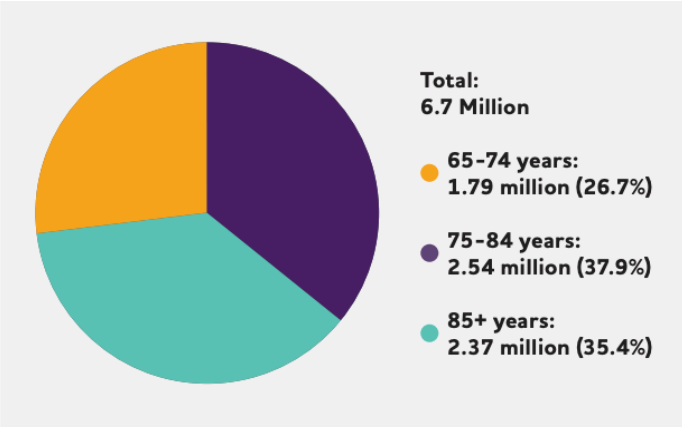

Figure 1. Number and Ages of People 65 or Older with Alzheimer's Disease (Alzheimer's Association, 2024)

The number of AD cases nationwide is expected to increase as the aging population continues to expand. Growth of the "over 65" population can be attributed to several causes, such as increasing life expectancy, better management of health-related risk factors, and introduction of the baby boom generation, who are currently ages 60-78 (Alzheimer's Association, 2024). As the number of aging individuals increases, so too will the incidence of AD, despite recent

advancements in the management of overall risk factors. A 2023 estimate, shown in *Figure 2*, predicts that by 2060, 13.8 million Americans aged 65 and older will be living with AD, barring the development of a cure or prevention method (Alzheimer's Association, 2024).

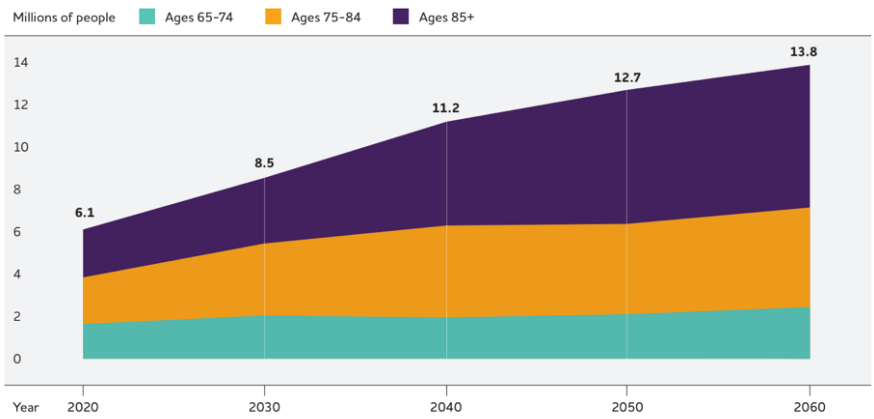

Figure 2. Projected Number of People 65 and Older (Total and by Age) in the U.S. Population with Alzheimer's Disease; 2020 to 2060 (Alzheimer's Association, 2024)

Age is not the only factor affecting AD prevalence in the United States. Studies show statistical differences in disease presence between various populations based on factors like gender and race. As of 2023, two thirds of AD cases over the age of 65 are in women (Alzheimer's Association, 2024). When looking at the total population of individuals over the age of 65, 12% of women are diagnosed with AD whereas only 9% of men are diagnosed (Alzheimer's Association, 2024). This discrepancy can be partially attributed to the overall longer life expectancy that women have over men, leading to a greater amount of time during which a diagnosis can occur. Nevertheless, we still see a discrepancy in overall lifetime risk for AD between men and women. *Figure 3* shows this discrepancy measured both at ages 45 and 65, suggesting a potential sex-mediated component to the disease.

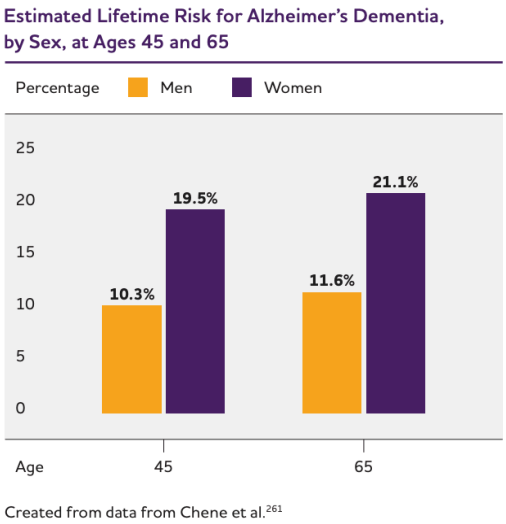

Figure 3. Estimated Lifetime Risk for Alzheimer's Disease, by Sex, (Alzheimer's Association, 2024)

More recent studies have shown that there might also be discrepancies among sexual and gender minorities, which are defined as members of the LGBTQ+ community and non-cisgender individuals (Alzheimer's Association, 2024). These groups face a greater risk of dementia due to the physiological effects of discrimination and healthcare disparities, which translates to a greater risk of AD. Some studies have also linked increased AD risk with HIV/AIDS, but the connection is not yet well understood (Alzheimer's Association, 2024). Race is yet another factor which plays a role in AD prevalence. As of 2023, there was a higher prevalence of AD in Black and Hispanic populations (19% and 14% of their respective populations over the age of 65) than in the Caucasian population (10% of their population over the age of 65) (Alzheimer's Association, 2024). Given that race is a social construct as opposed to a biological identification, genetic factors are not likely to provide significant enough evidence for this phenomenon. Most likely, these differences are due to socioeconomic factors and systemic barriers minority groups face in accessing equitable healthcare in the United States, as well as the physiological effects of stress due to systemic discrimination (Alzheimer's Association, 2024).

Symptoms of Alzheimer's Disease

AD is a progressive disease that can changes and worsen over time. In short, it is caused by neurodegeneration in specific areas of the brain, and symptoms arise and/or worsen as more

regions of the brain are affected. Most AD patients progress through multiple stages of the disease and symptom severity, as shown in *Figure 4*.

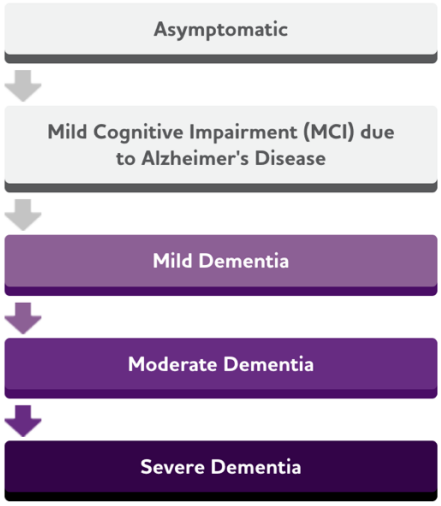

Figure 4. Stages of Neurodegeneration in Alzheimer's Disease (Alzheimer's Association, n.d.-c)

Asymptomatic patients can have physiological brain changes without any cognitive symptoms. This stage starts up to 20 years before the first symptoms arise. Mild cognitive impairments (MCIs) are defined as early stages of memory loss or cognitive impairment without losing ability to perform daily activities independently. Some cases of MCI will develop into dementia, and others will not. If the hallmark physiological changes of Alzheimer's are present at this stage, it can be considered early-stage AD. When a patient progresses from MCI to dementia, there are three major stages: mild, moderate, and severe. Individuals with mild dementia experience symptoms which interfere with some daily activities. Those with moderate dementia experience more pronounced symptoms which interfere with many daily activities. Finally, individuals with severe dementia have symptoms which interfere with nearly all daily activities (Alzheimer's Association, n.d.-c).

Diagnosis typically occurs around the MCI or mild dementia phases. On average, patients tend to live about 4-8 years after the initial diagnosis but can live up to 20 years (Alzheimer's Association, n.d.-c). During these years, AD patients experience a wide range of symptoms which affect their lives variably. These can include cognitive symptoms like progressive memory loss, difficulty remembering recent events or conversations, forgetfulness, difficulty expressing thoughts and emotions, lack of ability to multitask, difficulty concentrating, and decline in

decision making capabilities (Reitz et al., 2011). Symptoms might also include changes to personality and behavior such as depression, loss of interest in activities, social withdrawal, mood swings, distrust in others, anger or aggression, changes to sleep habits, wandering, loss of inhibitions, and increased delusions (Reitz et al., 2011). People dealing with AD might experience some or all of these symptoms depending on the areas of their brain affected by degeneration and other risk factors. Because of this, late-stage AD patients typically become partially or completely dependent on their caregivers (Reitz et al., 2011).

There is evidence to suggest that olfactory dysfunction might be one of the first clinical symptoms of AD (Zou et al., 2016). Studies show that patients with AD exhibit olfactory impairment and decreased odor identification capabilities compared to healthy elderly controls (Tkalčić et al., 2011). Damage to the olfactory bulb and tract have also been reported in many cases of AD (Zou et al., 2016). The role of olfaction in AD is still unclear but holds promise as a potential early marker of the disease. Overall, while there are multiple common symptoms, Alzheimer's Disease can develop and present variably between two individuals depending on their baseline health and cognitive engagement, making it difficult to compare experiences between two people facing the same condition.

Risk Factors of Alzheimer's Disease

Because of the disease's prevalence, the environmental and physiological risk factors for AD are well defined. It is widely understood among researchers that AD and its level of severity is a result of the combined genetic, biological, and environmental realities of each individual patient. Despite what many people tend to believe, less than one percent of AD cases are caused by genetic factors alone (Alzheimer's Association, 2024). Elements of an individual's environment, habits, and history play equally important roles in disease determination when it comes to AD.

When discussing genetic components of a disease, it is important to distinguish between risk genes and deterministic genes. Risk genes can increase the likelihood that a disease state will occur but does not guarantee it. APOE-e4 is the first and most common risk gene identified for AD with an estimated 40-65% of AD patients having it (Alzheimer's Association, n.d.-b). Having the APOE-e4 gene does not directly lead to AD, but can contribute, among other factors, to disease development. Deterministic genes on the other hand, will directly cause a disease

state. Thus far, researchers have identified deterministic genes being passed along in only a few hundred families, and these cases typically manifest as early onset AD. These genes are APP, Presenilin 1 (PS1), and Presenilin 2 (PS2) (Alzheimer's Association, n.d.-b). All the discovered deterministic genes affect beta-amyloid processing and production, which researchers believe is a key piece in understanding AD. Even though less than one percent of AD cases can be explained by deterministic genes alone, the discovery of these genes has led to a better understanding of the mechanisms and pathophysiology of AD (Alzheimer's Association, n.d.-b).

Age, while it does not directly cause disease development, is the most important non-modifiable risk factor for Alzheimer's Disease. The likelihood of AD increases as a person ages, with most diagnosed cases occurring after the age of 65. This phenomenon can be explained in several ways, such as decreased inflammatory and immune response and accumulation of amyloid plaques, which can affect AD development (Guerreiro & Bras, 2015). Sex is another non-modifiable risk factor which plays a role in AD. Statistically, women above the age of 65 are about twice as likely to develop AD as men of the same age. This is partially attributed to the greater life expectancy in women (79.3 years, in 2021) as opposed to men (73.5 years, in 2021) (CDC, 2023). The difference could also be attributed to sexual dimorphism in neurological development. For example, women tend to have more microglial cells, which could lead to increased neuroinflammation with age (Podcasy & Epperson, 2016). Neither age nor sex can be individually responsible for the development of AD but do tend to play important roles in disease development.

There are also several modifiable risk factors which affect an individual's propensity for developing AD. These include, but are not limited to lifestyle choices like diet, exercise, tobacco use, and alcohol consumption, cardiovascular disease, type 2 diabetes, and traumatic brain injuries (Reitz et al., 2011). Maintaining a healthy and active lifestyle while minimizing the consumption of tobacco and alcohol can decrease one's overall risk for AD. Cognitive stimulation is another key to decreasing AD risk. Incorporating regular mental exercises and socialization can aid in decreasing one's overall risk. It is currently unknown how the COVID-19 pandemic will affect the prevalence of AD, but it is hypothesized that the social isolation caused by intermittent lockdowns will likely increase the risk of AD for many people. Unfortunately, the true effects of the COVID-19 lockdowns will not be evident for several years (Alzheimer's

Association, 2024). Overall, no single factor can be attributed to causing Alzheimer's Disease in any single individual, and as research develops, so too does the list of potential risk factors.

Pathophysiology of Alzheimer's Disease

There are two main pathophysiological markers for Alzheimer's Disease: extracellular beta amyloid deposits and intracellular neurofibrillary tangles (National Institute of Health, 2022). The combination of these two accumulations causes neuronal and synaptic degradation, which then leads to brain atrophy starting in the temporal lobe (Barage & Sonawane, 2015). The specific mechanisms of these processes are not fully understood, though there are several proposed theories.

The most widely accepted theory is the amyloid cascade hypothesis, which describes the ways in which amyloid plaques build up in the brain and lead to AD pathogenesis. According to this hypothesis, amyloid precursor protein (APP) is first cleaved by the enzyme beta-secretase (BACE1), and the resulting protein product is cleaved again by a different enzyme, gamma secretase. The final protein formed in this process is amyloid-β_{42}, which accumulates in plaques between neurons, eventually leading to injury of the affected neurons and synapses (Barage & Sonawane, 2015). The injured neurons mechanisms for homeostatic maintenance are altered, leading to oxidative stress. This stress prompts changes in cell metabolism which induces formation of neurofibrillary tangles (NFTs). NFTs are an abnormal accumulation of tau protein, which is used normally neuronal support and stabilization, inside the cell (Barage & Sonawane, 2015). The presence of amyloid plaques promotes abnormal aggregation of these tau molecules, causing the formation of NFTs (Barage & Sonawane, 2015). The NFTs contribute to the neuronal death, synaptic loss, and neurotransmitter deficits which characterize AD pathology. *Figure 5* below provides a summarized overview of the amyloid cascade hypothesis.

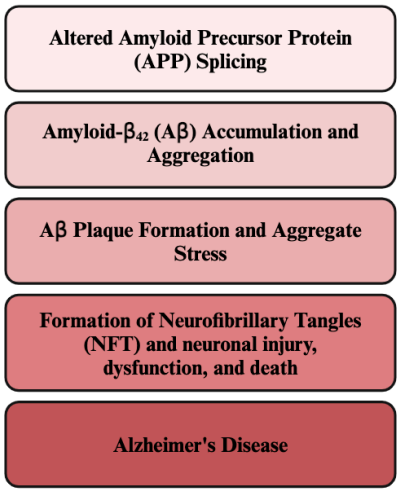

Figure 5. The amyloid cascade hypothesis suggests that alternative splicing of amyloid precursor protein (APP) causes the accumulation and aggregation of amyloid-β_{42}. Amyloid-β_{42} forms plaques and causes aggregate stress, which lead to the formation of neurofibrillary tangles, as well as neuronal injury, dysfunction, and death. This progression results in the clinical presentation of Alzheimer's Disease.

The amyloid cascade hypothesis is seen as one of the better theories for AD pathophysiology, but it still has several flaws. Firstly, much of what we know about the amyloid cascade is based on research performed with transgenic AD mice, and there is a question of whether this is an adequate model for translation into human pathogenesis. Especially in the pharmaceutical realm, certain drugs targeting the over production and accumulation of amyloid-β_{42} are shown to be successful in the mice models, but do not pass clinical trials in humans (Ricciarelli & Fedele, 2017). Many human based studies also show that amyloid-β_{42} accumulation does not always correlate with cognitive decline, which conflicts with the basic tenets of the hypothesis (Ricciarelli & Fedele, 2017). In general, the basic framework of the hypothesis holds up and is supported by research, but the validity of more specific details remains up for debate. Overall, the theory holds up in some AD cases, but there remain significant gaps in our understanding of AD and its underlying mechanisms.

Research also suggests that neuroinflammation plays an important role in the progression of Alzheimer's Disease. Neuroinflammation is the body's immune response to a buildup of amyloid plaques and NFTs (Barage & Sonawane, 2015). The nervous system has its own class of macrophages, called microglia, which respond to abnormal structures (like plaques and NFTs) which occur in the nervous system. Activation of these cells induces an immune response,

including release a of cytokines which induce inflammation. Repeated and consistent activation of microglia over time can cause functional and structural changes to neurons in the brain, which can contribute to AD development (Barage & Sonawane, 2015).

Current Treatments for Alzheimer's Disease

Currently, there is no available cure for Alzheimer's Disease. Physicians of patients with AD focus treatment regimens on maintaining or improving the quality of life for patients and their caregivers. This type of treatment might include maintaining cognitive health, managing behavioral symptoms as they arise, and slowing or delaying the development of new symptoms (CDC, 2020). There are also two major drug classes that have been approved by the FDA for treatment of Alzheimer's Disease: acetylcholinesterase (AChE) inhibitors and NMDA receptor antagonists (Alzheimer's Society, 2014). People with AD tend to have low levels of acetylcholine, which is a neurotransmitter contributing to intraneuronal communication. AChE inhibitors prevent the acetylcholinesterase enzyme from breaking down acetylcholine in the brain, allowing patients with AD to maintain the low levels of the neurotransmitters they do have (Alzheimer's Society, 2014). Some examples of commonly used AChE inhibitors are donepezil, rivastigmine, and galantamine, which all achieve the same effect using different mechanisms. There is currently one commonly used NMDA receptor antagonist, which is memantine. This drug works by blocking the effects of glutamate, which is a neurotransmitter that is overproduced in patients with AD (Alzheimer's Society, 2014). Another class of drugs used to treat AD relies on decreasing production of or removing amyloid-beta in the brain. The most used drug from this category is aducanumab, which is an amyloid-beta directed monoclonal antibody which removes amyloid-beta before it can form further plaques. The drug was approved by the FDA for treatment of AD in 2021 but will be discontinued by the manufacturer (Biogen) in early 2024 to reprioritize resources for AD research (Alzheimer's Association, n.d.-a). Nevertheless, the drug showed success in targeting amyloid beta production and accumulation as a viable treatment method for AD.

The Gut Microbiome and the Gut-Brain Axis

The Human Microbiome

The human gut microbiome is a system of bacteria, archaea, and eukaryotes which colonize the gastrointestinal tract and maintain a normally symbiotic relationship with the human host. There is estimated to be over 10^{14} microorganisms in the gut microbiome, which is nearly 10 times more than the number of cells in the human body, and the collective genome of the microbiome is 100x larger than the human host genome (Thursby & Juge, 2017). These microbes are acquired in several different ways, but through diet and environmental factors, making the gut microbiome relatively variable from person to person. Many ongoing projects, such as the MetaHit Project and the Human Microbiome Project, are working towards characterizing the total human microbiome. According to the data from these studies, there are 12 total phyla of microbes which colonize human guts, with four main groups making up 93.5% of all human gut microbes (Thursby & Juge, 2017). These four phyla are Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. These microbes aid in reinforcing gut and intestinal integrity, collecting energy from food sources, protecting the host from pathogens, and strengthening the immune system (Thursby & Juge, 2017). The microbiome even aids in the production, expression, and turnover of some neurotransmitters. In return, the microbes receive a safe environment to live and grow in. When something pushes the microbiome into dysbiosis though, the human host can experience detriments to their health.

Dysbiosis is characterized by alterations in the composition and function of the gut microbiome. This could mean decreased microbial diversity, a loss of beneficial microbes, an overgrowth of detrimental microbes, or some combination of the three (Hrncir, 2022). Several factors play a role in inducing gut microbiome dysbiosis, including genetics, overall health status (presence of infection or inflammation), and lifestyle choices such a diet, hygiene, and use of xenobiotics like antibiotics, drugs, and food additives. Rapid changes to the microbiota can result from changes to macronutrient ingestion. For example, dysbiosis can result from a high simple sugar diet, which slows metabolism, induces intestinal inflammation, and affects the intestinal barrier (Hrncir, 2022). The gut microbiome is also sensitive to a lot of the preservatives and artificial sweeteners commonly used in food production, and consistent ingestion of these products can be detrimental to the microbiome over time (Hrncir, 2022). While it is still unclear

whether microbiota dysbiosis causes certain diseases or reflects disease-induced changes, it is evident that the two are connected in determining human health.

The Gut-Brain Axis

The gut-brain axis (GBA) describes a bidirectional communication pathway between the enteric nervous system (ENS) and the central nervous system (CNS). As a unit, the gut-brain axis both maintains gastrointestinal homeostasis and influences high order neurological functions like motivation, affect, and cognition by linking neurologic and enteric systems (Carabotti et al., 2015). The GBA utilizes neuronal, hormonal, and immunological signaling pathways to execute its multiple functions. Clinical and experimental data also shows that the relationship is modulated by the gut microbiome in several ways. The microbiome can modulate neurological activity through production, expression, and turnover of neurotransmitters and neurotrophic factors, interacting with intestinal barriers, regulation of enteric sensory inputs, interacting with bacterial metabolites, and regulation of mucosal immunity. The GBA is a bidirectional pathway, and the brain can modulate microbiome and gut activity as well. It does so through alterations to mucus and biofilm production, motility, intestinal permeability, and immune function (Carabotti et al., 2015). Because the gut microbiome plays an important role in influencing the GBA, dysbiosis can have a significant effect on the hosts gastrointestinal and neurological health.

Studies performed in germ-free animal models show that bacterial colonization of the gut is indeed crucial ENS and CNS development and maturation (Carabotti et al., 2015). In these studies, researchers saw that a lack of microbial colonization was associated with alterations in neurotransmitter expression and turnover, as well as decreased sensory-motor functions in the gut. These deficits were corrected though, after the microbiome was restored (Carabotti et al., 2015). Similar studies have also shown that the microbiome plays an important role in mediating stress behaviors and anxiety in animal models (Carabotti et al., 2015). This connection is seen in humans as well, as research supports a connection between gut microbiome dysbiosis and several neurological disorders like anxiety, depression, autism spectrum disorder (ASD), and Alzheimer's Disease, which will be explored in the following sections (Carabotti et al., 2015).

The Gut Microbiome and Alzheimer's Disease

Many recent studies have linked AD to microbial dysbiosis. For example, fecal samples taken from patients with AD show decreased microbial diversity compared to age and sex matched control groups. More specifically, researchers saw decreased abundance of *Firmicutes* and *Bifidobacterium* and increased abundance of *Bacteroidetes* in the AD group fecal samples compared to the non-AD group fecal samples (Kowalski & Mulak, 2019). Studies performed in an AD mouse model showed similar deficits in microbial diversity (Kowalski & Mulak, 2019). There is also a potential link between AD and other gut mediated diseases such as irritable bowel syndrome (IBS), autism spectrum disorder (ASD), schizophrenia, and multiple sclerosis (MS), and poor dental hygiene, which affects the oral microbiome, has also been linked to AD (Kowalski & Mulak, 2019). The mechanisms underlying this connection though, is still not fully understood.

It is possible that, in some cases, direct migration of microbes from the gut to the brain along the GBA can influence AD. Autopsies performed on AD patients showed that certain microbes, such as Chlamydophila pneumoniae, Borrelia burgdorferi, and HSV-1, migrated to the brain (Kowalski & Mulak, 2019). Because these studies are performed post-mortem, these microbes cannot be directly linked to AD, but do provide evidence of a potential connection. There are a few theories on how these microbes can migrate to and penetrate the brain. One is through transmigration of infected monocytes and T-cells through an already compromised blood-brain barrier (Kowalski & Mulak, 2019). The microbes could also be entering through the olfactory nerves, which provide a direct pathway to the brain. In living AD patients, there has been an observed connection between AD and *H. pylori* infection in the brain (Kowalski & Mulak, 2019). The migration of microbes could play a role in the development and severity of AD symptoms, but it likely not the only mechanism for the relationship between the microbiome and disease development.

Dysbiosis as a causative agent for neuroinflammation is another mechanism which has been explored as a cause for then gut dysbiosis to AD connection. In all individuals, the microbiota influences peripheral immune cell activation and the cytokine profile, which in turn effects CNS inflammation and neurodevelopment. In elderly individuals, hyperstimulation of the immune system can eventually lead to some level of chronic, low-grade inflammation, which can be caused by age related alterations in the diversity and stability of the gut microbiota (Kowalski

& Mulak, 2019). These changes cause gut barrier breakdown, leading to increased cytokines and bacterial products in the bloodstream. These elements can affect the efficacy of the blood-brain barrier, and if foreign particles are able to cross into the brain, it can cause neuroinflammation. As addressed earlier, neuroinflammation is a risk factor and potential cause of AD.

Amyloid plaques are a key element in understanding both the general pathogenesis of AD, as well as how the gut microbiome might influence disease development. Amyloid-β_{42} is recognized as an antimicrobial peptide which contributes to the innate immune response in humans (Kowalski & Mulak, 2019). In a dysregulated state, such as in AD, this can be harmful. The microbiome itself is also a source of amyloids for the body. For example, the amyloid curli is produced by *E. coli* to aid in cell adhesion and aggregation (Barnhart & Chapman, 2006). These bacterial amyloids help microbial cells bind to each other through the production of a biofilm in the gut. Their tertiary structure is similar to amyloids utilized by the CNS, including beta amyloids (Kowalski & Mulak, 2019). Because of this similarity, exposure to bacterial amyloids primes the immune system's response to neuronal amyloids. Exposure of the immune system to bacterial amyloids in turn induces greater levels of neuroinflammation with the recognition of amyloid-β_{42} (Kowalski & Mulak, 2019). Additionally, bacterial lipopolysaccharides promote amyloid fibrillogenisis, which induces additional inflammation (Kowalski & Mulak, 2019). *Figure 6* summarizes the connections between the brain and the microbiome, and how that connection is involved in AD pathogenesis.

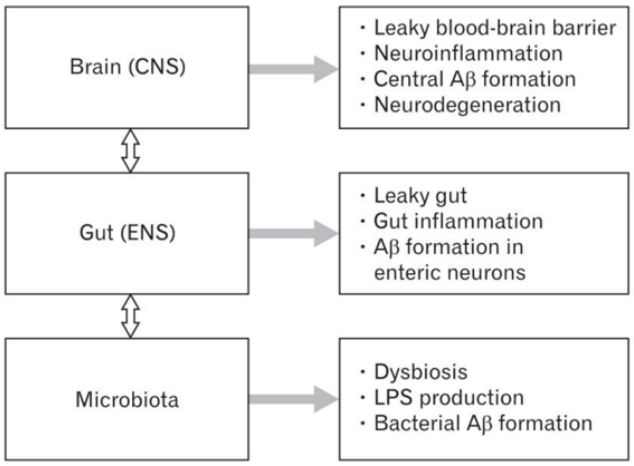

Figure 6. The gut-brain axis describes a bidirectional communication pathway between the enteric nervous system (gut) and the central nervous system (brain). All parts of this pathway affect AD pathogenesis, as shown in the figure above.

C. elegans

Caenorhabditis elegans, or *C. elegans*, is a nematode species which occurs naturally in temperate soil environments. These nematodes are small (1.5 mm adults), have a short life span (3 days at 20°C), are easy to maintain in a laboratory setting due to availability and low cost of their food source (*E. coli*), can produce abundant progeny (300-350 per individual worm), and are genetically and anatomically simple, making them ideal candidates for biomedical research, especially in fields such as developmental biology, genetics, neurobiology, and, importantly, neurodegeneration (Riddle et al., 1997).

The nervous system of *C. elegans* is relatively simple and has been fully mapped, making the species useful as a system for neurological research. There are 302 NS cells in hermaphrodites and 381 in males (Riddle et al., 1997). These cells are organized into ganglia in the head and tail, though most reside in the head (WormAtlas, n.d.). Chemosensory neurons make up about approximately ten percent of the total nervous system, and the chemosensory system is highly developed, as it is the worms' main way to interact with and gain information from its environment (Bargmann, 2006). *C. elegans* rely on chemosensation to find food, avoid harmful environments and cues, develop, and mate. Each worm has 16 bilaterally symmetric pairs of chemosensory neurons, shown below in *Figure 7* which respond to a vast array of odorants and stimuli.

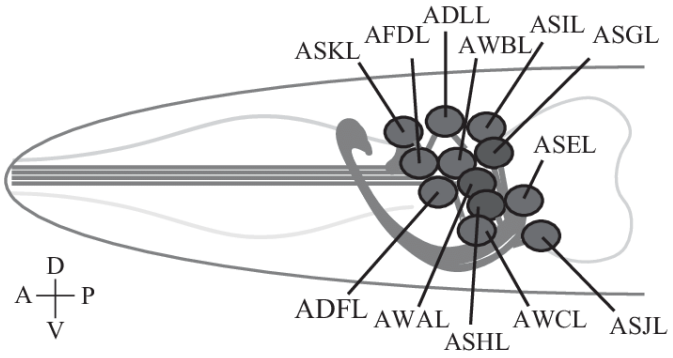

Figure 7. Map of Chemosensory Neurons in *C. elegans* (Maruyama, 2017)

Different odorants are sensed by different neurons, and therefore produce a wide range of responses. For example, odorants associated with food will be picked up by a certain set of neurons and will cause the animal to move towards the source of the scent, whereas an aversive odorant will be sensed by a distinct set of neurons, causing the animal to flee from the scent.

These behaviors are well characterized and can be utilized in behavioral assays to assess and monitor neurological function. In this study, the chemorepellent used for behavioral assays was copper (II) chloride ($CuCl_2$). $CuCl_2$ contains a heavy metal cation (Cu^{2+}) which is detected by the ASH and ADL chemosensory neurons, depicted in *Figure 7* (Wu et al., 2022). The behavioral response of *C. elegans* to this aversive chemical stimulant have been well documented in previous studies.

C. elegans as a Model for Alzheimer's Disease

Genes associated with human disease typically function as a part of evolutionarily conserved genetic pathways, meaning that they are often either the same or similar in simpler model organisms, such as *C. elegans*. The *C. elegans* genome is about 100 million base pairs long, fully characterized, and remarkably like the human genome, making it a powerful tool for studying molecular and genetic disorders in vivo (Markaki & Tavernarakis, 2010). An estimated 42% of human disease genes have orthologs in *C. elegans*, including Parkinson's Disease, spinal muscular atrophy, hereditary nonpolyposis colon cancer, and Alzheimer's Disease (Markaki & Tavernarakis, 2010).

Transgenic expression of human disease genes in *C. elegans* models allow for the study of neurodegenerative diseases like AD. As discussed earlier in the paper, significant evidence supports the role of amyloid-β_{42} as a causative agent of AD (Barage & Sonawane, 2015). This amyloid-β_{42} is derived from the proteolysis of a precursor, amyloid precursor protein (APP). *C. elegans* has a single APP gene called *apl-1* which is found on the X-chromosome and is required for developmental processes like molting and morphogenesis (Markaki & Tavernarakis, 2010). Unfortunately, the molecular machinery required for the splicing of APP to form amyloid- β_{42} does not exist in the *C. elegans* model. To address this issue, Dr. Link's Lab at the University of Colorado developed a transgenic line with the ability to express amyloid-β_{42} through regulation with a temperature dependent promoter. In this strain, a heat shock with a temperature above 20°C is required to initiate the production of amyloid-β_{42}. Without this regulatory promoter, the animals would indefinitely produce amyloid-β_{42}, and this progressive accumulation would cause paralysis. Studies with these strains of *C. elegans* show that expression of amyloid-β_{42} is correlated to deficits in olfaction, similar to the human model of AD. A previous MQP from

2021 further established deficits in olfaction and chemoreception in the AD models to the chemorepellent $CuCl_2$ (Tarantino, 2021).

C. elegans as a Model for Microbiome Research

C. elegans used in laboratory settings are microbially sterile and have only *E. coli* as a food source, therefore their gut microbiome is significantly different and less diverse than naturally occurring animals of the same species, whose microbiomes are rich and diverse due to their environment and food sources. This leaves them as a blank slate for the study of the gut microbiome and its effect on various aspects of health and development, including the microbiome's effect on neurodegeneration.

Gut microbiome research in animal models can often be made difficult because the use of synthetic microbial communities pose various issues. For example, many studies use an artificial choice of microbes which is not representative of the wild-type microbiome. Some studies will also rely on colonizing a host gut with human related microbes, which can be ineffective. The use of uncharacterized synthetic microbial communities also inhibits genetic traceability (Dirksen et al., 2020). For these reasons, researchers decided to better characterize the natural *C. elegans* gut microbiome and replicate it in a model system. Multiple studies characterized individual strains of bacteria present in the *C. elegans* microbiome using 16S rRNA sequencing, and this data was compiled for assessment. This data helped determine that the *C. elegans* microbiome contains over a dozen bacterial families, the most prevalent being Gammaproteobacteria and Bacteroidetes (Dirksen et al., 2020). From this compiled resource, a simplified yet accurate model *C. elegans* gut microbiome system was created, called the CeMbio mixture. The system is composed of twelve different bacteria from 9 families which represent the most significant parts of the *C. elegans* microbiome, based on the compiled data (Dirksen et al., 2020). Each utilized strain is easily culturable, can colonize in the animal's gut individually, and, when used together, form a robust microbial community which effects the host's life history (Dirksen et al., 2020). The table below lists each of the strains used in the CeMbio mixture and their respective taxonomies.

Table 1. The 12 Bacterial Strains Used in the CeMbio System.

Strain Name	Strain Taxonomy
CEenent1	*Enterobacter hormaechei*
BIGb0170	*Sphingobacterium multivorum*
BIGb0172	*Comamonas piscis*
BIGb0393	*Pantoea nemavictus*
MSPm1	*Pseudomonas berkeleyensis*
MYb10	*Acinetobacter guillouiae*
MYb11	*Pseudomonas lurida*
MYb71	*Ochrobactrum vermis*
JUb19	*Stenotrophomonas indicatrix*
JUb44	*Chryseobacterium scophthalmum*
JUb66	*Lelliottia amnigena*
JUb134	*Sphingomonas molluscorum*

Overall, the use of the CeMbio model provides a more ecologically relevant reflection of the natural *C. elegans* microbiome in a laboratory setting. Utilizing this resource in the context of neurodegeneration research provides a mechanism to study the relationship more accurately between neurodegenerative disorders like AD and gut microbiome dysbiosis.

Project Overview

A previous MQP project found that dietary supplementation with the complete CeMbio mixture improved chemosensation in a *C. elegans* model of AD, even when amyloid- β_{42} plaque accumulation was induced (Tarantino, 2021). This project aims to determine whether these results were a result of a specific bacterial colonization within the gut. Three strains of *C. elegans* were tested in this project, and those were a normal control strain (N2), a specific AD model control strain (CL2122), and an AD model strain (CL2355). The first goal was to establish the baseline chemoreception of each of the three *C. elegans* strains against the chemorepellent $CuCl_2$. Once this was established, the animals' diets were supplemented with individual bacteria species from the CeMbio mixture. Over the course of this project, six of the twelve CeMbio bacterial species were tested. Those bacteria were as follows: CEenent1, MSPm1, MYb10, MYb71, JUb66, and JUb134. Chemoreception to aversive stimuli was measured throughout the experiment using avoidance assays.

Methodology

Worm Strains and Maintenance

N2 (wildtype), CL2122 (*smg-1*ts; *dvIs15* [(pPD30.38) *unc-54*(vector) + (pCL26) *mtl-*2::GFP]), and CL2355 (smg-1(cc546); pCL45 [Psnb-1::human Amyloid beta 1-42::3' UTR (long); Pmtl-2::GFP]) were obtained from the Caenorhabditis Genetics Center (CGC).

The *C. elegans* strains were maintained on 60 mm nematode growth medium (NGM) plates seeded with the bacteria species corresponding to their experimental group (NGM: 3 g/L NaCl, 2.5 g/L peptone, 17 g/L agar, 25 mM KPO4 buffer [pH=7], 1mM MgSO4, 1mM CaCl2, 0.0129 mM cholesterol in ethanol, H2O to volume). Each plate was seeded with the appropriate food source using a micropipettor. Seeded plates were allowed to dry overnight before moving worms onto the plate. Stock strains were maintained on plates seeded with OP50 *E. coli* in LB media (LB: 10 g/L NaCl, 10 g/L tryptone, 5 g/L yeast, H_2O to volume). Microbiome supplementation groups were maintained on plates seeded with 100 uL of a single bacterial species at an OD of 1 in PBS. Worms were passed onto new seeded plates every 3-5 days by pick transfer to prevent starvation. The pick tool was sterilized between each worm transfer. All strains were maintained at 16°C for consistency, as the CL2122 and CL2355 strains were required to be kept in conditions to avoid activating their temperature sensitive transgenes.

Heat Shock Protocols

For all strains, 4-6 L4 stage worms were picked onto a plate containing the corresponding bacterial media of their experimental group as a food source. The worms were allowed to lay eggs for 48 hours at 16°C. After 48 hours, the plates, now containing eggs and L1 larvae, were transferred to a 25°C incubator for 72 hours. After 72 hours, the heat shock plates were removed from the 25°C incubator and were allowed to rest at room temperature for one hour. After resting at room temperature, the worms were used in avoidance assays.

CeMbio Bacterial Treatment

All 6 used CeMbio bacterial strains were obtained from the CGC on LB plates. Each bacteria was grown up in 10 mL of liquid LB for 24 hours at 25°C then was kept at 20°C until dilution. Each sample was diluted 1:10 by combining 100 uL of sample and 900 uL of PBS in a

cuvette. The optical density (OD) of each diluted sample at 600 nm was found using a Multiskan Spectrum spectrophotometer. Each sample was then diluted to an OD of 1 using PBS. 100 uL of each sample was seeded onto separate plates for gut microbiome supplementation experiments.

Avoidance Assay

Avoidance assays were used to observe the chemoreception behaviors of all three *C. elegans* strains under various experimental conditions. Unseeded NGM plates were used as assay plates. These plates were allowed to acclimate to RT and room humidity for about 30 minutes before experimentation. The experimental worm plates were also removed from the 25°C incubator and allowed to acclimate to RT for 1 hour before experimentation. Worms were washed from experimental plates with 1 mL of M9 buffer and transferred to a 1.5 mL microcentrifuge tube (M9: 3g /L KH2PO4, 6 g/L Na2HPO4 • 2H2O, 5 g/L NaCl, 1 mL 1M MgSO4, 1L H2O). The worms were allowed to settle, and the supernatant liquid was decanted. This was followed by an additional wash with 1 mL M9. The worms were again allowed to settle and the supernatant was decanted. The remaining liquid was transferred and split in relatively equal volumes to 3 unseeded NGM plates. Worms were given 5-10 minutes to acclimate to the new plates. After set-up, the worms were tested using the drop assay protocol found in Worm Book, which was originally described in experiments done by Hilliard, Bargmann, and Bazzicalupo (Hilliard et al., 2002). Assays were performed in humidity less than 40% and temperatures less than 25°C. The experimental set up and protocol is depicted in *Figure 8* below.

Drops were delivered to the plate using a mouth pipette fitted with a fine micro-needle tip made from pulling at 10 uL glass capillary tube over a flame. 10 worms from each plate were first tested with a solvent control by dropping approximately 5 nL of liquid behind the tail of a forward moving worm. After testing avoidance to the solvent control, lates were given 3-5 minutes to dry. The drop protocol was then repeated with 10 mM $CuCl_2$. Avoidance was quantified using the worms' response to a drop within the first five seconds. If the worm reversed direction over two body bends or made a 90° turn from its original direction, it was recorded as having avoided the stimulus. Avoidance index was then calculated for each assay plate by dividing the number of worms with avoidance behavior by the number of worms tested. In most cases, at least 9 assay plates were tested for each experimental condition.

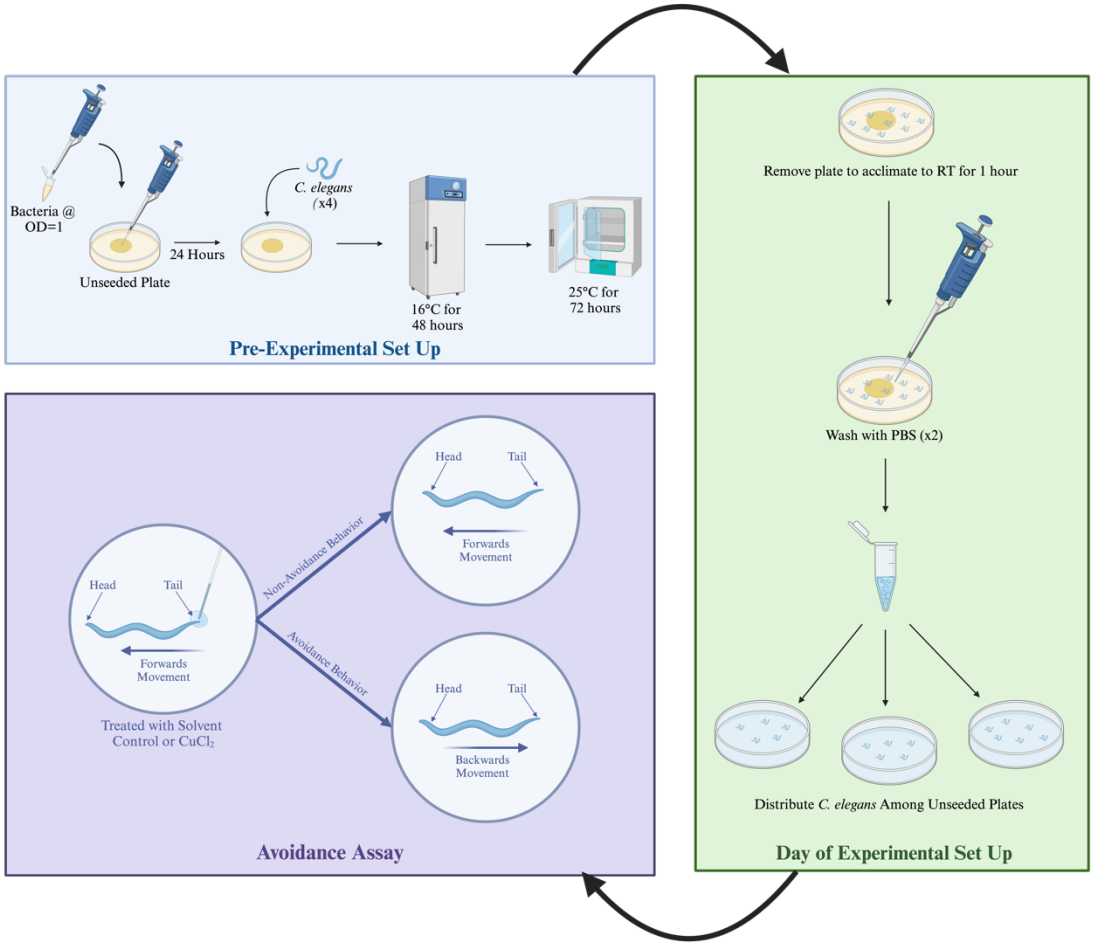

Figure 8. *C. elegans* grown with an OD50 food source at 16°C are transferred to plates seeded with one CeMbio bacteria strain and grown for 48 hours at 16°C. After 48 hours, the plates are heat shocked in an incubator at 25°C for 72 hours. The plates are then removed and acclimated to room temperature for 1 hour. The worms are then washed from the plate with PBS and distributed evenly to 3 unseeded plates. These unseeded plates with *C. elegans* are then used in an avoidance assay to determine the avoidance index.

Results and Discussion

AD Model Baseline Chemoreception to $CuCl_2$

An avoidance assay was done to characterize the baseline chemoreceptive behavior of all three *C. elegans* when exposed to an aversive stimulus. In each assay, N2 worms were used as a wildtype control and CL2122 worms were used as an AD model strain control. $CuCl_2$ was chosen as the chemorepellent for all test scenarios because it produced distinct behavioral results among the three test strains in previous MQP projects (Tarantino, 2021). All three strains were grown at 16°C and heat shocked at 25°C for 72 hours. During the avoidance assay, 10 animals were tested per each plate. Data points represent the average avoidance index for each plate. The results of the avoidance assay are shown in *Figure 9*. The average avoidance index of wildtype N2 was 0.63 ± 0.148, which is consistent with literature values (Hilliard et al., 2002). The average avoidance index of the AD model control CL2122 was 0.37 ± 0.242. The average avoidance index of the AD model CL2355 was 0.11 ± 0.0670.

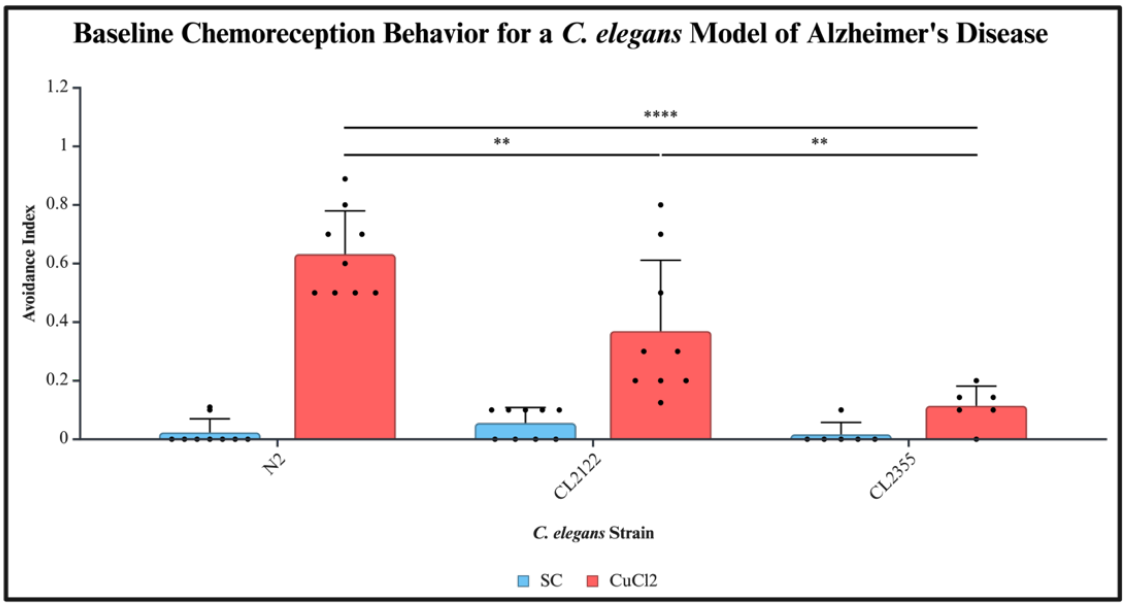

Figure 9. *C. elegans* model of AD is deficient in $CuCl_2$ avoidance responses. SC = solvent control. N2: n = 9 plates; CL2122: n = 9 plates; CL2355: n = 6 plates. Error bars are SD. Two-way ANOVA with Bonferroni multiple comparisons test. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Overall, the AD model strain CL2355 had a significantly lower avoidance index compared to its control strain CL2122, indicating that the strain had a notable deficiency in

chemosensation of $CuCl_2$. There was also a significant decrease in the avoidance index of CL2122 compared to the wild type N2 strain. Standard deviation for the CL2122 trials was relatively large in comparison to the other strains, which could contribute to the significant difference between the two control strains.

The significant differences in avoidance between the AD model and its controls support the assumption that CL2355 has a chemoreceptive deficiency to $CuCl_2$. This supports conclusions made in a previous MQP, which also found that $CuCl_2$ detection and avoidance were decreased in the AD model strain (Tarantino, 2021). These results reflect the interference of amyloid-β_{42} plaques on the olfaction process in CL2355s. This effect might be a direct consequence of the plaques themselves being physically present in the nervous system, or indirect consequences of plaque formation downstream, such as neuroinflammation or oxidative stress on the nervous system. In any case, amyloid-β_{42} plaque expression induces disruption to the olfactory pathways and thus alters chemosensory behaviors in the *C. elegans* AD model.

Avoidance to Chemorepellent with Microbiome Supplementation

After the baseline chemoreception of the AD model strain to $CuCl_2$ was established, supplementation of the gut microbiome was utilized to attempt reconstitution and the effects on chemosensory behaviors were observed through avoidance assays. To supplement the gut microbiome, all three *C. elegans* strains were fed one of six CeMbio bacterial strains from hatching to the time of the assay. The worms were fed each strain on seeded plates, which were used throughout worm hatching, growth, and heat shock.

Avoidance Assay with CEenent1 Supplementation

Avoidance assays were used to determine the effect of gut microbiome supplementation with CEenent1 on chemoreception of $CuCl_2$. During the avoidance assay, 10 animals were tested per each plate. Data points represent the average avoidance index for each plate. The results of these assays are shown below in *Figure 10* and compared to the baseline chemoreception for each strain. The avoidance index of the AD model strain CL2355 after supplementation with CEenent1 was 0.70 ± 0.121. This was significantly higher than the avoidance index of CL2355 without supplementation, which was 0.11 ± 0.0670. The avoidance index of the AD model strain

CL2122 after supplementation with CEenent1 was 0.71 ± 0.117. This was also significantly higher than the avoidance index of CL2122 before supplementation, which was 0.37 ± 0.242.

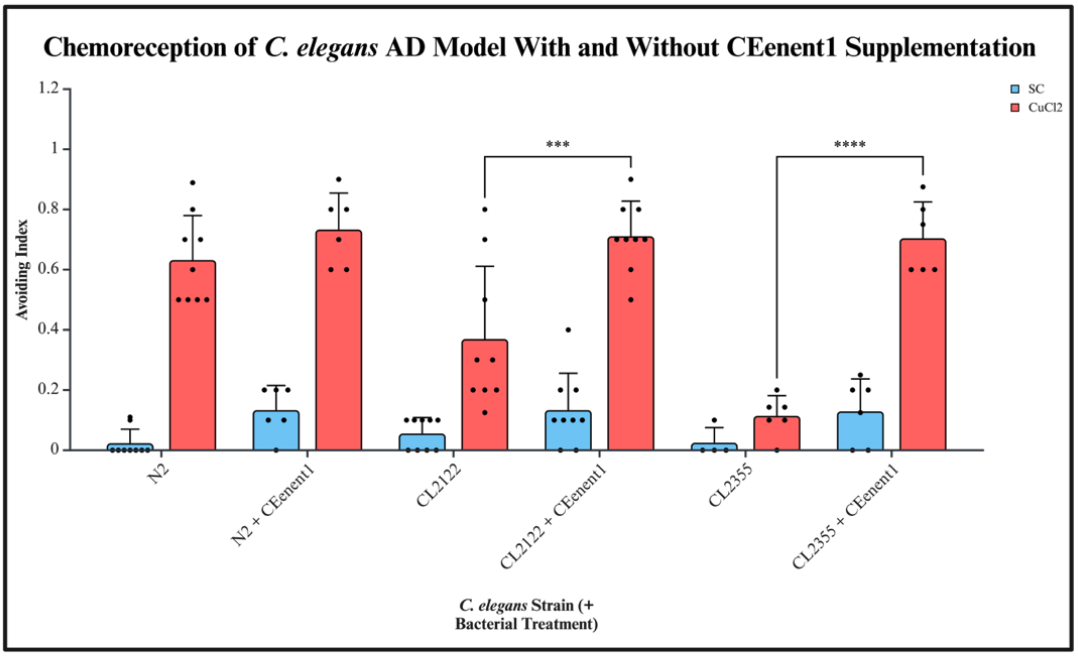

Figure 10. CEenent1 supplementation rescues avoidance behaviors towards $CuCl_2$ in the *C. elegans* model of AD. SC = solvent control. N2 + CEenent1: n = 6; CL2122 + CEenent1: n = 9; CL2355 + CEenent1: n = 6. Sample size of strains before supplementation are the same as in Figure 9. Error bars are SD. Two-way ANOVA with Tukey multiple comparisons test. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. No significant difference between N2 with and without CEenent1 supplementation.

The avoidance index of the AD model strain CL2355 was significantly increased following dietary supplementation with CEenent1. This means that, after supplementation with CEenent1, the animals' chemosensory ability to detect and avoid $CuCl_2$ were comparable to the wildtype, non-AD behavior. Reconstitution of the gut with CEenent1 likely interfered in some way with the pathophysiological pathways representing AD in the *C. elegans* model. This could mean interfering with the expression or formation of amyloid-β_{42} plaques, reducing the levels of neuroinflammation, or decreasing the toll of oxidative stress on the nervous system. The observed behavioral changes could be attributed to one or multiple of these potential explanations, and further exploration is needed to determine the specific cause. Overall, this data supports the conclusion that CEenent1 supplementation of the AD model strain from hatching

induces some level of gut microbiome reconstitution, and thus amelioration of chemosensory behaviors.

Avoidance Assay with MSPm1 Supplementation

Avoidance assays were used to determine the effect of gut microbiome supplementation with MSPm1 on chemoreception of $CuCl_2$. During the avoidance assay, 10 animals were tested per each plate. Data points represent the average avoidance index for each plate. The results of these assays are shown below in *Figure 11* and compared to the baseline chemoreception for each strain. The avoidance index of the AD model strain CL2355 after supplementation with MSPm1 was 0.66 ± 0.126. This was significantly higher than the avoidance index of CL2355 without supplementation, which was 0.11 ± 0.0670. The avoidance index of the AD model strain CL2122 after supplementation with MSPm1 was 0.67 ± 0.114. This was also significantly higher than the avoidance index of CL2122 before supplementation, which was 0.37 ± 0.242.

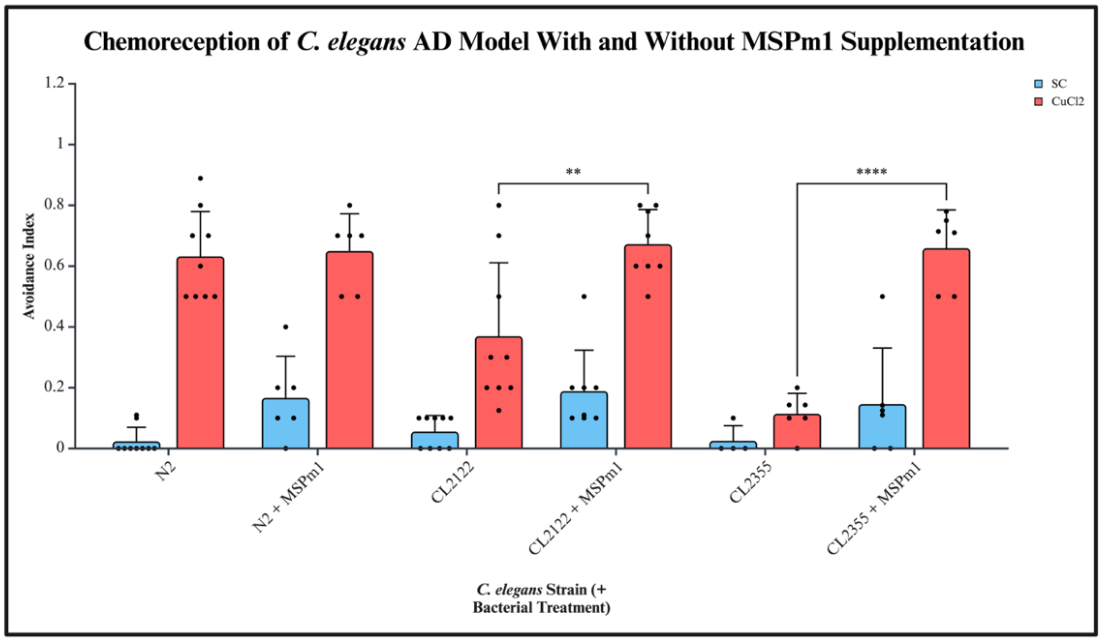

Figure 11. MSPm1 supplementation rescues avoidance behaviors towards $CuCl_2$ in the *C. elegans* model of AD. SC = solvent control. N2 + MSPm1: n = 6; CL2122 + MSPm1: n = 9; CL2355 + MSPm1: n = 6. Sample size of strains before supplementation are the same as in Figure 9. Error bars are SD. Two-way ANOVA with Tukey multiple comparisons test. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. No significant difference between N2 with and without MSPm1 supplementation.

Dietary supplementation with MSPm1 caused a significant amelioration of chemosensory behaviors in the *C. elegans* model of AD. This again can be explained by recognizing the way in which reconstitution of the gut microbiome likely influenced the pathophysiological expression of amyloid-β_{42} and therefore AD in the worm model (as described for CEenent1 supplementation). Overall, this data supports the conclusion that MSPm1 supplementation of the AD model strain from hatching induces some level of gut microbiome reconstitution, and thus amelioration of chemosensory behaviors.

Avoidance Assay with MYb10 Supplementation

Avoidance assays were used to determine the effect of gut microbiome supplementation with MYb10 on chemoreception of $CuCl_2$. During the avoidance assay, 10 animals were tested per each plate. Data points represent the average avoidance index for each plate. The results of these assays are shown below in *Figure 12* and compared to the baseline chemoreception for each strain. The avoidance index of the AD model strain CL2355 after supplementation with MYb10 was 0.67 ± 0.260. This was significantly higher than the avoidance index of CL2355 without supplementation, which was 0.11 ± 0.0670. The avoidance index of the AD model strain CL2122 after supplementation with MYb10 was 0.50 ± 0.0894. This was not significantly higher than the avoidance index of CL2122 before supplementation, which was 0.37 ± 0.242.

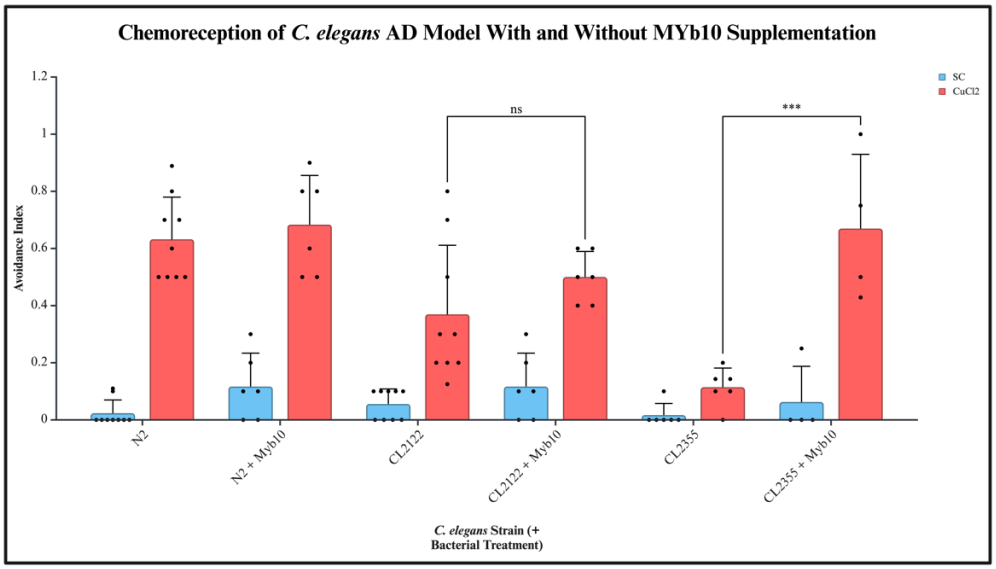

Figure 12. MYb10 supplementation rescues avoidance behaviors towards $CuCl_2$ in the *C. elegans* model of AD. SC = solvent control. N2 + MYb10: n = 6; CL2122 + MYb10: n = 6; CL2355 + MYb10: n = 4. Sample size of strains before supplementation are the same as in Figure 9. Error bars are SD. Two-way ANOVA with Tukey multiple comparisons test. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. No significant difference between N2 with and without MYb10 supplementation.

Dietary supplementation with MYb10 caused a significant amelioration of chemosensory behaviors in the *C. elegans* model of AD. This again can be explained by recognizing the way in which reconstitution of the gut microbiome likely influenced the pathophysiological expression of amyloid-β_{42} and therefore AD in the worm model (as described for CEenent1 supplementation). Overall, this data supports the conclusion that MYb10 supplementation of the AD model strain from hatching induces some level of gut microbiome reconstitution, and thus amelioration of chemosensory behaviors.

Avoidance Assay with MYb71 Supplementation

Avoidance assays were used to determine the effect of gut microbiome supplementation with MYb71 on chemoreception of $CuCl_2$. During the avoidance assay, 10 animals were tested per each plate. Data points represent the average avoidance index for each plate. The results of these assays are shown below in *Figure 13* and compared to the baseline chemoreception for each strain. The avoidance index of the AD model strain CL2355 after supplementation with MYb71 was 0.66 ± 0.126. This was significantly higher than the avoidance index of CL2355

without supplementation, which was 0.58 ± 0.0837. The avoidance index of the AD model strain CL2122 after supplementation with MYb71 was 0.67 ± 0.173. This was also significantly higher than the avoidance index of CL2122 before supplementation, which was 0.37 ± 0.242.

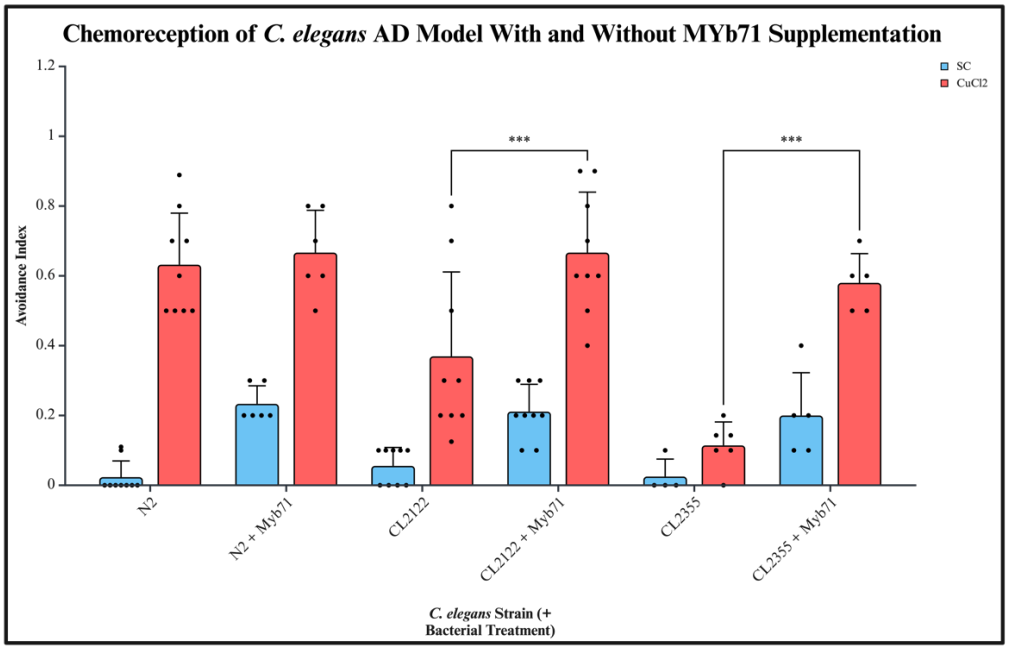

Figure 13. MYb71 supplementation rescues avoidance behaviors towards $CuCl_2$ in the *C. elegans* model of AD. SC = solvent control. N2 + MYb71: n = 6; CL2122 + MYb71: n = 9; CL2355 + MYb71: n = 5. Sample size of strains before supplementation are the same as in Figure 9. Error bars are SD. Two-way ANOVA with Tukey multiple comparisons test. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. No significant difference between N2 with and without MYb71 supplementation.

Dietary supplementation with MYb71 caused a significant amelioration of chemosensory behaviors in the *C. elegans* model of AD. This again can be explained by recognizing the way in which reconstitution of the gut microbiome likely influenced the pathophysiological expression of amyloid-β_{42} and therefore AD in the worm model (as described for CEenent1 supplementation). Overall, this data supports the conclusion that MYb71 supplementation of the AD model strain from hatching induces some level of gut microbiome reconstitution, and thus amelioration of chemosensory behaviors.

Avoidance Assay with JUB66 Supplementation

Avoidance assays were used to determine the effect of gut microbiome supplementation with JUB66 on chemoreception of $CuCl_2$. During the avoidance assay, 10 animals were tested per each plate. Data points represent the average avoidance index for each plate. The results of these assays are shown below in *Figure 14* and compared to the baseline chemoreception for each strain. The avoidance index of the AD model strain CL2355 after supplementation with JUB66 was 0.57 ± 0.216. This was significantly higher than the avoidance index of CL2355 without supplementation, which was 0.58 ± 0.0837. The avoidance index of the AD model strain CL2122 after supplementation with JUB66 was 0.60 ± 0.158. This was also significantly higher than the avoidance index of CL2122 before supplementation, which was 0.37 ± 0.242.

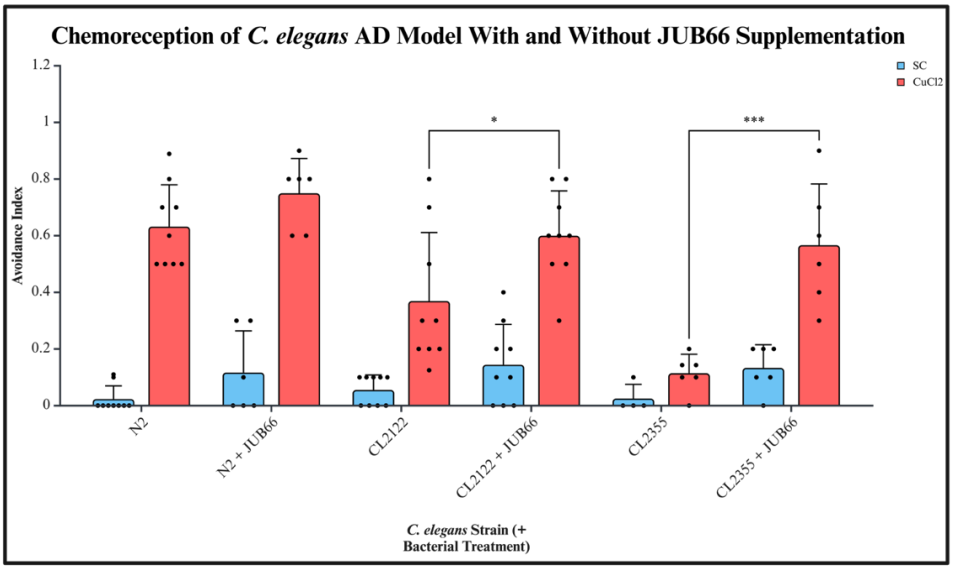

Figure 14. JUB66 supplementation rescues avoidance behaviors towards $CuCl_2$ in the *C. elegans* model of AD. SC = solvent control. N2 + JUB66: n = 6; CL2122 + JUB66: n = 9; CL2355 + JUB66: n = 6. Sample size of strains before supplementation are the same as in Figure 9. Error bars are SD. Two-way ANOVA with Tukey multiple comparisons test. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. No significant difference between N2 with and without JUB66 supplementation.

Dietary supplementation with JUB66 caused a significant amelioration of chemosensory behaviors in the *C. elegans* model of AD. This again can be explained by recognizing the way in which reconstitution of the gut microbiome likely influenced the pathophysiological expression of amyloid-β_{42} and therefore AD in the worm model (as described for CEenent1

supplementation). Overall, this data supports the conclusion that JUB66 supplementation of the AD model strain from hatching induces some level of gut microbiome reconstitution, and thus amelioration of chemosensory behaviors.

Avoidance Assay with JUB134 Supplementation

Avoidance assays were used to determine the effect of gut microbiome supplementation with JUB134 on chemoreception of $CuCl_2$. During the avoidance assay, 10 animals were tested per each plate. Data points represent the average avoidance index for each plate. The results of these assays are shown below in *Figure 15* and compared to the baseline chemoreception for each strain. The avoidance index of the AD model strain CL2355 after supplementation with JUB134 was 0.75 ± 0.354. This was significantly higher than the avoidance index of CL2355 without supplementation, which was 0.58 ± 0.0837. The avoidance index of the AD model strain CL2122 after supplementation with JUB134 was 0.77 ± 0.121. This was also significantly higher than the avoidance index of CL2122 before supplementation, which was 0.37 ± 0.242.

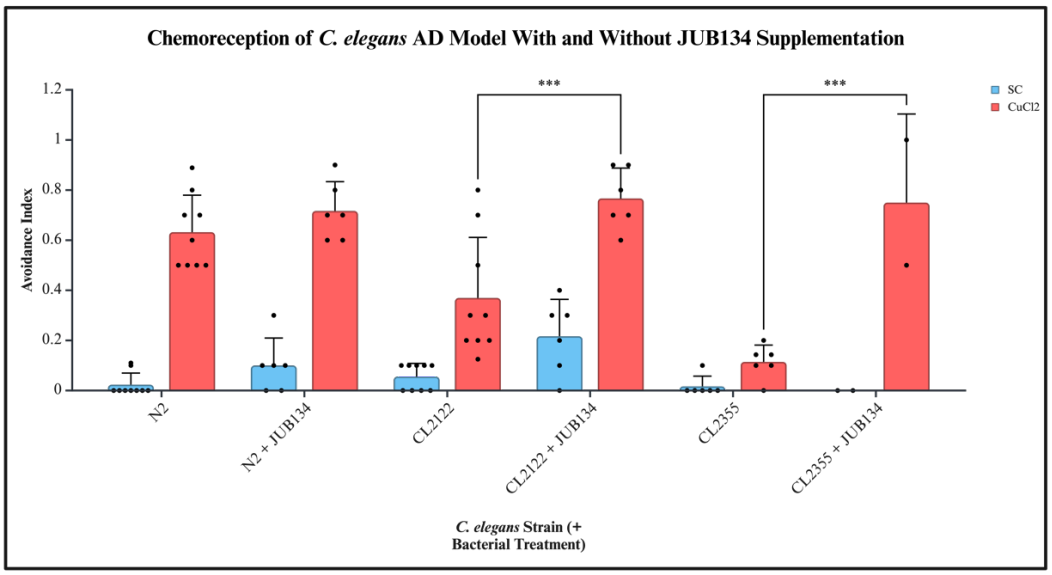

Figure 15. JUB134 supplementation rescues avoidance behaviors towards $CuCl_2$ in the *C. elegans* model of AD. SC = solvent control. N2 + JUB134: n = 6; CL2122 + JUB134: n = 6; CL2355 + JUB134: n = 2. Sample size of strains before supplementation are the same as in Figure 9. Error bars are SD. Two-way ANOVA with Tukey multiple comparisons test. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. No significant difference between N2 with and without JUB134 supplementation.

Dietary supplementation with JUB134 caused a significant amelioration of chemosensory behaviors in the *C. elegans* model of AD. This again can be explained by recognizing the way in which reconstitution of the gut microbiome likely influenced the pathophysiological expression of amyloid-β_{42} and therefore AD in the worm model (as described for CEenent1 supplementation). Overall, this data supports the conclusion that JUB134 supplementation of the AD model strain from hatching induces some level of gut microbiome reconstitution, and thus amelioration of chemosensory behaviors.

In five of the six experiments (excluding MYb10), there was an observed statistically significant difference between the unsupplemented AD control strain (CL2122) and the supplemented AD control strain. This can partially be attributed to variability within the baseline chemoreception data for the CL2122 strain, which had a relatively large standard deviation compared to the average. This could have potentially skewed the value towards a lower value. Additionally, there are significant genetic differences between the AD control strain and the wildtype N2 strain which might also help account for some of the observed behavior. The AD control strain, CL2122, is a specific control for the AD model strain, CL2355. The main difference between these two strains is that CL2355 has a gene for expression of amyloid-β_{42}, and CL2122 does not. All other modifications to the wildtype strain that are present in the AD model strain are also present in the AD control strain, and these modifications potentially influence the behavior being observed. Finally, it is possible that the changing of the food source itself is enough to improve the health and chemosensation of the AD control strain. Prior to the supplementation protocol followed for each experiment, the only food source each of the strains was exposed to was *E. coli*, specifically the strain OP50. It is possible that exposure to a new bacterial strain which is found naturally in the *C. elegans* gut microbiome had positive benefits to the health of the worm strains, potentially causing some of the observed behavioral changes. In humans, the development and progression of AD is complex and influenced by several different risk factors, including genetics, environment, and health behaviors. This is also true for the *C. elegans* AD model.

Conclusions and Future Directions

The first goal of this project was to establish how the expression of amyloid-β_{42} in the *C. elegans* AD model affects chemoreception of $CuCl_2$, an aversive stimulus. The chemoreceptive behaviors were tested using avoidance assays, and it was found that the AD strain had significantly decreased chemosensory abilities compared to either control strain. This suggests that expression of amyloid-β_{42} is responsible for a deficit in overall chemoreception sensitivity. It was also determined that the AD control strain had significantly lower chemoreceptive capabilities than the wildtype strain. This suggests that the expression of amyloid-β_{42} is not the only determinant of chemoreceptive ability, especially in the AD models. There are likely several genetic or environmental factors outside of the expression of amyloid-β_{42} which influence olfactory function in *C. elegans*.

The second goal was to determine the therapeutic effect of gut microbiome reconstitution in the AD model with any or all the CeMbio. It was found that all six of the tested CeMbio bacteria strains caused amelioration of chemosensory behaviors in the AD model strain, CL2355. It is likely that dietary supplementation with CeMbio bacteria in the *C. elegans* AD model allows for partial reconstitution and diversification of the gut microbiome. This reconstitution aids in repair of the gut-brain axis, which influences factors effecting the development of AD, specifically the accumulation and formation of amyloid-β_{42} plaques. It is unclear though, whether these supplementations caused reconstitution or full recolonization of the gut. Because of this, further genetic testing is needed to characterize the gut microbiome differences between the controls and the AD strain before and after supplementation. A potential avenue for this testing would be 16S rRNA sequencing, which would allow for bacterial classification and the identification of relative bacterial abundance (Armanhi et al., 2016).

Another immediate next step for the continuation of this project would be to repeat the protocols utilized during this project to test the remaining six CeMbio bacteria. This will be key in determining whether there are specific bacteria strains within the CeMbio mixture which are responsible for ameliorating worm chemosensory behaviors. If these experiments show that most or all the 12 CeMbio bacteria strains cause chemosensory amelioration, then a next step will be to perform a similar set of experiments utilizing non-CeMbio bacteria strains to determine whether the behavioral changes seen in the AD strain are specific to the CeMbio bacteria or general to any bacterial or microbial sources.

Another consideration is that all bacterial treatments were applied from hatching through the experiment. This means the worms were exposed to the treatment for their entire development. It is possible that the bacterial supplementation is providing some level of neuroprotection before the induction of amyloid plaque expression, which does not happen until later phases of development. If this is true, that means that the supplementation is working as more of a preventative measure, as opposed to a retroactive treatment for AD. Preventative measures are extremely important, but in a disease like AD where clinical symptoms are not obvious until many years after the beginning of biochemical changes, a preventative measure may not be as effective or widely used compared to a retroactive treatment. For this reason, it would be useful to perform a set of experiments testing the effects of gut microbiome supplementation after the induction of amyloid plaque production to determine whether there is potential for retroactive therapeutic effects.

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