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# Exploring the Potential Role of *Lactobacillus plantarum* in the Reversal of Induced Cognitive Long-term Memory Impairment

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Long-term Memory Formation (LTM)

*Pseudomonas aeruginosa*

*Escherichia coli*

*Aeromonas hydrophila*

*Lactobacillus plantarum*

**ABSTRACT**

Long-term Memory (LTM) is formed by sequential changes in the different brain regions due to synaptic plasticity changes. This synaptic plasticity changes formed in the brain due to the acquittance of unexposed information and its retrieval due to learning and memory formation (LMF). In a normal condition, LMF uses RNA and protein synthesis machinery to form LTM, which lasts till the end of an organism's lifetime. Formed LTM shows sequential changes happening in the presynaptic and postsynaptic neurons. Stated sequential changes are initiated when the released neurotransmitter binds with the postsynaptic neuronal receptors and activates the brain's ERK - CREB neuronal signaling pathway. Based on the previous findings, the present study was designed to study the interrelationship between cognitive impairment and oral/gut dysbiosis with the help of a probiotic strain (*Lactobacillus plantarum*). Two phases of behavioural analysis (first and second phase) were used to identify the effect of oral microbial infusions on impaired LTM formation and its reversal using restoration of dysbiosed gut/oral microbiota. The first phase of behavioural analysis (FPBA) reported that oral microbial infusion plays a major role in developing oral/gut dysbiosis, which results in impaired cognitive functions. Further, formed oral/gut microbiota dysbiosis was reversed with the help of probiotic strain in the second phase of behavioural analysis (SPBA). Thus, a comparative two-phase behavioural analysis revealed that probiotics may play a significant role in reversing induced cognitive decline. The outcome of the present study also proved that probiotic treatment might play a major role in the reversal of dysbiosed microbiota in the oral cavity and the gut.

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## 1 Introduction

Microorganisms are tiny creatures of our nature present throughout the globe. These microorganisms have beneficial and harmful effects on all host systems, including humans. Fermented food products like cheese, yogurt, and other canned products contain some beneficial microorganisms which may promote beneficial effects in humans through the production of some primary/secondary metabolites by these microorganisms (Rezac et al. 2018; Xiang et al. 2019; Maraz and Khan 2021; Zapaśnik et al. 2022; Hakim et al. 2023; Icer et al. 2023). These microorganisms are categorized as beneficial microorganisms, and various lactobacillus species like *Lactobacillus casei*, *L. curvatum*, *L. delbrueckii*, *L. bulgaricus*, *L. plantarum*, *L. sakei* are also included in this (Lorenzo et al. 2018; Rezac et al. 2018; Hakim et al. 2023; Icer et al. 2023). These lactobacillus species play an essential role in developing oral/gut homeostasis mechanisms within the host. Disruption in this oral/gut homeostasis mechanisms results in oral/gut dysbiosis, which further results in systemic diseases like neurodegenerative disorders (Radiac and Kapila 2021; Hou et al. 2022; Deandra et al. 2023; Ma et al. 2023; Mukilan 2023).

Neurodegenerative (ND) disorders are complex progressive disorders that affect aged persons, especially those aged above sixty years. Several health parameters are associated with the development of this ND disorder; among others, improper oral hygiene plays a major role in the development of cognitive decline (CD). This CD is one of the characteristic features of ND disorders, which progress through memory loss (Gómez- Gómez and Zapico 2019; Alvarenga et al. 2021; Guo et al. 2022; Hou et al. 2022; Lamptey et al. 2022; Gu et al. 2023). Recent reports have shown that poor oral hygiene plays a significant role in the initiation of cognitive decline during mild cognitive impairment/long-term memory formation. This poor oral hygiene may be a result of pathogenic microbial colonization, which affects the normal oral microflora of the oral cavity (Chung and Chan 2023; Gu et al. 2023; Kulkarni et al. 2023; Mukilan et al. 2024; Pruntel et al. 2024). Imbalances in the oral microbiota further result in gut dysbiosis through the transmission/transport of formed pathogenic colonizes from the oral cavity to the gut. Imbalances in this gut microbiota may reduce the transmission of neurotransmitter precursor (NP) compounds from the gut to the brain through the vagus nerve (Park et al. 2021; Yu et al. 2021; Lu et al. 2023). Reduced amount of NPC compound results in reduced synthesis and release of neurotransmitters from presynaptic neurons (PrSN). Released neurotransmitter further binds with postsynaptic neuron membrane receptors and activates a reduced amount of cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), and enzyme-regulated kinase – 1/2 (ERK-1/2) through reduced calcium influx happening inside the postsynaptic neuron (PoSN). Less expressive cAMP, PKA, and ERK-1/2 may reduce the phosphorylation of cAMP response element binding protein – 1 (CREB-1). Further, reduced phosphorylation of CREB - 1 down-regulates the expression of immediate early genes and postsynaptic density proteins (Ganesh et al. 2012; Olguín et al. 2016; Mukilan et al. 2015; Sivasangari and Rajan 2020; Rajan 2021; Mukilan 2023). Reduced expression of this neuronal molecule may further result in cognitive impairment (CI) initiation. Formed CI resulted from reduced long-term memory formation under dysbiosed conditions (Országhova et al. 2021; Mukilan 2022; Mukilan 2023). In the present study, we examined the unavoidable role of probiotic strain (*L. fermentum*) in reversing formed LTM impairment with the help of two-phased reward-based learning paradigms (RBLP).

## 2 Materials and Methods

### 2.1 Study animals (SA)

Commercially available healthy naïve goldfish (*Carassius auratus*) with a body length of 6.5-8 cm and 6-15 g weight were purchased from a local aquarium shop in Coimbatore, Tamil Nadu, India. Purchased animals were shifted from the aquarium to the laboratory with utmost care to prevent the formation of stress. Once shifting was over, study animals (SA) were housed in a standard rectangular glass tank with a length, breadth, and height of 42 X 30 X 21 inches for five days during the assimilation process. During assimilation, SA was maintained at standard controlled laboratory conditions with a photoperiod of 12 (light):12 (dark) hours, a controlled temperature range between 28 ± 2º C, and continuous aeration. SA fed with commercially available food resources (dry food pellets – Taiyo Pet Products Pvt Ltd, India) three times a day with a time interval of five hours in their assimilation environment (9.00, 14.00, and 19.00 h). The water quality was measured to maintain the home tank water with the needed amount of dissolved oxygen content during the experimental period (EP). The home tank was continuously replaced with fresh water on alternative days till the end of EP to maintain a dust-free environment. All experimental study designs follow the institutional animal care guidelines of the institution (Sri Ramakrishna Group of Institutions, Coimbatore, Tamil Nadu, India).

### 2.2 Study Design

A reward-based learning paradigm (RBLP) was used in this study design (SD) to study the effect of normal and dysbiosed microbial flora on LTM formation. SA underwent three phases of RBLP (exploration, training, and testing) in a glass experimental chamber (GEC) having a length, breadth, and height of 42 X 30 X 21 inches. This GEC contains two feeding chambers (FC) and one central chamber (CC). The designed GEC, CC, and FCs were differentiated based on the length and breadth size. CC acts as an entry space for the SA in the GEC with a size of 30 (length) X 20

(breadth) X 21 (height) inches. Compared to CC, both the FCs have a size of 6 (length) X 5 (breadth) X 21 (height) inches and serve as either positive/negative reward chambers. The commercially available dry food pellets were given as a reward for learning the cue in positive FC, not negative FC (Mukilan 2023).

**2.3 Collection and Analysis of Oral Swab Sample**

The oral swab samples were collected from ten healthy individuals (without dental plaques – aged between 18-21 years), six diseased individuals (without dental plaques – aged between 18-24 years) from the Department of Biotechnology, Sri Ramakrishna College of Arts & Science (SRCAS), Coimbatore, Tamil Nadu, India. After determining their concerns, oral swab samples were taken from healthy and diseased individuals. All oral samples were collected using a sterile cotton swab by swabbing the teeth and their associated surface. Collected samples were spread on nutrient agar plates under aseptic conditions in laminar airflow. Spread plates were incubated at 37º C for 24 - 48 hours. Later, incubated grown plates were used to isolate the desired microorganisms using pure culture techniques from both samples. Isolated microorganisms (M1, M2, and M3) were infused into EGs 2-5, and their impact on LTM formation was studied using RBLP behavioural scores. Behavioural scores were calculated based on the amount of time spent by the EA in the LC, CC, and RC. RC and LC act as positive and negative reward chambers. After RBLP analysis, three oral-infused cultures were identified using biochemical test results.

**2.4 Biochemical Characterization**

Isolated microorganisms (M1, M2, and M3) were characterized using biochemical characterization tests like indole, methyl-red, catalase, voges-proskauer, and oxidase tests. Biochemical characterization tests were performed according to the standard conditions (Alghamadi 2021).

**2.5 Oral Infusion Mixture Preparation**

The grown overnight cultures were used to prepare the oral infusion mixture with the help of phosphate buffer saline (PBS) for its infusions in the four experimental groups (EGs) except the control. Experimental group 1 (EG–1) did not receive any oral infusion as it was designed as a control, while EGs – 2, 3, and 4 received oral infusions of culture M1, M2, and M3 in the ratio of 50:50 (contains 50% desired microorganism and 50% PBS) as a single dose of 500 microlitres, and EG – 5 received oral infusion mixture ratio of 20:20:20:40 [contains M1, M2, and M3 cultures (each in every 20%) and PBS (40%)]. Formulated blends were orally infused into the specific EGs as a single dose. Following oral infusions, all EGs were provided 24 hours to recover from the handling stress before the first phase of behavioural analysis (FPBA). Followed by FPBA, the second phase of behavioural analysis (SPBA) was carried out with the help of a probiotic strain *L. plantarum* (MTCC No. 12921) acquired from MTCC, IMTECH, Chandigarh, Punjab, India. The acquired probiotic culture was used to prepare an oral probiotic mixture in a ratio of 50:50 (Mukilan et al. 2024).

**2.6 Behavioural Analysis**

Behavioural analysis was carried out in two consecutive phases, i.e., the first and second phases, to study the effect of desired cultures on LTM impairment and its reversal using a probiotic strain, *L. plantarum*. In FPBA, SAs were separated into four different experimental groups (EGs) according to the needs of the study. The EGs consist of experimental group – 1 (control), experimental group – 2 (infused with isolate M1), experimental group – 3 (infused with isolate M2), experimental group – 4 (infused with isolate M3), and experimental group – 5 (infused with M1, M2, and M3). Before infusions, experimental animal activities were confirmed in the exploratory phase. After the exploratory phase, microbial oral infusions were given to the EG – 2, 3, 4, and 5. Followed by infusions, training, and testing were performed in the FPBA. In SPBA, all four experimental groups (EGs – 2 – 4 and 5) receive probiotic oral infusions before taking the training and testing phases in RBLP. In RBLP, behavioural responses were calculated based on the time spent in the LC, CC, and RC. Other than RBLP, open field test (OFT), and predator exposure test (PET) were also performed to test the effect of microbial oral infusions on the development of anxiety-like behaviour, and fear memory formation in all EGs.

**2.7 Predator Exposure Test**

The predator exposure test (PET) was performed after completion of FPBA to identify the development of fear memory formation in the infused and non-infused EGs. For PET, we have used rectangular glass tanks for the creation of three different compartments [complete fear (CF), mid fear (MF), and No fear (NF) zones] within a single behavioral experimental chamber having a size of 42 X 30 X 21 inches (length, breadth, and height). Inside the NF zone, a separate chamber is created to hold the predator in an isolated manner. *Pseudotropheus demasoni* (cichlid fish) was used as a predator for this study. Behavioral responses were calculated based on the time spent in the CF, MF, and NF zones during the 900 seconds of exposure to the predator (Thangaleela et al. 2018).

**2.8 Open Field Test**

Following PET, all EGs were allowed to perform an open field test (OFT) in the designed GEC with the equal-sized individual box (10 x 5 cm) diagram placed below the bottom of the GEC. After completion of PET, EGs were introduced to the behavioral setup (BS) for 900 seconds, and their mobility was recorded based on the time spent in the inner compartment (TSI) and time spent in the

outer compartment (TSO). Behavioural responses of the EGs were used for identifying the presence/absence of anxiety-like behavior in the BS (Horka et al. 2024).

### 2.9 Data Analysis and Graphical Representation

Behavioural scores of all experimental groups were recorded in a single Microsoft Excel Sheet according to the behavioural parameters like IPBS exploration, training, and testing for IPBS and SPBS, amount of time spent in CF, MF, and NF zones for PET, and TSI, and TSO for the OFT. Recorded scores of consecutive days were used to calculate the mean value (MV), standard deviation, and standard error (SE) for the statistical analysis. MV and SE were used to create bar diagrams.

## 3 Results

### 3.1 Effect of Microbial Oral Culture Infusions on Long-Term Memory Retrieval

The present study uses FPBA to explore the role of pathogenic/non-pathogenic oral infusions on cognitive learning and memory formation during LTM retrieval with the help of RBLP in a habituated environment.

#### 3.1.1 Identification of Induced Cognitive Memory Impairment

Initially, IPBS was used to study the role of oral microbial infusions in the induction of cognitive memory impairment. In IPBS, all five EGs (EGs 1 -5) underwent a habituation (H) process in the home tank for seven days (Days 1-7). All EGs were maintained in the home tank between days 1-7 during the H process to adapt to the laboratory setup. Following the process of H, the exploration phase (E) was carried out for all EGs in the ES between days 8-10 for 15 minutes/day. Behavioural scores of the exploration E phase showed that all EGs' animals were active, and ES did not provide any stressed environment for the habituated EGs (Figure 1). Following the E phase, four experimental groups (EGs 2-5) received oral infusions of desired microorganisms in pure (EGs 2-4) and mixed forms (EG 5). After receiving oral infusions, EGs were maintained in separate tanks for transportation and colonization in the gut for three days (Days 11-13).

Microorganisms present on the collected oral swab samples were grown on a nutrient agar medium under aseptic room conditions for 24-48 hours. After incubation, unknown microbial colonies were picked up from the mother plate, and their purity was confirmed by the quadrant streaking method (Figure 2). After purity confirmation, a quadrant individual colony was used to prepare the respective overnight cultures. Grown overnight cultures were named M1, M2, and M3 and infused orally into designated EGs in pure and mixed forms. The result of biochemical characterization tests (indole, methyl red, catalase, Voges-Proskauer, and oxidase tests) showed that isolated culture M1 was identified as *P. aeruginosa* based on the positive results of catalase and oxidase tests, culture M2 showed positive results for methyl red and catalase tests and characterized the presence of *E. coli*, and isolated culture M3 showed positive reaction for all four tests except methyl red which shows the presence of *A. hydrophila* (Figure 3 & Table 1).

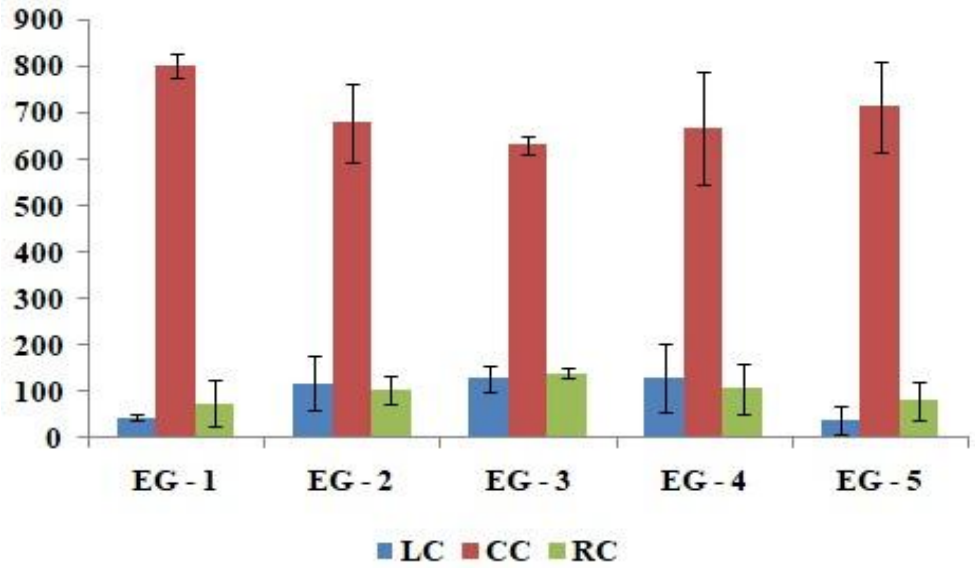


Figure 1 Exploratory phase of the first behavioral analysis phase showed that all experimental animals were active and adapted to the experimental setup. The amount of time spent in the left chamber (LC), central chamber (CC), and right chamber (RC) were used for the preparation of the bar diagram with standard error and mean (SEM) values

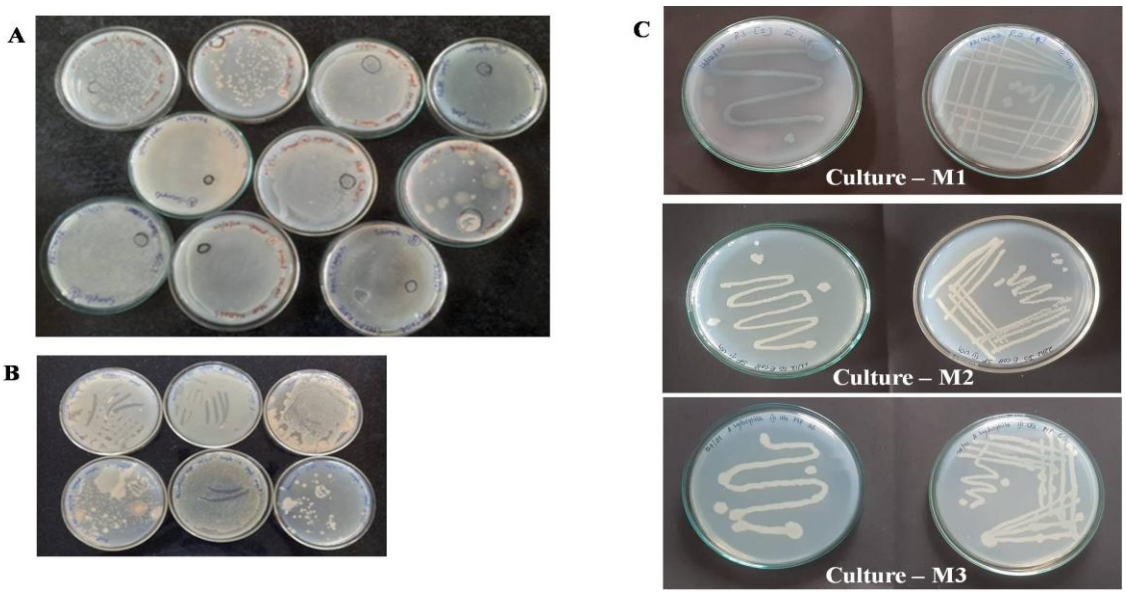


Figure 2 Representative plate pictures isolated from the collected oral swab samples (A – healthy individuals, B – diseased individuals), (C) Based on their colony morphology, three different bacterial colonies were picked up and grown on nutrient agar medium and named as culture – M1, M2, and M3

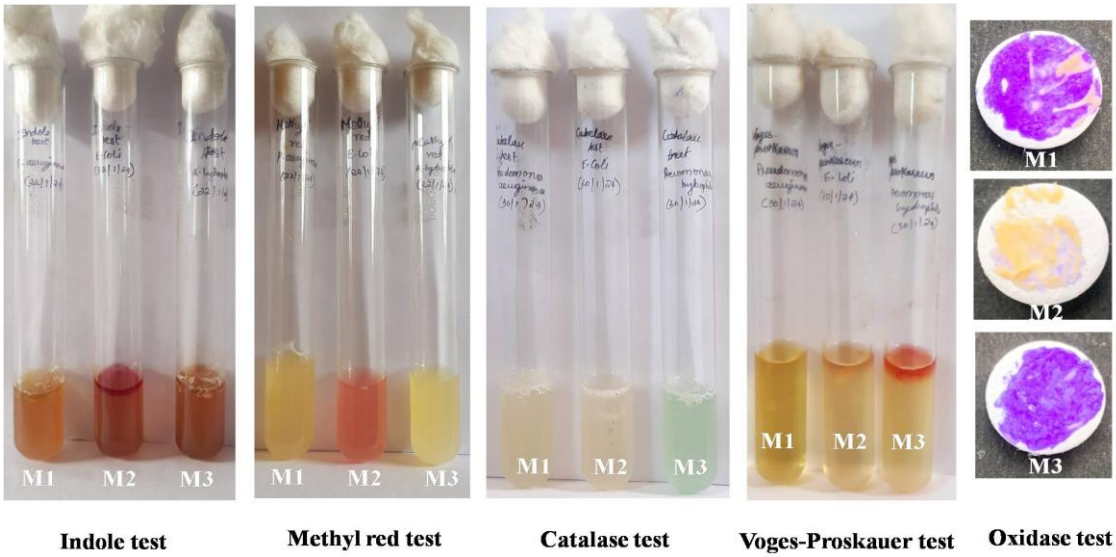


Figure 3 Biochemical characterization results showed that selected desired cultures – M1, M2, and M3 were identified as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Aeromonas hydrophila*

Table 1 Results of biochemical characterization test performed in this study

| Name of the biochemical test | *Pseudomonas aeruginosa* | *Escherichia coli* | *Aeromonas hydrophila* |
|---|---|---|---|
| Indole | - | - | + |
| Methyl red | - | + | - |
| Catalase | + | + | + |
| Voges-proskauer | - | - | + |
| Oxidase | + | - | + |

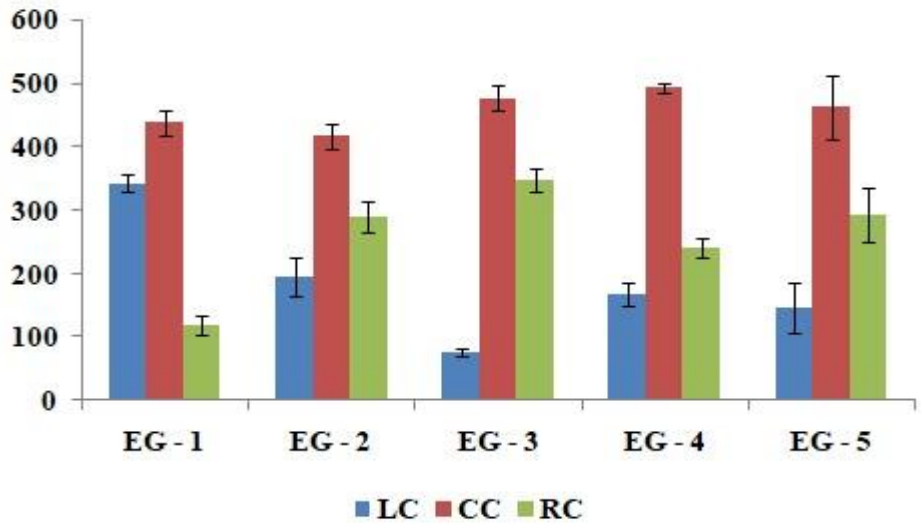


Figure 4 The first phase of behavioural training showed that all experimental animals were trained in the experimental setup with the help of positive and negative reward conditioning. The amount of time spent in the left chamber (LC), central chamber (CC), and right chamber (RC) were used for the preparation of the bar diagram with standard error and mean (SEM) values

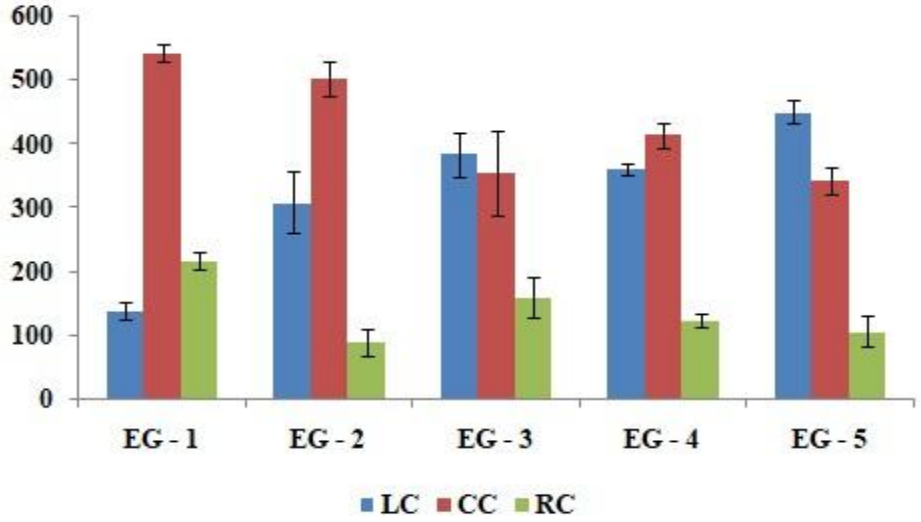


Figure 5 The first phase of behavioural testing proved that microbial oral infusions induced impaired cognitive memory retrieval. The amount of time spent in the left chamber (LC), central chamber (CC), and right chamber (RC) were used for the preparation of the bar diagram with standard error and mean (SEM) values

After infusions, the training (Tr) phase was carried out in ES for three days between days 14-17 for all EGs (EG 1-5). Behavioural responses of the Tr phase showed that microbial oral infusions did not impact EGS 2-5 compared to EG-1 (did not receive any infusion). Obtained scores also proved that microbial oral infusions showed no impairment in the Tr phase of FPBA. It also proved that the infused oral microbial mixture did not involve gut dysbiosis formation during the Tr phase (Figure 4). Further, the testing (Te) phase was carried out after a seventy-two-hour interval of the Tr phase (Days 21-23). Consolidated behavioural scores of the Te phase showed that oral microbial infusions had a varying range of memory impairment in EGs 2-5 during the memory retrieval in FPBA compared to EG-1 (Figure 5). The results showed that EG 3, 2, 4, and 5 gradually developed memory impairment compared to the non-infusive group (EG – 1). Observed results proved that pathogens like *P. aeruginosa* and *A. hydrophila* play a significant role in developing cognitive memory impairment through gut dysbiosis compared to commensal microorganisms like *E. coli*. The outcome of the FPBA showed that oral microbial infusions play a major role in the development of cognitive memory impairment compared to the impact of memory formation on non-infusive EG (Figure 6).

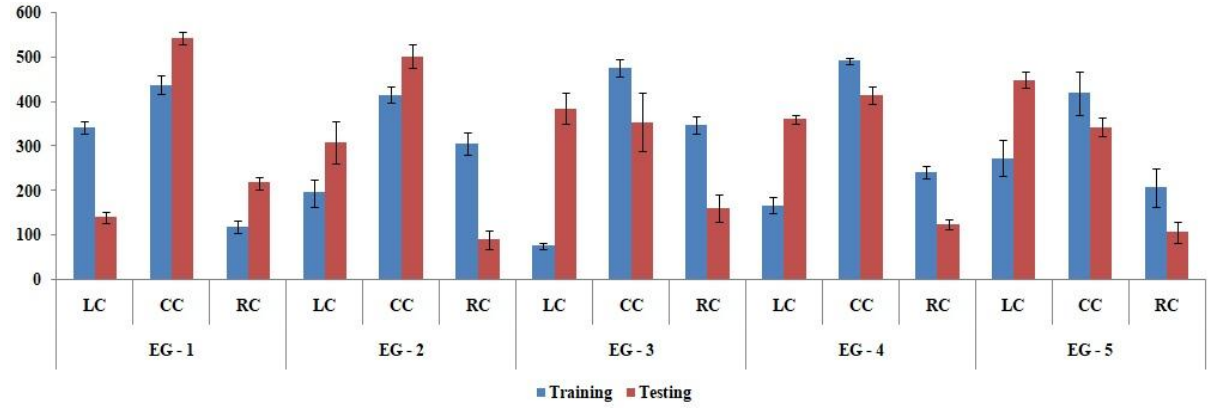


Figure 6 Comparative analysis of the first phase of behavioural training and testing proved that microbial oral infusions had a greater impairment on memory retrieval compared to the training scores [left chamber (LC), central chamber (CC), and right chamber (RC)]. SEM value was represented in the bar diagram

### 3.1.2 Impact of Oral Microbial Infusions on Stress Formation

Before SPBA, experimental animals were allowed to perform the predator exposure test (PET), and open field test (OFT) to identify the presence/absence of fear memory formation/stress among the infused and non-infused EGs. Initially, PET was performed to determine the amount of fear memory developed in EGs after FPBA. PET Scores showed that fear memory development was not induced in the infusive groups (in both infusions, i.e., pure and mixed form) compared to the non-infusive group. Behavioural responses of the infused EGs (EG – 2, 3, 4) showed that oral microbial infusions of *P. aeruginosa*, *E. coli*, and *A. hydrophila* will develop the least amount of fear memory formation compared to the non-infused EG (EG – 1). However, mixed cultures of *P. aeruginosa*, *E. coli*, and *A. hydrophila* cause higher levels of memory of fear in the EG – 5 (Figure 7). Following PET, OFT was performed to identify the effect of microbial oral infusions on motor behaviour and stress development in the experimental animals. Behavioural responses showed that microbial oral infusions do not impact the motor behaviour of the EGs. Still, it showed the development of anxiety-like behaviour in the infused group (EG - 2, 3, 4, and 5) compared to the non-infusive experimental group (EG -1). Obtained results showed that microbial oral infusions might play a major role in the development of anxiety-like behaviour due to oral/gut dysbiosis. They also proved that experimental animals were physiologically active and could explore the provided stimuli. Behavioural scores of the infusive EGs (EG – 2, 3, 4, and 5) in pure and mixed forms showed a higher amount of time spent in the outer compartment, which shows the development of anxiety-like behaviour compared to the non-infusive group (EG -1). Responses of EG -1 (non-infusive group) showed a higher amount of time spent in the inner compartment, which proves the absence of anxiety-like behaviour development. The observed pattern of the non-infusive group (EG

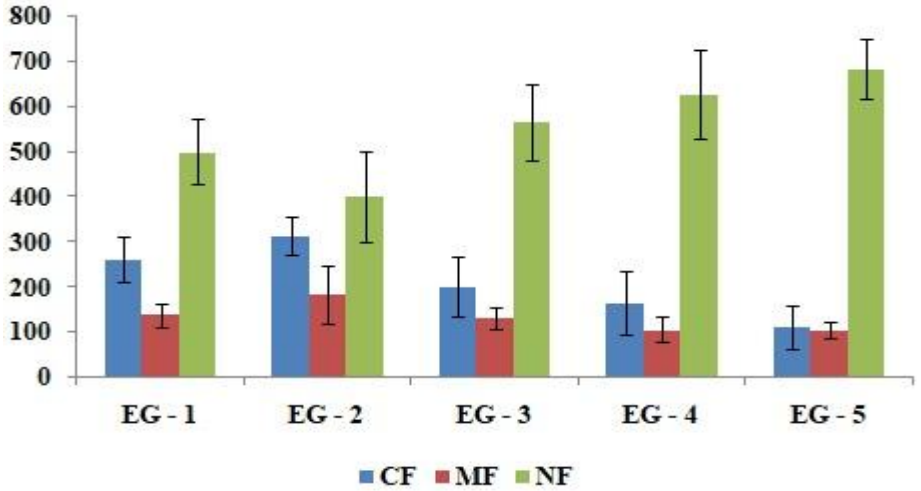


Figure 7 Behavioural scores of the predator exposure test showed that microbial oral infusions do not show any fear memory development (No fear (NF) zone) compared to mid fear (MF) zone, and complete fear (CF) zone. The represented bar diagram was created using the average mean value and standard error value presented in the form of a pin

– 1) was vice-versa in the infusive groups (EG – 2, 3, 4, and 5). The outcome results of the OFT stated that microbial oral infusions induce anxiety-like behaviour in the infused group as a result of dysbiosed oral and gut microbiota. Dysbiosed oral and gut microbiota are further involved in the production of the stress hormone cortisol in the host, which may affect cognitive memory retrieval (Figure 8).

### 3.2 Impact of probiotic strain *L. plantarum* on the reversal of cognitive impairment

Following FPBA, SPBA was performed to understand the impact of probiotic strain (*L. plantarum*) on the reversal of oral/gut dysbiosis-induced LTM impairment.

#### 3.2.1 Reversal of Induced Cognitive Impairment by the Reversal of Dysbiosed Microbiota

After completion of OFT and PET, the Tr and Te phase was carried out in the SPBA to test the effect of probiotic strain on the reversal of cognitive impairment. After FPBA, probiotic oral infusions were given to all EGs (EGs 2, 3, 4, and 5) in pure and mixed form. After probiotic oral infusions, a three-day time interval (28-30 days) was given to all EGs. Following a time interval, the SPBA Tr phase was carried out between days 31-33 to identify the impact of probiotic oral infusion (POI) on information acquisition. Behavioural scores of the Tr phase showed that POI enhanced higher learning abilities in EGs – 2, 3, and 4 compared to EG -5 (Figure 9). Observed results also showed that POI may

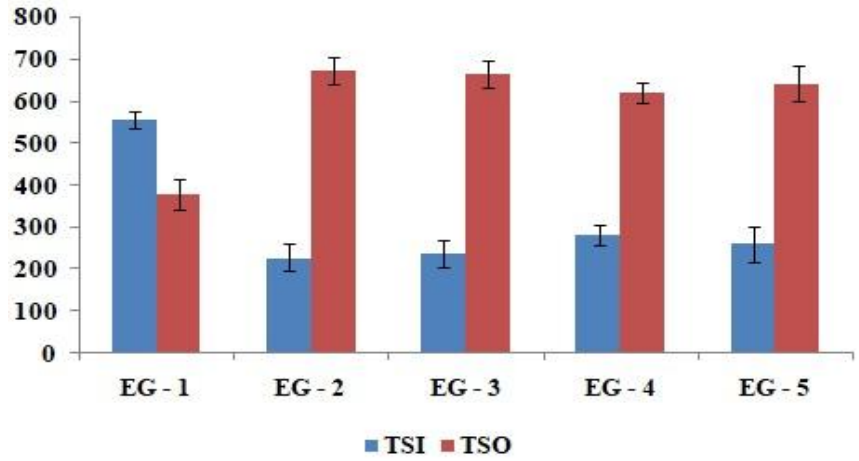


Figure 8 Behavioural responses of open field test (OFT) showed the effect of oral microbial infusions in the development of anxiety-like behaviour Represented bar diagram showing the SEM value in the form of a pin on bars (TSI – time spent in the inner compartment; TSO - time spent in the outer compartment)

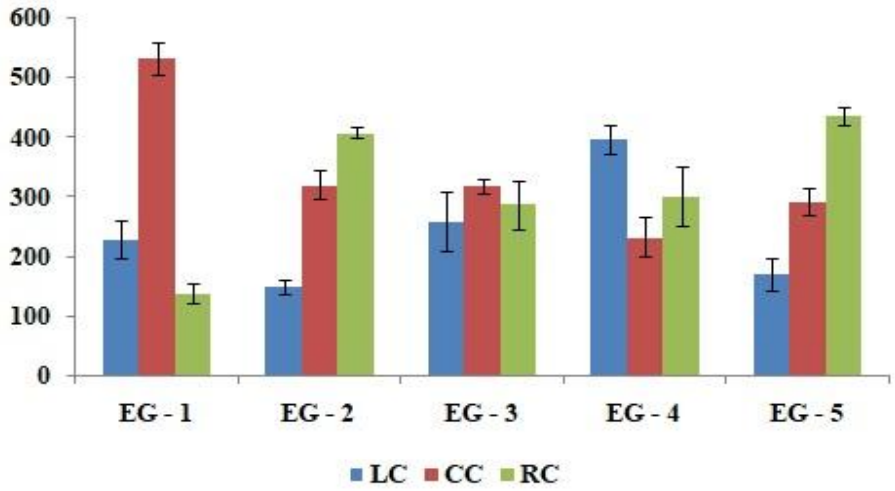


Figure 9 Behavioural responses of second phase training showed that probiotic oral infusions may take part in the reversal of oral/gut dysbiosis through the restoration of dysbiosed microbiota in experimental groups – 2, 3, 4, and 5 compared to the experimental group - 1. [left chamber (LC), central chamber (CC), and right chamber (RC)]. SEM value was represented in the bar diagram.

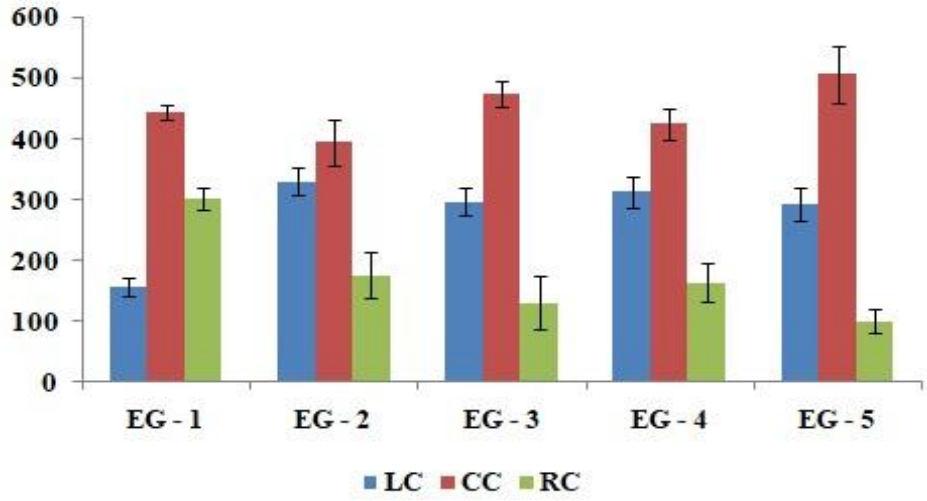


Figure 10 Behavioural responses of second phase testing proved that probiotic oral infusions completely restored dysbiosed microbiota in infused experimental groups – 2, 3, 4, and 5 compared to non-infused experimental group - 1. [left chamber (LC), central chamber (CC), and right chamber (RC)]. SEM value was represented in the bar diagram

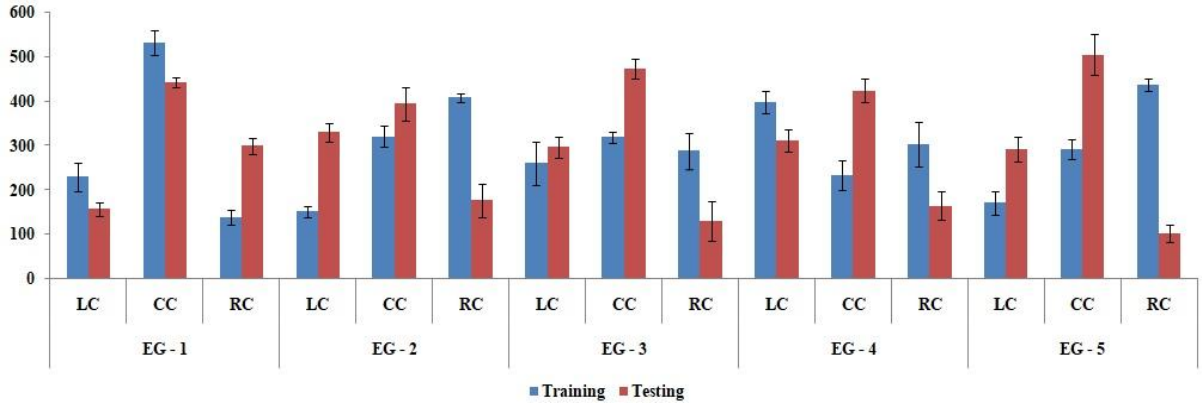


Figure 11 Comparative analysis of the second phase of behavioural training and testing showed that infused experimental groups able to retrieve learned information after receiving probiotic oral infusions. [left chamber (LC), central chamber (CC), and right chamber (RC)]

reverse the dysbiosis-induced production of neurotransmitter precursor compounds in the gut, which results in its increased transmission to the central nervous system (CNS). After three days (Days 34-36), the Te phase of SPBA was carried out between 37-39 days. Behavioural responses of the Te phase proved that POI reversed the cognitive impairment in an increased manner in EG – 2, 3, 4, and 5 compared to the control group EG – 1 (Figure 10). Comparative analysis of FPBA and SPBA showed that microbial infusion of the oral pathogen plays a major role in the formation of cognitive impairment through the aberration of beneficial microbiota in the oral cavity/gut. This dysbiosed oral/gut microbiota may play a major role in the development of cognitive impairment. The formed cognitive impairment in FPBA was reversed with the help of probiotic microorganisms used in SPBA as an oral mixture. Reversal of cognitive impairment may result from restoration of normal oral/gut microbiota or reduction of pathogenic load in the oral cavity/gut (Figure 11).

## 4 Discussion

The present study identified the impact of pathogenic microbial colonization in the development of impaired cognitive memory formation through the microbiome-gut-brain (MGB) axis. This MGB plays a major role in the development of cognitive health. Recent studies have shown that gut microbial dysbiosis may play a major role in the development of neurodegenerative disorders (ND) like Alzheimer's disease (AD), Parkinson's disease (PD), mild cognitive impairment (MCI), etc (Dai et al. 2022; Zhang et al. 2022; Krishaa et al. 2023; Mukilan 2023). In ND, gut dysbiosis (GD) may take part in various dysfunctions like decreased production of neurotransmitters, increased intestinal permeability, development of brain lesions/neuroinflammation, and oxidative stress (Ma et al. 2019; Chidambaram et al. 2022; Hou et al. 2022; Intili et al. 2023; Varesi et al. 2023). These conditions may play a major role in developing imbalances in the brain homeostasis

mechanism (BHM). Imbalances in BHM may result in developing brain cognitive dysfunctions associated with LTM formation. This GD was also progressed by various conditions like poor diet, poor oral hygiene, matrix metalloproteinase expressions, infections, and antibiotic treatment (Chung and Chan 2023; Gu et al. 2023; Kulkarni et al. 2023; Pitchaikani et al. 2024). Among others, poor oral hygiene plays a major in the formation of cognitive impairment during the period of childhood and adolescence. This poor oral hygiene was caused by the attachment and colonization of unwanted pathogenic microorganisms in the oral cavity (OC). Colonization of these microorganisms in OC results in the development of tooth decay, periodontal diseases, and tooth loss. Dysbiosis in oral flora further transports highly colonized pathogens to the gut through the mucosal lining (Mukilan 2023; Wei et al. 2023; Pruntel et al. 2024). As a result, disruption of gut microflora takes place in the gut, showing the least dysbiosed amount of beneficial flora. This dysbiosis initially results in intestinal inflammatory disorders (IID) development. Further, this IID results in increased intestinal permeability, which results in the least transmission of neurotransmitter precursor compounds from the gut to the brain through the blood-brain barrier (BBB) (Ma et al. 2019; Rutsch et al. 2020; Kandpal et al. 2022; Santana et al. 2022).

Besides BBB, the enteric nervous system (ENS) also plays a major role in the development of LTM due to its interconnection with the central nervous system (CNS). In a healthy state, ENS transports secreted NPC from the gut to the brain for the synthesis of neurotransmitters in presynaptic neurons, followed by its release into the synaptic cleft (SC) (Martande et al. 2020; Fleming II et al. 2020; Orr et al. 2020; Dicks 2022). Once released into SC, it binds with postsynaptic neuronal receptors and activates neuronal molecules involved in the extracellular regulated kinase (ERK) - cAMP response element binding protein (CREB) signaling pathway in the brain. This ERK-CREB signaling pathway uses adenyl cyclase (AC), protein kinase A (PKA), immediate early genes (IEGs – *Egr-1*, *C-fos*, and *C-jun*), and postsynaptic density proteins (PSDs like PSD-95) for the formation of LTM (Mukilan et al. 2018a, b; Sivasangari and Rajan 2020; Rajan 2021; Mukilan 2022; Mukilan 2023). In the present work, we tried to elucidate the impact of a diseased/dysbiosed state on LTM formation with the help of oral microbial infusions. Our results showed that oral microbial infusions have a more significant effect on the development of cognitive decline by down-regulating the expression of neuronal signaling molecules involved in the ERK-CREB signaling pathway (Mukilan 2023). Our data also showed that oral microbial infusion may have a greater impact on the development of oral/gut dysbiosis through the secretion of LPS, bacterial toxins, and antibiotics over the beneficial flora. Dysregulated oral/gut flora may result in the decreased secretion and transportation of NPC from the gut to the presynaptic neuron through the BBB and ENS. As a result, impaired LTM was formed in the brain regions. In this study, impaired LTM was also reversed with the help of the probiotic strain *L. plantarum*.

**Conclusion**

The outcome of the present study showed the role of *P. aeruginosa*, *E. coli*, and *A. hydrophila* microbial infusions on oral/gut dysbiosis and also results in impaired LTM formation. Further, this study also proved that microbial colonization in the oral cavity/gut may result in the aberration of normal beneficial flora in those regions. Experimental results showed that the entry of pathogens into the oral passage through poor oral hygiene may play an important role in developing imbalanced oral/gut microbiota. Further, it results in impaired LTM formation validated by FPBA. FPBA results showed a higher level of impairment in the EGs who received oral infusions of *P. aeruginosa* and *A. hydrophila* than in the EGs who received oral infusions of *E. coli*. It also proved that cognitive decline is higher in the EG who received mixed oral mixture than other EGs (received pure oral mixture). Thus, FPBA elucidated that all three isolates showed a high level of impaired memory formation in its complex form. Later on, impaired LTM was reversed with the help of probiotic strain in SPBA. SPBA results showed that cognitive reversal was high in EGs (received pure oral infusions) and low in EGs received complex mixtures. Comparative behavioural analysis proved that induced oral/gut dysbiosis may reversed with the help of probiotics in this pioneering study. Thus, the present research findings support the use of traditional fermented foods in treating memory loss or cognitive reversal in ND.

**Authors Contributions**

MM performed the conceptualization, research design, funding acquisition, original investigation, draft preparation, review, and manuscript editing. VE, MV, and MD performed the experiments and data collection.

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**Conflict of Interest**

Authors report no conflicts of interest in this work

**Data Availability**

Research data is available with the authors and shall be provided upon request.

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