Probiotic Strain *Lactobacillus fermentum* as a Potential Agent for the Reversal of Non-Periodontal Microorganisms Induced Cognitive Dysfunctions

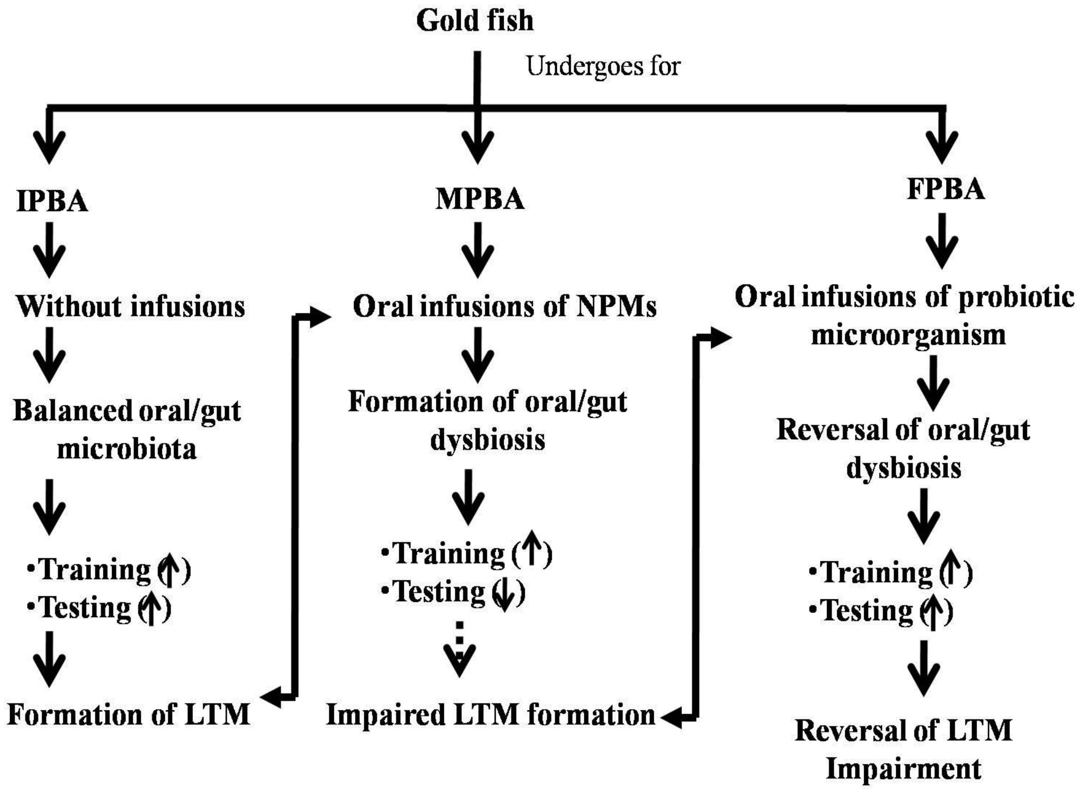
Murugan Mukilan
Department of Biotechnology, Sri Ramakrishna College of Arts & Science, Coimbatore 641 006, Tamil Nadu, INDIA
mukilan@srcas.ac.in

Abstract

The gastrointestinal tract of mammals consists of trillions of microorganisms including beneficial and non-beneficial floras termed as gut microflora. These beneficial gut microfloras are responsible for maintaining homeostasis mechanism of physiological functions including cognitive health. However, disturbance in this gut microflora results in the formation of gut dysbiosis. Gut dysbiosis results in the reduced synthesis and transport of neurotransmitter precursor compounds from the gut to the brain which causes impairment in the synthesis of neurotransmitters. The reduced synthesis of neurotransmitters further results in impaired cognitive memory formation. Formed cognitive impairment was reversed with the help of probiotic microorganisms. The present study explored the positive role of the probiotic strain Lactobacillus fermentum in the reversal of non-periodontal microorganisms (NPMs) induced cognitive impairment.

For identifying the effect of L. fermentum on the reversal of cognitive impairment, a three-phased behavioral analysis was used in the study. Behavioral studies were carried out for all experimental groups in different scenarios including a serene habituated environment with NPMs infusions and with L. fermentum infusions. Behavioral responses showed that a stress-free habituated environment plays a major role in the development of cognitive memory during the initial phase of behavioral analysis (IPBA). In mid phase of behavioral analysis (MPBA), IPBA-formed cognitive memory was impaired by the infusions of NPMs. Collective behavioral analysis proved that probiotic oral infusions in final phase of behavioral analysis (FPBA) played a major role in the reversal of induced cognitive impairment in experimental groups that received oral infusions of NPMs.

Keywords: *Lactobacillus fermentum*, Learning, Memory, *Pseudomonas aeruginosa, Streptococcus pneumonia, Staphylococcus aureus.*



Graphical Abstract

Introduction

Intestinal microbiota (IM) confers a huge number of benefits including gut protection, nutrient and energy absorption and prevention of viral disease progression. This IM consists of beneficial and pathogenic flora in a balanced state. In a healthy state, this IM is able to influence gut health, inflammation, cognition and disease progression through the regulation of host homeostasis mechanism[17,19,25,31,40]. In the regulation of gut homeostasis, beneficial flora (probiotics) plays an unavoidable role in the maintenance of gut health and secretion of neurotransmitter precursor compounds (NPCs). These secreted NPCs are further transmitted to the brain through the blood-brain barrier (BBB). Further, NPC transmission results in the formation of bi-directional communication between the gut and the brain which results in the development of microbiome-gut-brain (MGB) axis[2,9,32,51,53]. The formed MGB axis plays a major role in the development of cognitive functions through the process of learning and memory formation (LMF). This LMF is a result of the restructuring of existing neuronal connections present within the brain through the formation of brain plasticity changes[10,20,33,39,45].

Formed plasticity changes are shown in the form of information acquaintance and its storage in different brain regions including the olfactory bulb, hippocampus and amygdala. The information is for exposure to a repeated stimulus for a limited time, which stores the learned information in the form of short-term memory (STM) and long-term memory (LTM)[3,30,34]. This STM and LTM formation uses two different strategies for information storage and retrieval. Both differ from each other in using already existing protein molecules and RNA-dependent protein synthesis machinery for memory formation. Formed STM lasts for less than 48 hours compared to LTM which lasts till the death of the organisms. Thus the formed LTM plays a major role in the development of proper cognitive health with the help of MGB axis[11,13,28,34].

During the process of LTM formation, gut microbiota produced NPCs were transported to the brain in the form of metabolites from the enteric nervous system (ENS). Transported NPCs further reach the brain through the BBB. Once reaching the presynaptic neurons in the brain, NPCs result in the production and release of neurotransmitters into the synaptic cleft[1,22,48]. After release, neurotransmitters bind with the specific post-synaptic neuronal receptors which result in increased calcium influx. Increased calcium influx later on results in the activation and phosphorylation of neuronal molecules cyclic adenosine monophosphate (cAMP), protein kinase A (PKA) and cAMP response element binding protein (CREB)] involved in the activation of immediate early genes (IEGs) like early growth response gene -1 (*Egr-1*), *C-fos* and *C-jun*.

Activation of IEGs inhibits the stimulation of the negative regulator (MicroRNA-148a) involved in cognitive memory formation which also results in the initiation of post-synaptic density protein production for the development of LTM[15,16,30,35-38,44]. Thus the formed LTM depends on the MGB axis for its initial induction in a healthy/normal condition. During pathogenic colonization, imbalance in the GM results in the least production and transportation of NPCs to the brain which may act as an inducer for impaired cognitive memory formation[1,22,34,48]. The present study tried to elucidate the impact of the probiotic microorganism *Lactobacillus fermentum* in the reversal of NPM induced cognitive memory impairment.

Material and Methods

Study Animals: Commercial adult naïve goldfish (*Carassius auratus*) were purchased from the local aquarium in Coimbatore, Tamil Nadu, India. Fishes were immediately transferred to laboratory conditions in a stress-free environment after purchase with an ambient temperature and dissolved oxygen content. Once reaching the laboratory, animals were transferred to a rectangular glass tank (RGT) of 42 X 30 X 21 inches (Length, Breadth and Height) in the laboratory aquarium. RGT has a controlled temperature (26 ± 2º C), light and dark cycle (12:12 hours) and also with continuous air circulation (24 X 7/day). All fish were fed with dry round food pellets (Taiyo Pet Products India Private Ltd., India) thrice a day during the time intervals of 9.00, 14.00 and 18.00 hours to meet their energy needs.

Throughout the study, water was changed on alternative days to maintain a debris/dust-free environment in the home and experimental tanks. The purchased animals were maintained in this setup for seven days for their adaptation to the laboratory conditions. Once the adaptation was over, body length and body weight were measured for all experimental fishes (EFs). EFs having a body weight and body length of 7.5-9 cm and 6-15 g were selected for forming the study groups (n = 6/group). During the experiment, behavioral work flow followed the institutional ethical guidelines of Sri Ramakrishna Institutions, Coimbatore[31].

Behavioral Study Apparatus: Formed study groups (EGs) received a reward-based learning paradigm (RBLP) with the help of an experimental rectangular glass tank (ERGT) having a length, breadth and height of 42 X 30 X 21 inches. This ERGT consists of three different areas/chambers including two feeding chambers (FCs) and one central chamber (CC). These two FCs were acting as positive and negative chambers with a color cue. The positive chamber contains a blue color cue (with a food reward) and the negative chamber contains a red color cue (without a food reward). A designed behavioral study apparatus was used in the three different phases of the behavioral analysis[34].

Strains used and its Purity Confirmation: Three isolates (*Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Staphylococcus aureus*) were availed from the PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India. Probiotic strain (*Lactobacillus fermentum*) was

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acquired from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, Punjab, India. Acquired isolates and probiotic strain were quadrant streaked on nutrient agar and *Lactobacillus* MRS agar plates for their purity confirmation and individual colony identification. Obtained individual colonies from quadrant plates were used for the arousal of overnight cultures. The grown overnight culture was used for the preparation of an oral infusion mixture along with phosphate buffer saline (PBS) in a ratio of 50:50[34].

Behavioral Study: Behavioral analysis was carried out for four different study groups (EGs) in all three different stages (without infusions, with NPM infusions and with probiotic infusions). Differentiated study groups are study group (EG) - 1, EG - 2, EG - 3 and EG - 4. The experimental time line is shown in figure 1.

Initial Phase of Behavioral Analysis: In the initial phase of behavioral analysis (IPBA), behavioral studies were carried out in a habituated serene environment with the help of ERGT. IPBA was carried out to study the impact of a stress-free environment without any oral infusions for all EGs (EGs – 1, 2, 3 and 4). IPBA is carried out with three different behavioral parameters (exploration, training and testing).

Mid Phase of Behavioral Analysis: After completion of the IPBA, mid-phase behavioral analysis (MPBA) was carried out on the same experimental set-up after the oral infusions of NPM. All four EGs used in this MPBA are EG -1 (control without infusion), EG - 2 (infused with *P. aeruginosa*), EG - 3 (infused with *S. pneumonia*) and EG - 4 (infused with *S. aureus*). After infusions, 3 days (72 hours) time interval was given for the infused group for the settlement of NPM in the gut, which was followed by training and testing behavioral parameters in the experimental setup.

Final Phase of Behavioral Analysis: The final phase of behavioral analysis (FPBA) was carried out for all four EGs after the completion of MPBA. After MPBA, EG -2, EG -3 and EG - 4 received oral infusions of *L. fermentum* except EG - 1. FPBA was performed to identify the reversal of NPM-induced cognitive impairment with the help of a probiotic strain *L. fermentum*. In FPBA, behavioral responses were calculated based on the training and testing parameters after 3 days of probiotic oral infusions.

Open Field Test: Open field test (OFT) was used in this study to know the effect of NPM/probiotic oral infusions on the presence/absence of anxiety-like behavior. OFT was performed in the rectangular glass tank (RGT) with a size of 42 X 30 X 21 (Length, Breadth and Height) inches. The bottom of the RGT was separated into 36 boxes (10 X 5 cm/each) with the help of box diagrams. OFT was done after the completion of MPBA and FPBA.

Statistical Representation: Behavioral scores of all three behavioral paradigms (IPBA, MPBA and FPBA) were used for the preparation of the bar diagram using the Microsoft Excel Program.

Results

The present study uses three different behavioral paradigms (IPBA, MPBA and FPBA) to understand the effect of residential microflora (RM), RM dysbiosis and reversal of RM dysbiosis on cognitive memory formation.

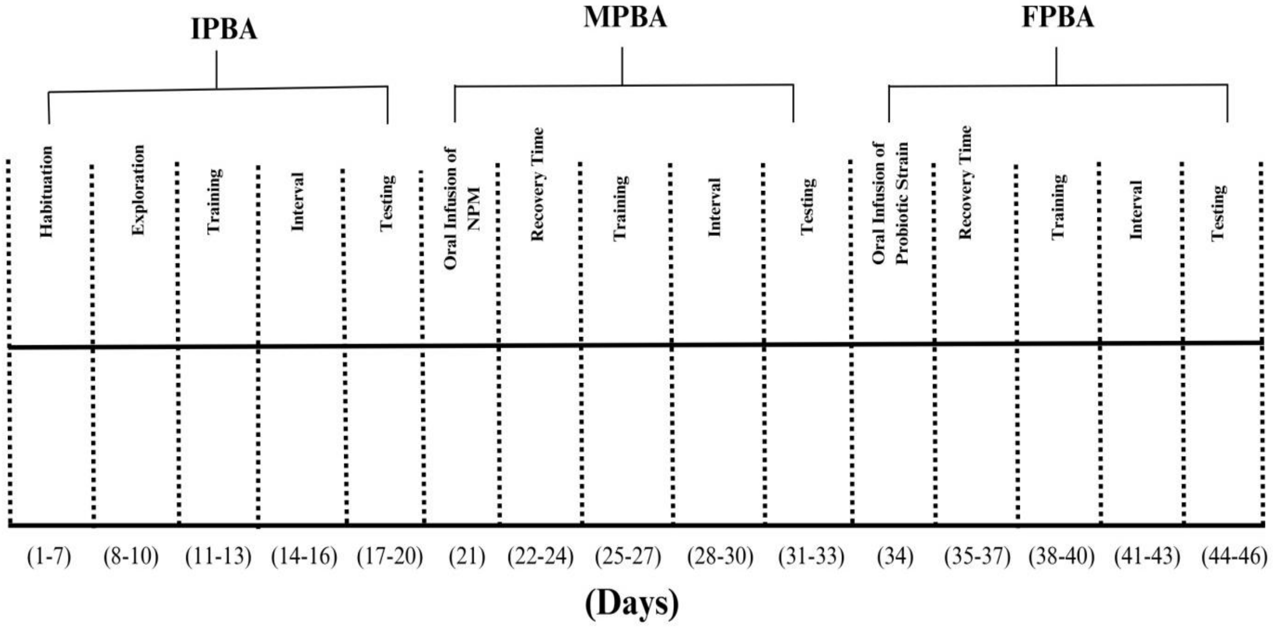

Figure 1: Line diagram showing the experimental time line of the behavioral studies carried out in the present study [IPBA - initial phase of behavioral analysis; MPBA - mid phase of behavioral analysis; FPBA - final phase of behavioral analysis]

Role of Oral/Gut Residential Microflora on the Development of Cognitive Memory: In the IPBA, experimental animals of all four EGs including EGs - 1- 4 were allowed to perform the three different behavioral phases (exploration, training and testing) between days 8 - 20. Before IPBA, all EGs underwent for habituation process for seven days in the laboratory aquarium. The habituation process acts as a preparatory phase for the adaptation of all EGs to the laboratory conditions in a stress-free manner. Followed by the habituation process, IPBA starts with the exploration task. During exploration, each EG was allowed to expose the ERGT for 15 minutes on three consecutive days with all experimental chambers (CC and FCs). In this stage, FCs presented without any color cues.

Behavioral responses of the exploration phase showed that EGs actively explored all three chambers of ERGT and spent more amount of time in CC compared to the other two compartments (LC and RC). The amount of time spent in CC was gradually reduced on days 9-10 compared to the initial day (Day 8). The outcome of the exploratory phase proved that all experimental animals explored the experimental set-up in a stress-free and active manner during the provided times (Fig. 2).

Following the exploration phase, the training phase was carried out for all EGs during days 11-13. During the training phase, initially on day 11, more amount of time was spent on CC compared to the LC and RC by the experimental animals. Only a very few experimental animals entered the positive chamber (RC) and got the reward. On days – 12 and 13, the amount of time spent in CC was gradually reduced and the number of entries to RC and LC was increased gradually. Increased entry of LC also showed the animal's ability to learn the negative stimuli which are followed by grasping the food reward by learning the positive color cue (stimuli) in the ERGT.

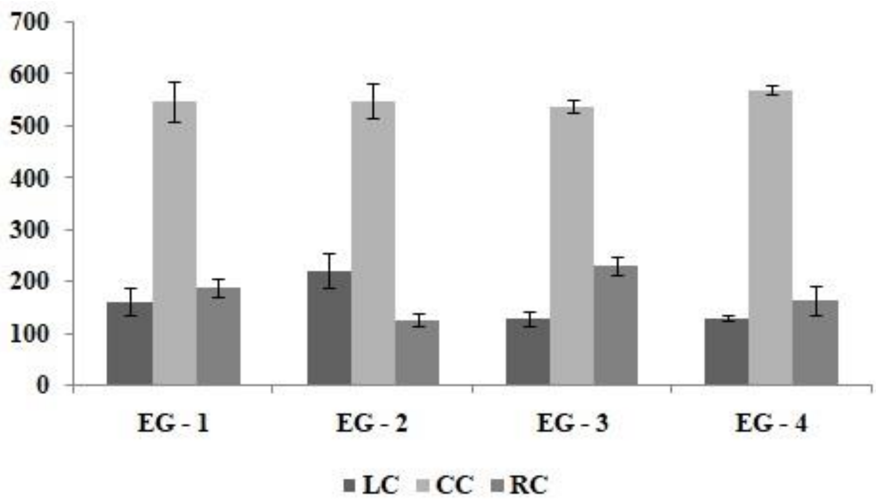

Figure 2: Behavioral scores of the exploration phase showed that all experimental animals were active and explored all three chambers of the experimental setup in a gradual manner

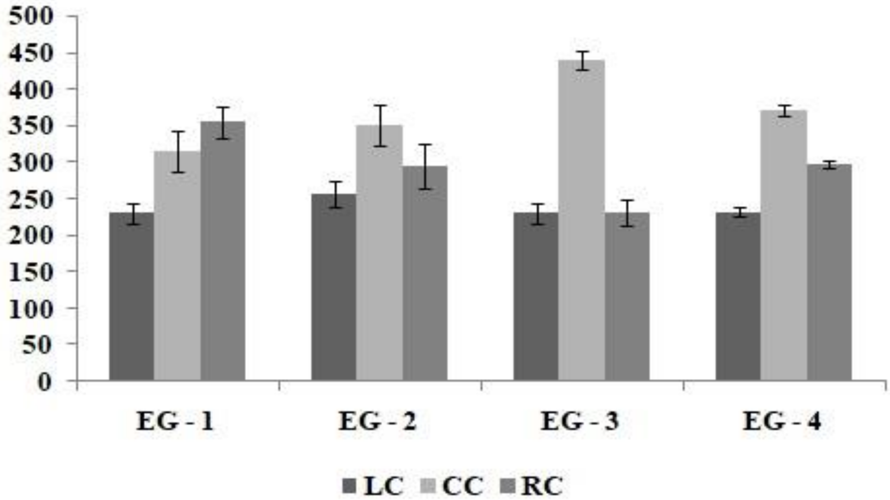

Figure 3: Behavioral scores of the IPBA training phase showed the number of attempts to the positive and negative chambers. Obtained behavioral responses showed that the number of attempts to the positive reward chamber (right chamber) was increased in the consecutive days compared to the negative reward chamber (left chamber)

Behavioral scores of the training phase showed that all EGs tried to enhance their learning eligibilities through exploring the positive color cues with the reward of food pellet and also proved that negative color cues may also act as an inducer for learning positive stimuli with a food reward (Fig. 3). At the end of the training phase, three days time interval (Days 14 – 17) was given for all EGs for the storage of learned information within the brain regions. This time interval may also result in the structural reformation of synaptic plasticity changes in the post-synaptic neurons.

The testing phase (Days 17-20) was carried out after the completion of 72 hours (3 days) of memory consolidation. The testing phase shows the level of information acquaintance that happened in the EGs during and after the training phase. Behavioral responses of EGs showed that information retrieval was high in all EGs (EGs – 1, 2, 3 and 4) as a result of memory storage in the brain. It also proved that the number of entries to the positive chamber was high due to formed plasticity changes in the different brain regions (Fig. 4).

Comparative analysis of IPBA training and testing phases showed that experimental animals learnt about the food reward associated with the positive stimuli during the training phase and stored information was used for the retrieval of learned information in a short period during the testing phase. It also showed that the number of attempts to RC was high during the process of testing compared to the training phase. During the testing phase, more amount of time was spent in RC compared to LC and CC which showed the formation of cognitive memory in a stress-free habituated environment with the help of native microflora present in the oral cavity/gut (Fig. 5).

Effect of NPM Oral Microbial Infusions on the Development of Impaired Cognitive Memory Formation: Following IPBA analysis, the purity of the obtained three NPMs (*P. aeruginosa*, *S. pneumonia*, *S. aureus*) was confirmed with the help of simple and quadrant streak methods. After purity confirmation, individual colonies were taken from the quadrant nutrient agar plates and were used for the preparation of overnight culture with the specified volume of 2 ml (Fig. 6).

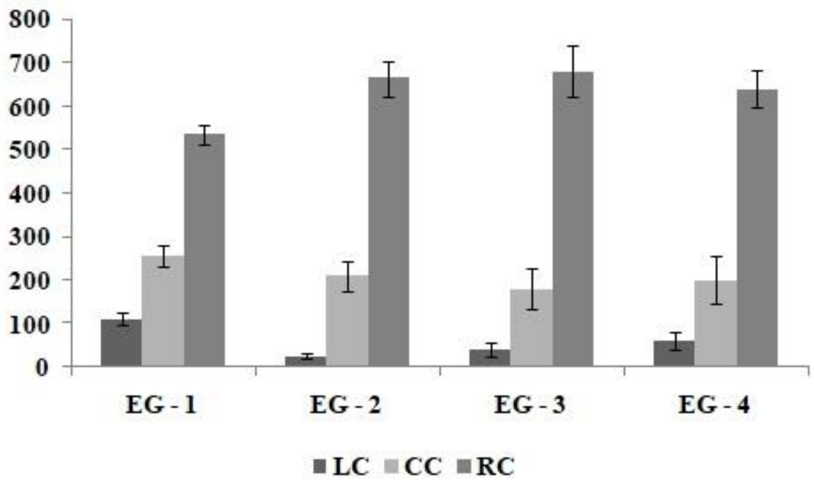

Figure 4: Behavioral scores of the IPBA testing phase showed the retrieval of learned information in an increased manner compared to the IPBA training phase. Obtained behavioral responses showed that the number of attempts to the positive reward chamber (right chamber) was increased compared to the testing score of IPBA

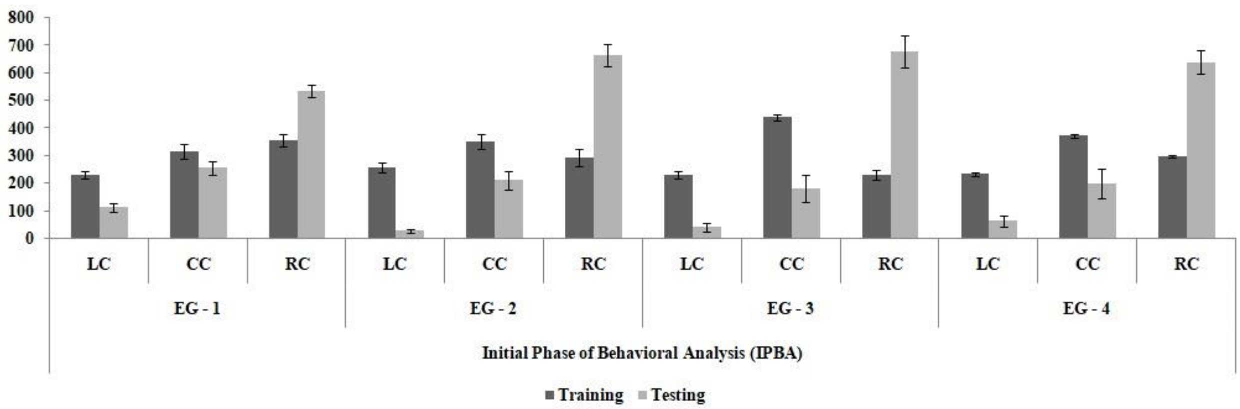

Figure 5: Comparative analysis of IPBA training and testing phases showed that experimental animals learned about the food reward associated with the positive stimuli during the training phase and retrieved stored information within a short time during the testing phase

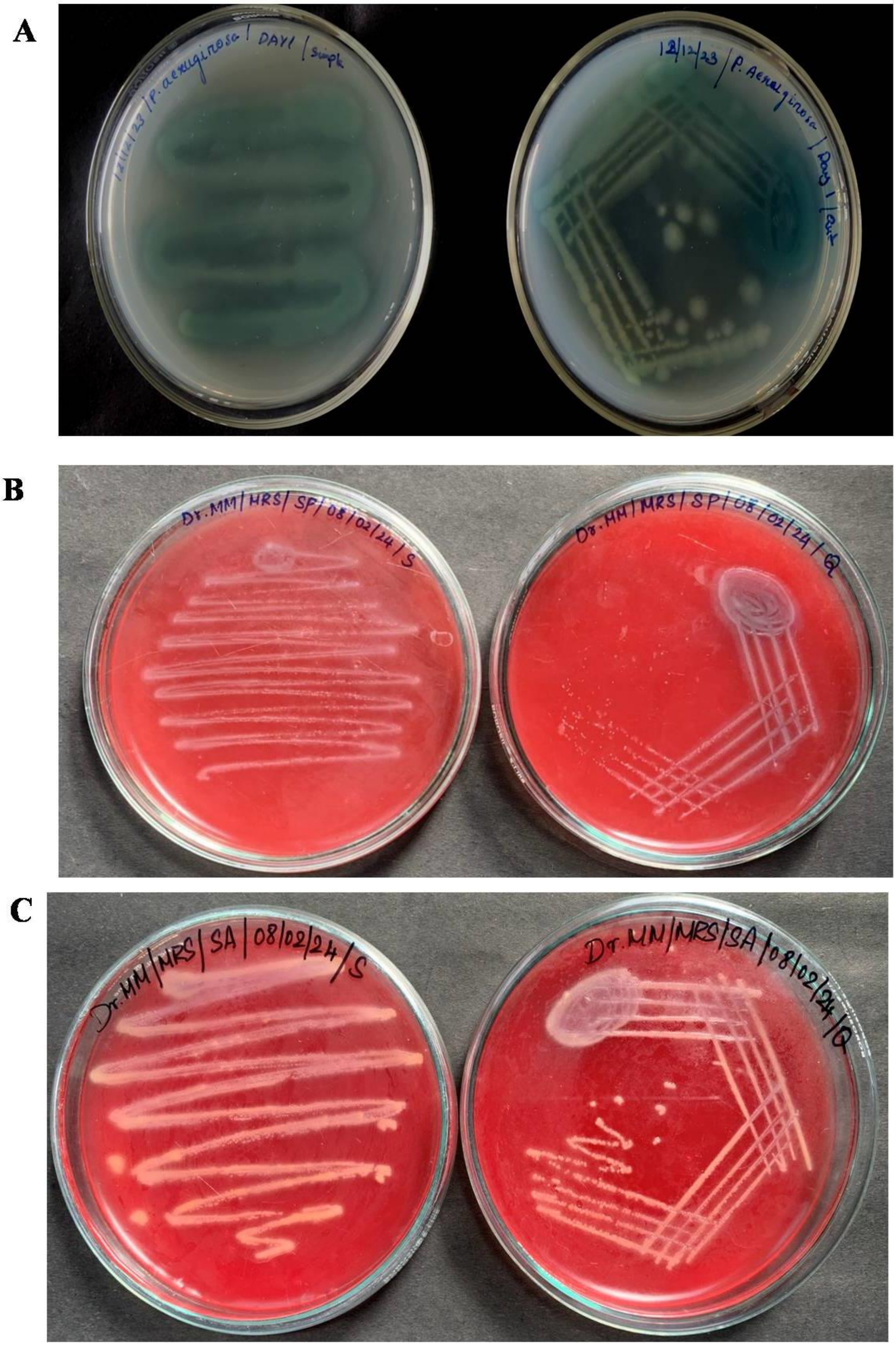

Figure 6: Representative plate pictures showing the purity of the obtained non-periodontal cultures (*P. aeruginosa, S. pneumonia, S. aureus*) with the help of simple and quadrant streak methods

Prepared overnight culture was used for the preparation of oral infusion mixture (OIM) in the ratio of 50:50 along with PBS. Prepared oral microbial infusion mixture was infused into their respective EGs with the help of an oral gauge. Prepared OMI was infused on day 21 to the EG – 2, 3 and 4 provided with 72 hours (days 22-24) of time interval for the transportation of OIM to the gut.

MPBA was performed after oral microbial infusions of NPM during days 25 – 33 in the ERGT with the help of positive and negative color cues. In MPBA, two different behavioral tasks (training and testing) were performed by all EGs to study the effect of NPM oral microbial infusions on cognitive memory formation. All experimental animals were allowed to perform MPBA training between days 25 - 27. Behavioral scores of the MPBA training phase showed that OMI did not show any imbalance in the learning abilities of EGs compared to the training phase of IPBA (Fig. 7). Obtained results also showed that OMI does not have any impact during the process of MPBA training. Followed by MPBA training, the MPBA testing phase was carried out after three days interval on days 31-33.

Behavioral responses of the MPBA testing phase showed that OMI had an impact on the retrieval of learned information from the formed plasticity changes due to oral/gut dysbiosis. Behavioral scores validated that most of the infused EGs showed a decreased number of correct choices compared to IPBA training. Thus, the obtained results proved that OMI showed a greater level of memory decline during the testing phase of MPBA (Fig. 8). Comparative analysis of the training and testing phases of MPBA showed that NPM oral microbial infusions were not involved in the information acquaintance/destruction of brain homeostasis mechanism in the experimental animals but they had a greater impact on memory retrieval in infused EGs (Fig. 9).

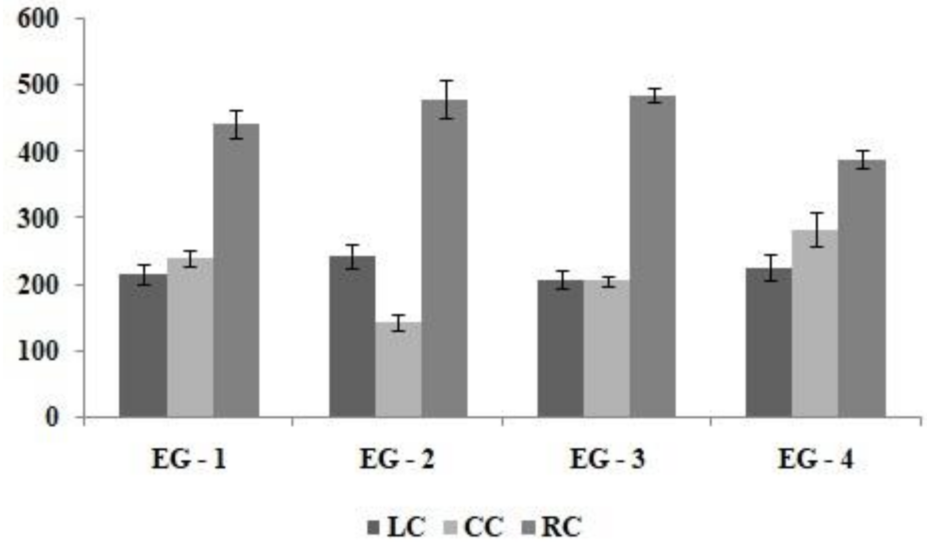

Figure 7: Behavioral scores of the MPBA training phase showed the learning ability was not hindered after receiving NPM oral microbial infusions. Obtained behavioral responses showed that the number of attempts to the positive reward chamber (right chamber [RC]) was high compared to the number of entries to the central chamber (CC) and left chamber (LC)

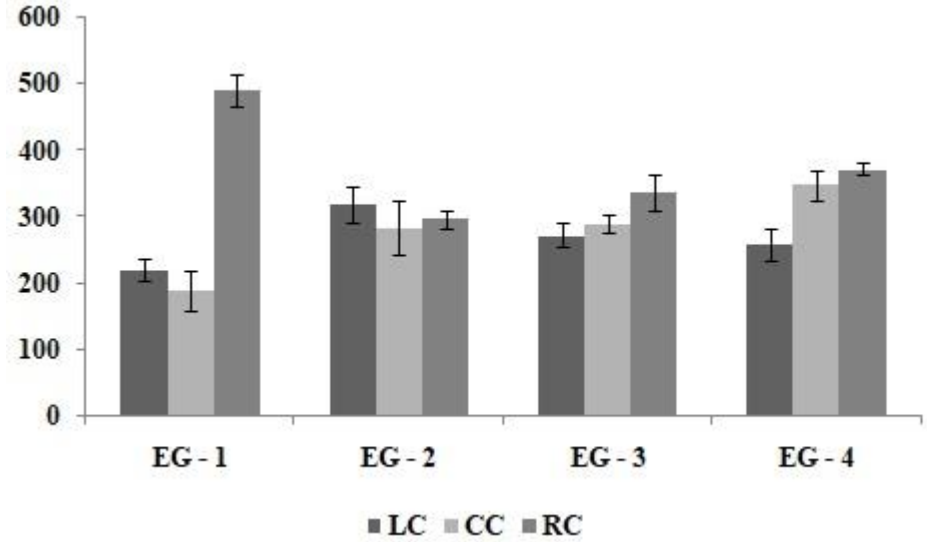

Figure 8: Behavioral responses of the MPBA testing phase showed impaired information retrieval in the NPM-infused group compared to the control (EG – 1). Obtained behavioral scores showed that the number of attempts to the positive reward chamber (right chamber [RC]) is reduced in infused groups (EGs – 2, 3 and 4) compared to the number of correct entries in the EG – 1.

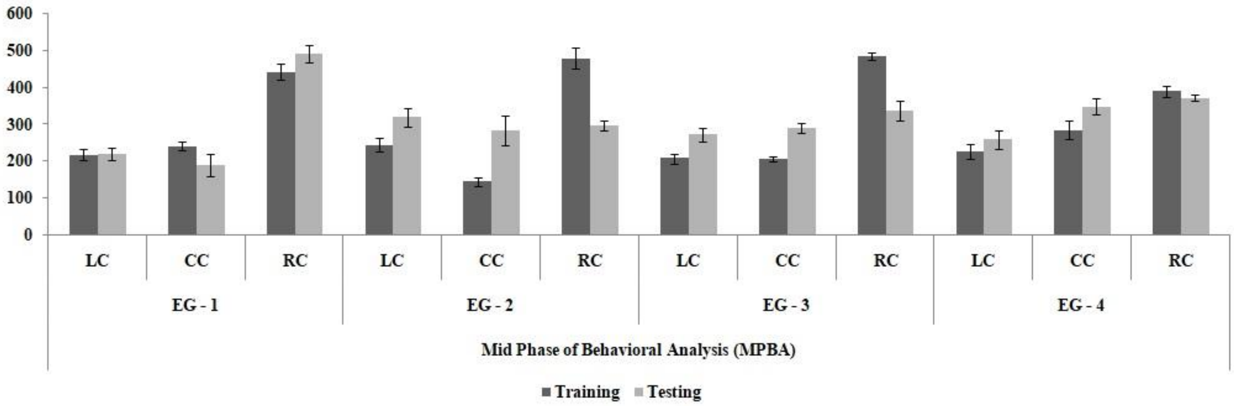

Figure 9: Comparative analysis of training and testing phases of the MPBA, showed that the NPM oral microbial infusions were not involved in the destruction of the brain homeostasis mechanism but they had a greater impact on memory retrieval in infused EGs

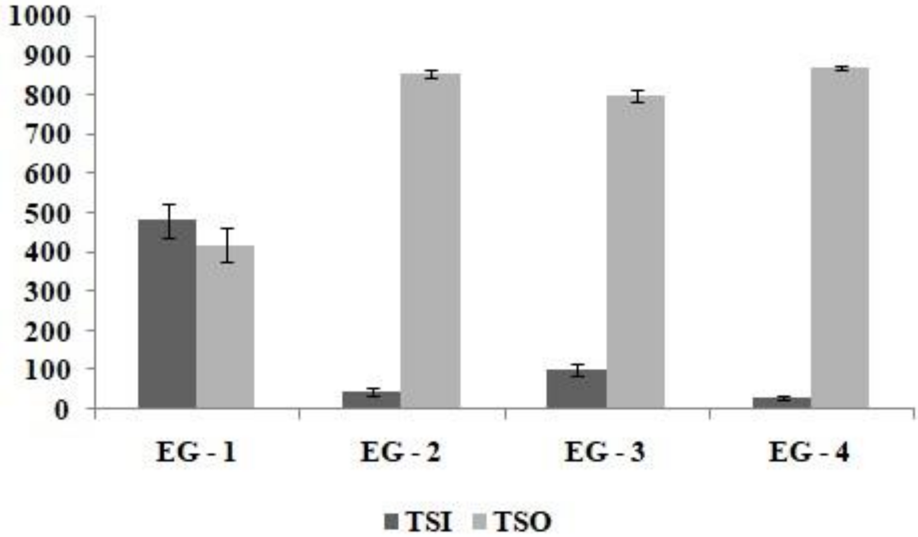

Figure 10: Behavioral responses of the open field test showed the development of anxiety like behavior in the NPM-infused groups (EGs – 2, 3 and 4) compared to the non-infused group (EG – 1) [TSI – time spent in the inner compartment; TSO - time spent in the outer compartment]

After completion of MPBA testing phase, MPBA open field test (OFT) was performed to determine the effect of NPM OMI in the presence/absence of anxiety like behavior. Behavioral scores of MPBA OFT showed the development of anxiety like behavior in the NPM-infused groups (EG s – 2, 3 and 4) compared to non-infused groups (EG -1). NPM-infused groups spent more amount of time in the outer compartment (TSO) compared to time spent in the inner compartment (TSI). It also proved that NPM infusions may alter the HPA axis via oral/gut dysbiosis and may result in the development of anxiety like behavior in infused groups (Fig. 10).

Impact of Probiotic Strain (*L. fermentum*) in the Reversal of NPM-Induced Cognitive Impairment: The prepared probiotic oral microbial infusions were infused on day 34 to the EGs - 2, 3 and 4 after completion of MPBA. After three days (days 35-37) of infusion, the training and testing phase was carried out on FPBA between days 38 - 46. After three days of recovery, training was carried out between days 38 - 40. Behavioral scores of the SPBA training phase showed that probiotic infusions enhanced the learning abilities of NPM-infused groups (EGs – 2, 3 and 4) compared to the training data of MPBA. It also showed that probiotic infusions may have a greater impact on the learning abilities of infused groups in an increased manner (Fig. 11). Followed by memory consolidation interval (days 41 – 43), testing was carried out between 44-46 days after FPBA training phase.

Behavioral responses of all EGs showed that probiotic-infused EGs witnessed higher level of responses towards the positive stimuli, which also showed that probiotics reversed the impact of NPM oral microbial infusions on the FPBA testing phase (Fig. 12). Comparative analysis of FPBA training and testing phase showed that probiotic oral infusions played a major role in the reversal of NPM induced cognitive impairment in infused EGs compared to EG – 1 (control) and also stated the enhanced activity of probiotics in the acquaintance of new information from the experimental setup after MPBA (Fig. 13). FPBA OFT was performed to identify the effect of probiotic oral infusions on the development of anxiety like behavior. Behavioral scores of FPBA OFT showed the reversal of anxiety-like behavior in the NPM-infused groups (EGs – 2, 3 and 4) compared to non-infused groups (EG -1).

All EGs spent more amount of time in the inner compartment (TSI) compared to time spent in the outer compartment (TSO). Thus the obtained results showed that the probiotic microorganisms were involved in the regulation of anxiety like behavior through the HPA axis (Fig. 14). Comparative analysis of the IPBA, MPBA and FPBA training scores showed that plasticity changes occur during both healthy and diseased states. The formed plasticity changes were strengthened due to probiotic infusions in infused EGs compared to the non-infused ones. It also proved that oral/gut dysbiosis never blocks the acquaintance of new information through the neuronal circuit changes in the brain (Fig. 15).

However, NPM infusions result in the development of impaired cognitive memory through the formation of oral/gut dysbiosis. The formed oral/gut dysbiosis played a major role in the development of cognitive impairment and it reversed with the help of probiotic intake which is validated by the comparative analysis of the testing scores of IPBA, MPBA and FPBA (Fig. 16). Overall results of the present study proved the significance of probiotic strain in the reversal of oral/gut dysbiosis.

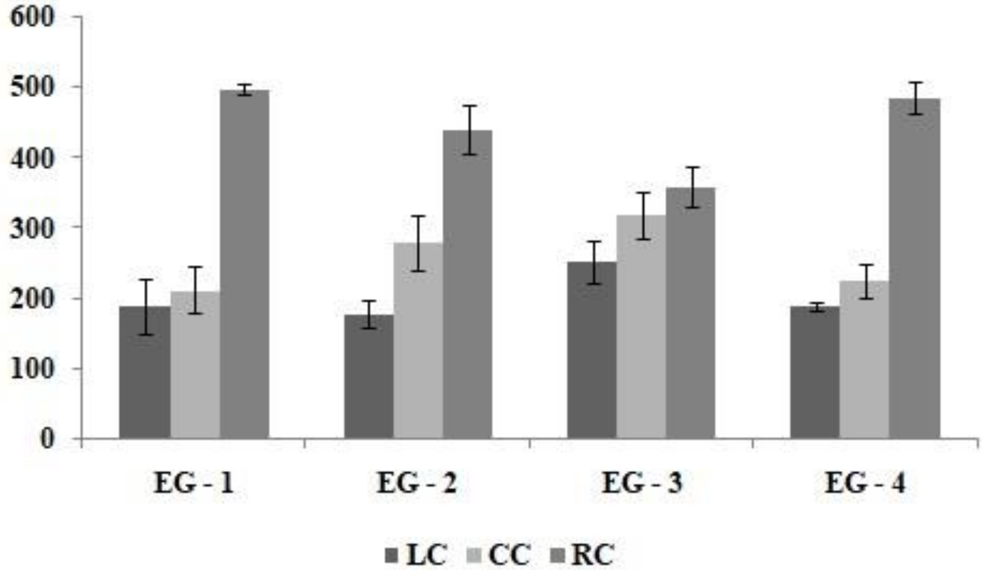

Figure 11: Behavioral scores of the FPBA training phase showed that the probiotic oral infusions may take part in the strengthening of formed synaptic plasticity in the infused groups compared to MPBA training

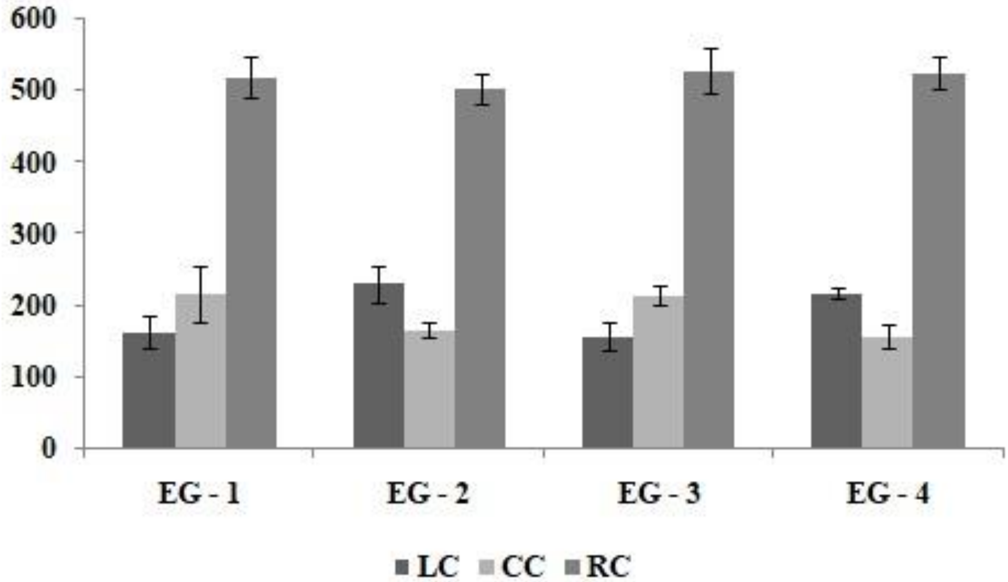

Figure 12: Behavioral scores of the FPBA testing phase showed that the probiotic oral infusions reversed the impaired cognitive memory formation in the infused groups compared to the control

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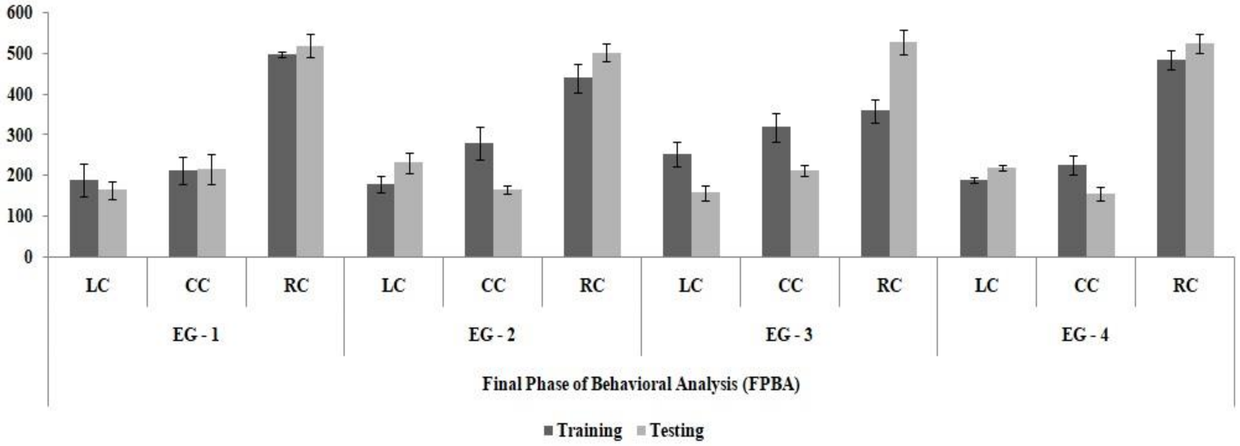

Figure 13: Comparative analysis of the FPBA training and testing phase showed that probiotic oral infusions played a major role in the reversal of NPM-induced cognitive impairment in infused EGs compared to the EG – 1 (control)

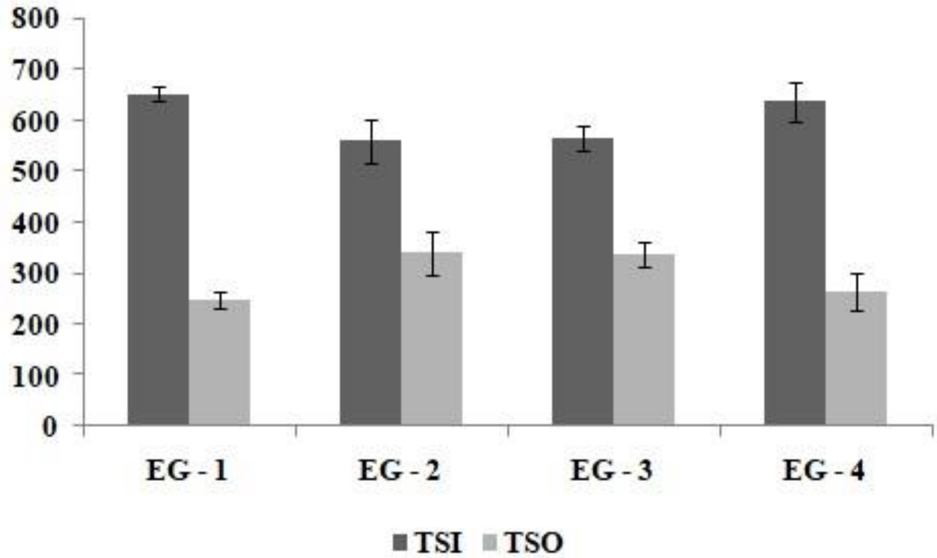

Figure 14: Behavioral scores of the FPBA open field test showed that probiotic oral infusions reduced the level of anxiety-like behavior development in the NPM-infused groups (EGs – 2, 3 and 4) compared to the non-infused group (EG – 1) [TSI – time spent in the inner compartment; TSO - time spent in the outer compartment]

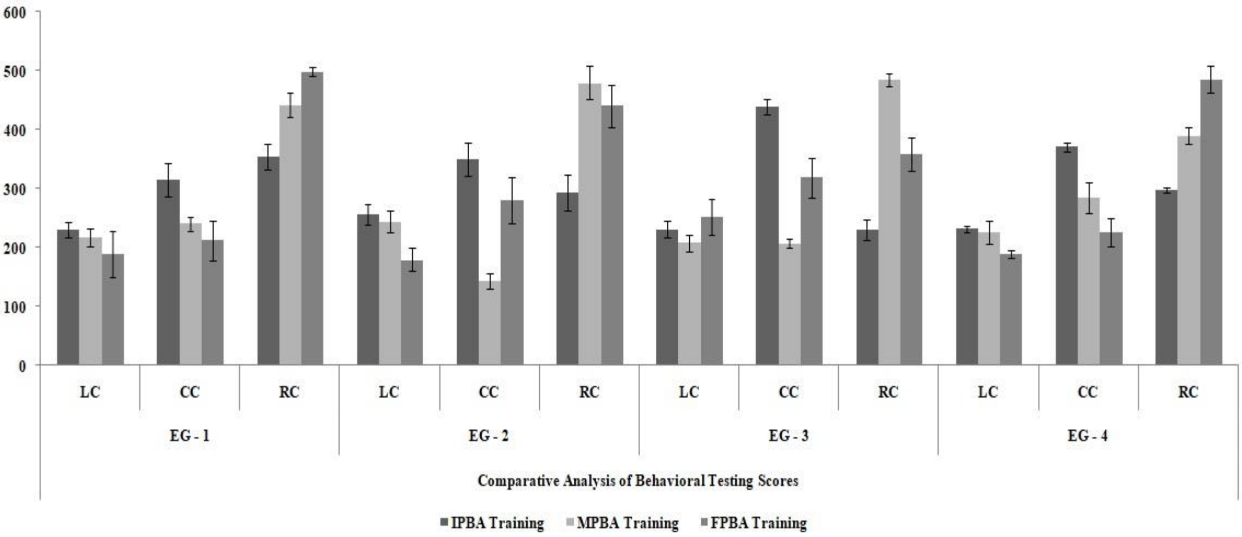

Figure 15: Comparative analysis of the training scores of IPBA, MPBA and FPBA showed that plasticity changes occur during healthy, NPM-infused (diseased) states. However, formed plasticity changes were strengthened due to probiotic infusions in infused EGs

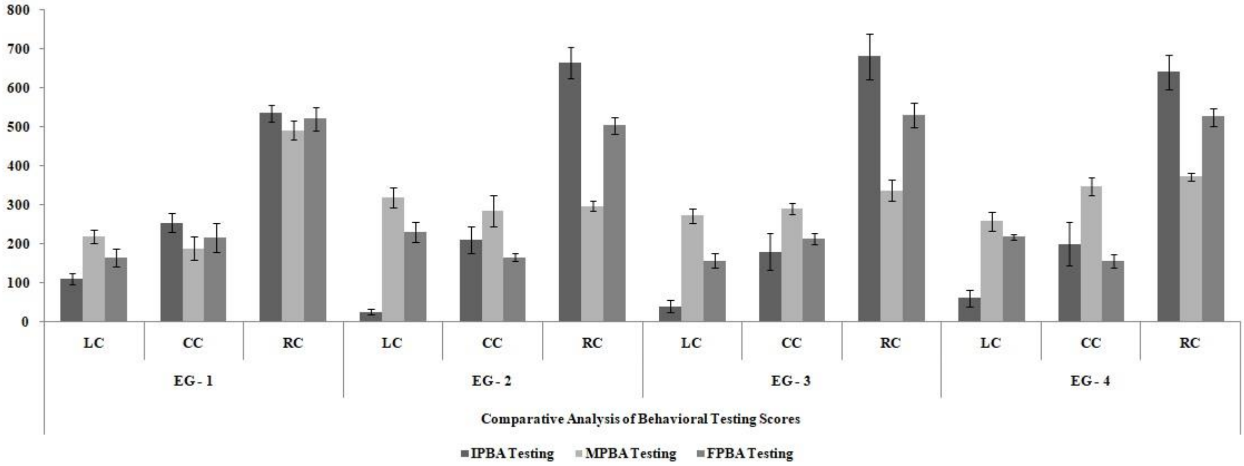

Figure 16: Comparative analysis of the testing scores of IPBA, MPBA and FPBA showed that oral/gut dysbiosis played a major role in the development of cognitive impairment. Formed cognitive impairment was reversed with the help of probiotics in the NPM-infused EGs

Discussion

The microbiome-gut-brain (MGB) axis consists of a complex interaction between the oral/gut microflora, enteric and central nervous system. This MGB links the cognitive centers of the brain (hippocampus) with the intestinal functions. In these intestinal functions, oral and gut microbiotas play a major role in the synthesis and regulation of NPCs production within the gut[5,7,24,43,49]. Further produced NPCs are transported to the brain for the formation of cognitive memory. Formed cognitive memory was a result of neurotransmitter production in the presynaptic neuron followed by the activation of neuronal signaling molecules within the postsynaptic neurons.

Activation of these neuronal signaling molecules results in the formation of LTM during healthy conditions which is supported by recent studies[9,23,34]. However, the formation of this LTM may be hindered during the state of diseased conditions due to pathogenic microbial colonization in the oral cavity and gut. Few reports showed that there is a relationship between periodontal microorganisms and neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, mild cognitive impairment etc.[27,34,50] Major causes of these impairments were stated as poor oral hygiene, oral/gut dysbiosis, food habits, lifestyle changes, etc. Compared to other conditions, poor oral hygiene plays a major role in the aberration of normal oral/gut microflora through the periodontal and non-periodontal pathogens[4,34,47,52].

The present study tried to explore the effect of NPM in the induction of cognitive memory impairment and its reversal with the probiotic strain. The present study showed the impact of *L. fermentum* in the reversal of NPM-induced cognitive memory decline through the MGB axis. Recent reports showed the involvement of probiotic strains in the reversal of cognitive impairment through the MGB axis. Probiotic microorganisms use a specific rehabilitation process to maintain the normal beneficial flora present in the oral cavity and gut. Intake of oral probiotic infusions results in the proliferation and maintenance of oral and gut microflora through the production of metabolites/precursor molecules like short-chain fatty acids (SCFAs)[2,6,26,42].

The produced SCFAs were responsible for establishing the reversed homeostasis connection between the brain and the gut and were also involved in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. The regulated HPA axis may further result in the decreased production of cortisol which may act as a negative regulator of cognitive memory formation[14,18,41,46]. Initially, probiotic microorganism intake may regulate neurotransmitter production through normalized production and transportation of NPCs to the brain from the gut. Transported NPCs may result in the production of neurotransmitters in the presynaptic neurons. The produced neurotransmitter binds with the post-synaptic neuronal receptors present in the synaptic cleft and results in the increased calcium influx[8,12,29].

Increased calcium influx results in the activation of IEGs through the phosphorylation and activation of CREB, PKA and cAMP in the brain neuronal connections. Activation of IEGs results in LTM formation through the production of PSDs during the reversal of oral/gut dysbiosis with the help of probiotic microorganisms[21,31,34,44]. The present study tried to explore the specific role of *L. fermentum* in the reversal of NPM-induced oral/gut dysbiosis. Initially, cognitive memory formation was tested in a stress-free habituated environment with the help of RBLP during IPBA.

Results of IBPA showed that residential microflora of the oral cavity and gut may be involved in the proper cognitive memory formation through the MGB axis. Formed cognitive memory was impaired during NPM-induced microbial dysbiosis which results in the reduced/impaired cognitive memory formation in the MPBA. Formed MPBA memory impairment was reversed with the help of probiotic strain infusion in the NPM-infused groups (EGs - 2, 3 and 4) during the FPBA. The obtained experimental results proved that probiotic oral infusions played a major role in the reversal of NPM-induced cognitive impairment during the formed oral/gut dysbiosis. It also proved that oral/gut dysbiosis formation shows the impact of poor oral hygiene on cognitive memory formation.

Conclusion

The present study made an initial attempt to show the effect of a probiotic strain (*L. fermentum*) in the reversal of NPM-induced cognitive impairment. To prove the effect of probiotic strain, I have used a comparative three-phased behavioral paradigm which consists of the initial, mid and final phases of behavioral analysis with the use of RBLP. Experimental results of IPBA and MPBA showed the effect of NPM (*P. aeruginosa*, *S. pneumonia, S. aureus*) OMIs on cognitive memory formation in a stress-free habituated environment. Formed NPM-induced cognitive impairment was reversed with the help of probiotic oral microbial infusions in the NPM-treated groups compared to control (EG – 1) in FPBA.

The outcome of the IPBA showed the impact of native oral/gut microflora on cognitive memory formation. In MPBA, formed cognitive memory impairment in the EGs - 2, 3 and 4 reversed with the help of probiotics treatment in FPBA. Thus the present work laid down a path to identify the impact of NPM infection on brain neuroinflammatory reactions involved with cognitive decline/memory loss. It also proved that other than periodontal microorganisms, NPM may also be involved in the initiation and proliferation of neurodegenerative disorders along with oral/gut dysbiosed state in diseased/aged individuals.

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