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Original Research Article

Anti-inflammatory and Neuroprotective Effects of Lactobacillus Strains Contribute to its Antidepressant-Like Property Against Chronic Unpredictable Stress-Induced Behavioural Abnormalities in Mice

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ABSTRACT

Depression is one of the most worrisome disorders of the central nervous system (CNS). Recently, more researchers have delved into studying the links between the gut microbiota and psychiatric disorders such as depression, anxiety, and cognitive impairments such as amnesia. With this novel angle came the research into probiotics, the gut microbiota-modifying bacteria. This study was therefore designed to evaluate the neuroprotective effect of specific probiotic strains on chronic unpredictable stress induced depressive symptoms in mice. Thirty-five mice (20-30 g) were shared into five groups randomly. Groups 1 and 2 received vehicle (10 mL/kg), groups 3 and 4 received probiotics (10 mL/kg), and group 5 received fluoxetine (10 mg/kg). Only groups 2, 3 and 5 were exposed to an unpredictable order of chronic mild stress daily for twenty-one (21) days. Afterward, behavioural, and biochemical assays were carried out. The results revealed that chronic unpredictable stress caused significant ($p<0.05$) elevation in depressive-like behaviours, increased the production of proinflammatory cytokines and even signalled hippocampal neurodegeneration. However, a significant ($p<0.05$) reversal of these effects was observed in the probiotics-treated groups as effectively as the fluoxetine group. It can be concluded that anti-inflammatory and neuroprotective effects of lactobacillus strains may contribute to its antidepressive-like action against CUS-induced depression in mice.

Keywords: Probiotics, Inflammation, Stress, Depression, Cytokines

Introduction

Depression is an emotional state characterized by low mood, anhedonia, feelings of hopelessness, insomnia and even loss of appetite. It is considered one of the main types of mood disorders.[1] Anxiety is another emotional state with clinical features of fear, worry, tension, and even physical changes such as palpitations and tremors without an obvious cause.[1] The Institute of Health Metrics and Evaluation[2] described both diseases as the most common mental disorders in human health with over 280 million people in the world suffering from these disorders. As the number of sufferers rises, so does the need to explore more efficient therapies for the management of mood disorders. Aside the conventional approach (that is, the currently used medications), there are indications that gut-microbiota play a major role in the regulation of mood and behaviours.

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Therefore, in recent times, attention has gradually shifted towards the constitution of gut microbiome in relation to behaviours and brain molecular disruptions. Several studies have reported a bidirectional communication between the gut and the brain, and how gut microbiota affects mood and behaviours, hence the existing evidence that probiotics can alter the constitution and condition of the gut microbiome.[3] Trillions of microorganisms in the intestines have been shown to regulate the gut-brain axis with the presence of compelling evidence of specific microorganisms affecting behaviours and molecular abnormalities in the brain.[4]

Probiotics are live non-pathogenic microorganisms, which can confer health benefit(s) on the host by stimulating the host's immune response to invading pathogens.[5-7] They are most found in dairy products, breast milk, and fermented vegetables.[7] Several preclinical studies have demonstrated the benefit of probiotics in mood and cognitive dysfunctions. Li *et al*. [8] described an improvement in spatial memory in rats after probiotics supplementation. In addition, Bravo and colleagues [9, 10] reported the beneficial effect of probiotics on depression and anxiety in rodent models. Also, probiotics caused a significant reduction in the indices of depression and anxiety and re-established brain chemistry. [11] Earlier, certain probiotics formulation significantly ameliorated neuroinflammation in aged rats.[12] The anti-inflammatory effect demonstrated by probiotics in the gut and central nervous system has been attributed to its ability to limit the production of pro-inflammatory cytokines (tumour necrosis factor alpha, TNF- α; interleukin-12, IL-12; and interferon gamma, IFN-γ) [13] which have been implicated in the pathophysiology of mood disorders.

In the same vein, Ng *et al*. [14] highlighted the therapeutic potential of probiotics in the treatment of some psychiatric disorders. Consistently,

Marotta et al. [15] reported that probiotics significantly enhanced mood and physiological well-being. More recently, reports on the mood-enhancing and health-improving effects of probiotics via the host's gut-brain axis have emerged. [1,16] Therefore, the present study sought to leverage on the current knowledge about the beneficial effects of probiotics on specific aspects of mood (depression and anxiety) while investigating the neuroprotective effect of lactobacillus strains against chronic stress-induced behavioural abnormalities in animals.

Materials and Methods

Preparation of Probiotic strains
Five strains of lactobacillus (*Lactobacillus plantarum* A014, *Lactobacillus rhamnosus* A072, *Lactobacillus pseudomensenteroides* A064, *Lactobacillus pentosus* A044 and *Lactobacillus plantarum* A084) were collected and isolated from human breast milk and faecal samples. The five strains were cultured in MRS–cysteine medium and incubated for 24 hours at 37ºC under anaerobic conditions. The Gaspak Jar (BBL, USA) inserted with Anaero Gen™ 3.5L (Thermo Scientific, Oxoid, Japan) were used during the incubation process. [17] Each probiotic strains (with approximate cell counts of 1 x 10^8 CFU/mL) was collected and centrifuged for 10 minutes at 4,000 rpm. They were later washed twice and re-suspended in 10 mL of sterile phosphate-buffered saline (PBS) to produce a kind of probiotic cocktail.

Experimental animals
Thirty-five male Swiss mice (20–30 g) were acquired from the University of Ibadan Central Animal House, Ibadan, Nigeria. The animals were allowed to acclimatize in Afe Babalola University Animal House for two weeks under standard conditions. The animals were given standard animal feed (pellet) and water daily. The experimental research was approved by the Afe Babalola University Ethical Committee (AB/EC/20/22/08) and the animals were handled following the guidelines outlined in the Guide for use and care of laboratory animals.[18]

Experimental procedure
Chronic unpredictable stress (CUS) was applied to mice according to Antoniuk *et al*. [19] The thirty-five mice were randomly divided into five groups (n = 7). Groups 1 and 2 received vehicle (10 mL/kg), groups 3 and 4 received probiotics (10 mL/kg), and group 5 received fluoxetine (10 mg/kg). All groups were exposed to chronic stress daily except group 1, which served as the normal control, and group 4 that received probiotics only. All animals were housed singly and the following stressors were applied for three weeks: water withdrawal (12 hrs), cage tilt at 45º (7 hrs), moist beddings (7 hrs), overnight illumination (12 hrs), background noise (30 mins), food withdrawal (12 hrs), moist beddings (12 hrs), tail pinch (1 min), cage tilt at 45º (12 hrs), foot shock (30 sec), overnight illumination (12 hrs) and final cage tilt at 45º(7 hrs).[20-22] In order for the protocol to truly be unpredictable, no same stressor was applied on two consecutive days.

Behavioural assays
All animals were subjected to behavioural tests twenty-four hours after the last treatment and CUMS procedure.

Open field test
The open field test was carried out using an open field apparatus (60 x 60 x 30 cm^3) with sixteen squares (15x15 cm^3). Each mouse was placed in one of the central squares and locomotor activity was measured by counting the number of squares the animal moved over for5 mins. The apparatus was cleaned with diluted alcohol (70%) before each new mouse was tested.[23]

Forced Swim Test
The forced swim test was carried out by following the procedure that was described by Porsolt.[24] Each mouse was allowed to swim in a cylindrical container (10cm diameter and 25cm height) which was filled with water for 6 mins. The mobility time (time spent in active swimming, floating, struggling) and immobility was recorded during the last 4 mins using a stopwatch.

Tail Suspension Test
The tail suspension test was carried out as described by Steru and others. [25] Each mouse was suspended by the tail on a wire attached to a tripod stand at a height of 50 cm with the help of an adhesive tape. Total time for the test was 6 mins but duration of immobility was recorded for the final 4 mins.

Sucrose Splash Test
Sucrose splash test was carried out at the beginning and at the end of each CUMS procedure. Anhedonic symptoms were assessed in the animals by splashing 10% sucrose solution on the dorsal coat of each mouse. The grooming time was recorded for 5 mins.

Biochemical assay for cytokines
The day after behavioral tests, the mice in all groups were anesthetized and subsequently sacrificed by decapitation. The whole brains were rapidly removed and placed in cold media. Soon after, the prefrontal cortex (PFC) and hippocampus of each mouse brain were removed. Thereafter, one half of the brain samples were homogenized with cold PBS and centrifuged at 10,000rev/min for 15 mins. The supernatant separated from the centrifuged brain sample was used to assay IL-12, and IFN-γ concentrations using ELISA kits from BioLegend® USA. The procedure was carried out following the manufacturer's instructions.

Golgi Staining
Golgi staining was carried out on the hippocampus and prefrontal brain slices as modified by Zhang and colleagues. [26]

Statistical analysis
The data was analysed by using one-way analysis of variance (ANOVA). The Tukey's multiple comparison post-hoc test was used to analyse and compare the differences between the various groups. The data were presented as mean ± standard deviation (SD) at $p<0.05$ level of significance. Results are presented in graphs and bar charts.

Results and Discussion

Probiotics attenuates chronic unpredictable stress-induced depressive-like behaviours in mice
Psychiatric disorders are worsened by the exposure to chronic stress from external sources. This is because such stressors can cause permanent damage to certain areas of the central nervous system (CNS).[27,28] In preclinical psychiatric studies, the chronic unpredictable stress (CUS) model of depression is most used because of its established effectiveness.[29,30] In other studies, some behaviours were associated with depression such as an increase in immobility time in the tail suspension and forced swimming tests.[31]
In the present study, effect of probiotics on the behavioural abnormalities displayed by mice following exposure to chronic unpredictable stress (CUS) was evaluated using forced swim test (FST), tail suspension test (TST) and sucrose splash test (SST). Following one-way ANOVA analysis, the data showed significant difference in the immobility time in the FST [$F_{(4, 21)} = 18.85$, $P<0.0001$]; TST [$F_{(4, 24)} = 8.186$, $P=0.0003$] and sucrose preference index (grooming time) in SPT [$F_{(4, 22)} = 7.870$, $P=0.0004$]. Furthermore, post hoc revealed that the group of animals exposed to stress (CUS group) significantly ($p< 0.05$) recorded increased immobility time in the FST (Figure 1) and TST (Figure 2) and decreased sucrose preference (i.e., decreased grooming) in the SST (Figure 3) compared with the control. However, treatment with probiotics significantly ($p<0.05$) reversed these depressive-like behaviours by decreasing immobility time in FST and TST and increasing grooming in SST compared with the CUS-exposed animals. This agrees with the study by Akkasheh et al. [32] which revealed clinically diagnosed depressed adults scored lower than the placebo group on the Beck Depression Inventory, and Steenbergen et al.'s study [33] where depressed patients treated with probiotics had significantly lower cognitive reactivity to sadness and aggression. Also, several studies, over the years, have agreed that interventions targeted at the gut microbiota may most likely be effective treatment for neuropsychiatric disorders.[34-37]

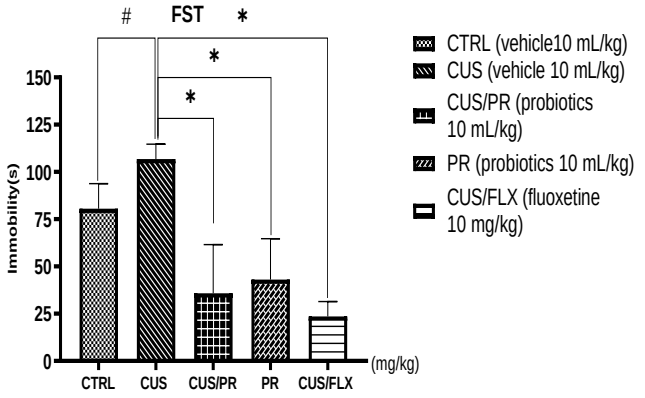

Figure 1: Effect of probiotics on CUS-induced depressive-like behaviours

Data represent mean ± SD (n=5-6).

# p< 0.05 when compared with control group; *p< 0.05, when compared with CUS group

CTRL- control; CUS- chronic unpredictable stress; PR- probiotics; FLX- fluoxetine

CUS significantly increased immobility time in FST compared to control.

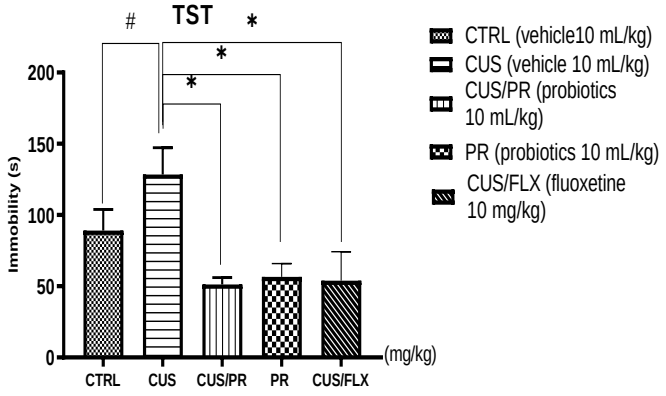

Figure 2: Effect of probiotics on CUS-induced depressive-like behaviours

Data represent mean ± SD (n=5–7).

# p< 0.05 when compared with control group; *p< 0.05, when compared with CUS group

CTRL- control; CUS- chronic unpredictable stress; PR- probiotics; FLX- fluoxetine

CUS significantly increased immobility time in TST compared to control.

Probiotics attenuates CUS-induced pro-inflammatory cytokines (IFN-γ and IL-12) in mice brains

Inflammation plays a key role in the pathogenesis of depression and cytokine have been implicated in the pathology of depression. [38] In agreement with previous studies, animals exposed to chronic stress exhibited behavioural abnormalities accompanied by elevated proinflammatory cytokines.[39,40] There was significant difference in the level of IL-12 [$F_{(4, 20)}$ = 18.51; P<0.0001) and IFN-γ [$F_{(4, 24)}$ = 23.28; P<0.0001] among the groups. Tukey's multiple comparison tests further revealed that CUS significantly ($p < 0.05$) increased brain levels of IL-12 (Fig. 4) and IFN-γ (Figure 5) compared with control. However, the mice pre-treated with probiotics showed a significant decline in the concentration of IL-12 and IFN-γ compared with CUS treated animals. This finding is consistent with the recent report that probiotics demonstrate anti-inflammatory effect against pro-inflammatory biomarkers in CUS-induced depressive-like model in mice.[39] The outcome of this study also agrees with other studies that post-stress probiotics treatment caused a reduction in TNF-α and IFN-γ levels,[40,41] and Chong *et al.*[42] reported reduced levels of proinflammatory cytokines following *Lactobacillus plantarum* DR7 administration.

Probiotics offers neuroprotection against CUS-induced neurodegeneration in hippocampus and prefrontal cortex

Structural and functional alteration in the brain regions like hippocampus and prefrontal cortex are strongly associated with depression.[43] Evidence of reduced hippocampal volume of depressed patient has been reported.[44] In this study, histology of the prefrontal cortex and hippocampal regions of the brain revealed clear neurodegeneration of dendrite and neuronal cells in the groups exposed to stress. The pyramidal cells and dendrites were significantly depleted in the group exposed to stress alone compared to control (Figure 6). However, this depletion was significantly reduced by the treatment with probiotic. This is indicative of its neuroprotective effect against chronic stress. Existing studies have reported beneficial effects of probiotics on the neurodegenerative process by restoring neuronal health, [45] and brain weight in 6-month old 3xTg-AD mice.[46] Other probiotic strains (*Lactobacillus pentosus* A4C, *Lactobacillus pentosus* B1B, *and Lactobacillus rhamnosus* 12) have also shown neuroprotective effect on hippocampus region of the brain.

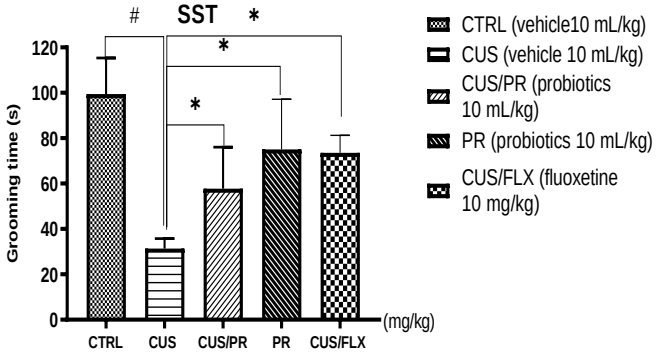

Figure 3: Effect of probiotics on CUS-induced depressive-like behaviours

Data represent mean ± SD (n=5-6).

# p< 0.05 when compared with control group; *p< 0.05, when compared with CUS group

CTRL- control; CUS- chronic unpredictable stress; PR- probiotics; FLX- fluoxetine

CUS significantly decreased grooming time in SST compared to control.

Conclusion

Probiotics attenuated chronic stress induced behavioural dysfunction in mice. It can therefore be said that its anti-inflammatory and neuro-protective activities against impairments linked to chronic stress may also contribute to its antidepressant-like action. However, further studies are needed to affirm its potential benefit as adjunct therapy in the management of CNS disorders.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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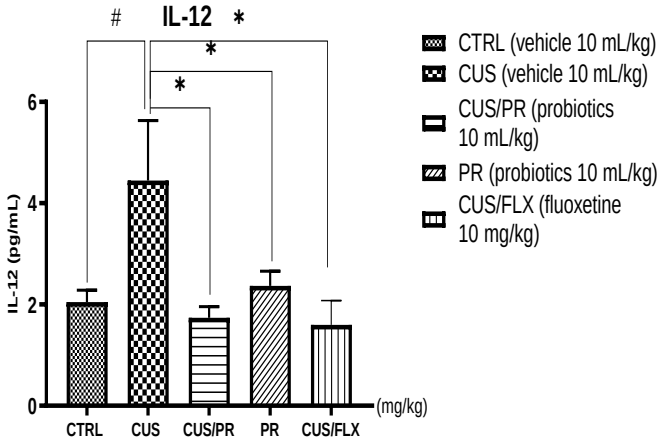

Figure 4: Effect of probiotics on the brain level of pro-inflammatory cytokines in mice subjected to CUS model of depression.
Data represent mean ± SD (n=5).
# p< 0.05 when compared with control group; *p< 0.05, when compared with CUS group
CTRL- control; CUS- chronic unpredictable stress; PR- probiotics; FLX- fluoxetine
CUS significantly increased concentration of IL-12 compared to control.

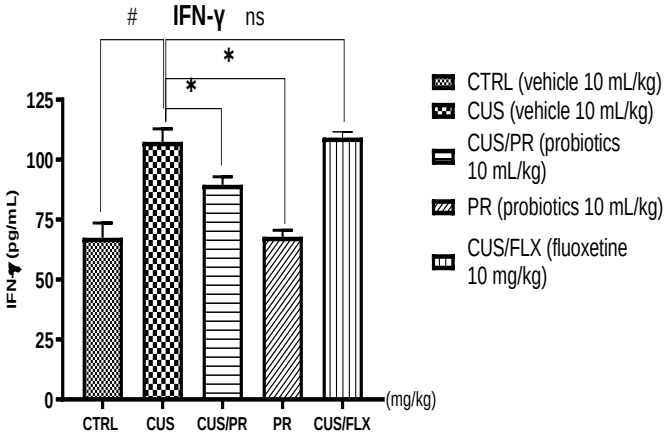

Figure 5: Effect of probiotics on the brain level of pro-inflammatory cytokines in mice subjected to CUS model of depression
Data represent mean ± SD (n=5-6).
# p< 0.05 when compared with control group; *p< 0.05, when compared with CUS group
CTRL- control; CUS- chronic unpredictable stress; PR- probiotics; FLX- fluoxetine
CUS significantly increased concentration of IFN- γ compared to control.

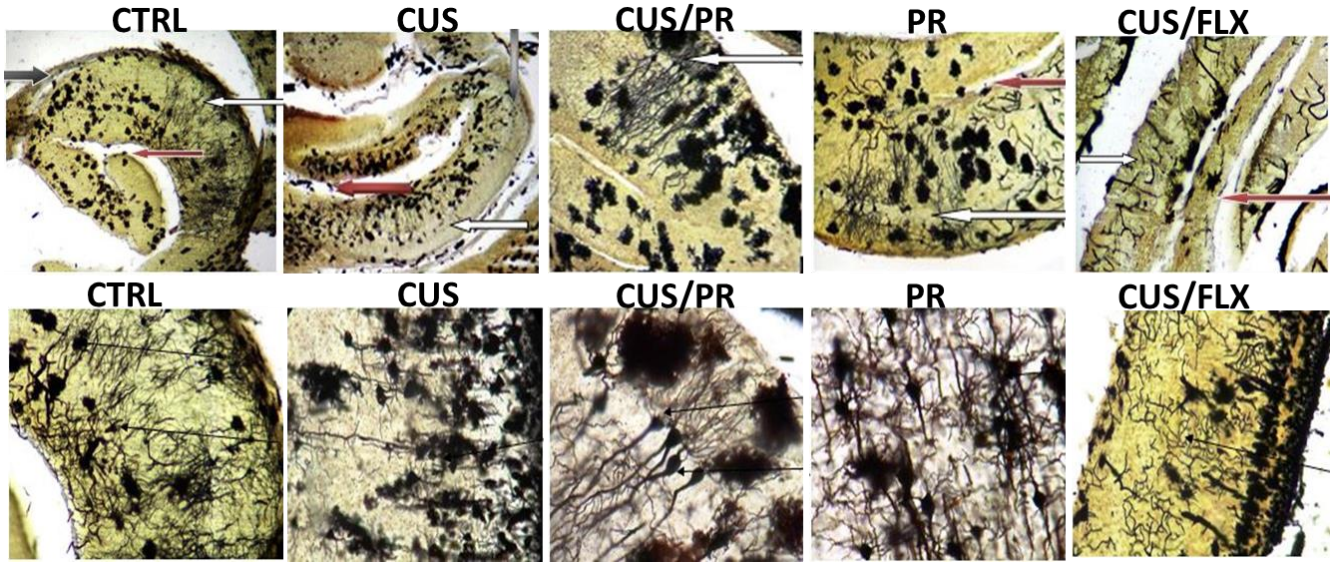

Figure 6: Representative Golgi-stained sections of hippocampus (top) and prefrontal cortex (bottom) of CUS exposed animals. In CUS group, dendritic arborisation appears reduced. No observable difference in the pattern of the dendritic arborisation in CUS/PR, PR only and CTL groups. Magnification- X100
CTRL- control; CUS- chronic unpredictable stress; PR - probiotics; FLX- fluoxetine

References

1. Chao L, Liu C, Sutthawongwadee S, Li Y, Lv W, Chen W, Yu L, Zhou J, Guo A, Li Z, Guo S. Effects of probiotics on depressive or anxiety variables in healthy participants under stress conditions or with a depressive or anxiety diagnosis: a meta-analysis of randomized controlled trials. Front Neurol. 2020; 11(421):1-10.
2. Institute of Health Metrics and Evaluation. Global Health Data Exchange (GHDx). http://ghdx.healhdata.org/gbd-results-tool?params=gbd-api-2019-permalink/d780dffbe8a381b25e1416884959e
3. Zheng P, Wu J, Zhang H, Perry SW, Yin B, Tan X, Chai T, Liang W, Huang Y, Li Y, Duan J, Wong ML, Licinio J, Xie P. The gut microbiome modulates gut-brain axis glycerophospholipid metabolism in a region-specific manner in a nonhuman primate model of depression. Mol Psychiatry. 2021; 26:2380–2392.
4. L-In V, Saengha W, Karirat T, Konsue A, Wangkahart E, Katisart T. Probiotics from Thai fermented foods reduced anxiety and enhanced neuroplasticity in a Wistar rat model. Trop J Nat Prod Res. 2022; 6(6):910-914. Doi: 10.26538/tjnpr/v6i6.15

5. Ajayi AS, Ogunleye BO, Oluwasola MA, Okediya CK, Akpata OB, Akinnola OO. Screening of probiotic characteristics of lactic acid bacteria isolated from some fermented Nigerian food products. Trop J Nat Prod Res. 2020;4(12):1166-1169
6. Applegate JA, Walker CLF, Ambikapathi R, Black RE. Systematic review of probiotics for the treatment of community-acquired acute diarrhea in children. BMC Public Health 2013; 13(Suppl 3):S16.
7. Food and Agriculture Organization (FAO) of the United Nations and World Health Organization (WHO). Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. FAO and WHO Expert Consultation Report 2021.
8. Li W, Dowd SE, Scurlock B, Acosta-Martinez V, Lyte M. Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. Physiol Behav.2009; 96:557–567.
9. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Nat Acad Sci USA. 2011; 108:16050–16055.
10. Bravo JA, Julio-Pieper M, Forsythe P, Kunze W, Dinan TG, Bienenstock J, Cryan JF. Communication between gastrointestinal bacteria and the nervous system. Curr Opin Pharmacol. 2012; 12:667–672.
11. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 2016; 165:1332-1345.
12. Distrutti E, Cipriani S, Mencarelli A, Renga B, Fiorucci S. Probiotics VSL#3 Protect against development of visceral pain in murine model of irritable bowel syndrome. PLoS One 2013; 8:e63893.
13. Leta V, Ray Chaudhuri K, Milner O, Chung-Faye G, Metta V, Pariante CM, Borsini A. Neurogenic and anti-inflammatory effects of probiotics in Parkinson's disease: A systematic review of preclinical and clinical evidence. Brain, Behavior, and Immunity 2021;98: 59-73. Accessed using https://doi.org/10.1016/j.bbi.2021.07.026
14. Ng QX, Peters C, Ho CYX, Lim DY, Yeo WS. A meta-analysis of the use of probiotics to alleviate depressive symptoms. J Affect Disord2018; 228:13–19.
15. Marotta A, Sarno E, Del Casale A, Pane M, Mogna L, Amoruso A, Felis GE and Fiorio M. Effects of Probiotics on Cognitive Reactivity, Mood, and Sleep Quality. Front Psychiatry. 2019; 10(164):1-11. Accessed using Doi: 10.3389/fpsyt.2019.00164
16. Noonan S, Zaveri M, Macaninch E, Martyn K. Food & mood: a review of supplementary prebiotic and probiotic interventions in the treatment of anxiety and depression in adults. BMJ Nutrition, Prevention & Health 2020; 3(2):351-362.
17. Rajoka MSR, Jin M, Haobin Z, Li Q, Shao D, Jiang C, Huang Q, Yang H, Shi J, Hussain N. Functional characterization and biotechnological potential of exopolysaccharide produced by Lactobacillus rhamnosus strains isolated from human breast milk. LWT 2018;89:638-647
18. National Research Council. Guide for the care and use of laboratory animals. National Academies Press 2010
19. Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J. Chronic unpredictable mild stress for modeling depression in rodents: meta-analysis of model reliability. Neurosci Biobehav Rev. 2019; 99:101–116. Accessed using doi: 10.1016/j.neubiorev.2018.12.002
20. Akinluyi ET, Edem EE, Dakoru KM, Nnodim CJ, Oladipupo MA, Adeoluwa OA, Obisesan AO, Ben-Azu B, Adebayo OG. Psychobiotic interventions attenuate chronic alcohol use-mediated exacerbation of posttraumatic stress disorder in rats: the role of gut-liver axis response. J Complement Integr Med. 2022;20(1):92-105
21. Yang CR, Bai YY, Ruan CS, Zhou FH, Li F, Li CQ, Zhou XF. (2017). Injection of anti-proBDNF in Anterior Cingulate Cortex (ACC) reverses chronic stress-induced adverse mood behaviors in mice. Neurotox Res. 31: 298–308.
22. Nikolova YS, Misquitta KA, Rocco BR, Prevot TD, Knodt AR, Ellegood J, Voineskos AN, Lerch JP, Hariri AR, Sibille E, Banasr M. Shifting priorities: highly conserved behavioral and brain network adaptations to chronic stress across species. Transl. Psychiatry 2018; 8(1):1-13.
23. Kraeuter AK, Guest PC, Sarnyai Z. The open field test for measuring locomotor activity and anxiety-like behaviour. In: Guest PC (Eds) Pre-Clinical Models. Methods in Molecular Biology, vol 1916. Humana Press, New York, 2018.
24. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Euro J of Pharmacol. 1978;47(4):379-391
25. Steru L, Chermat, R., Thierry, B., Simon, P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl.) 1985;85:367–370.
26. Zhang X, Bearer EL, Perles-Barbacaru AT, Jacobs RE. Increased anatomical detail by in vitro MR microscopy with a modified Golgi impregnation method. Magnetic Resonance in Medicine 2010; 63(5):1391-1397
27. Weber K, Giannakopoulos P, Herrmann FR, Bartolomei J, Digiorgio S, Ortiz Chicherio N, Delaloye C, Ghisletta P, Lecerf T, De Ribaupierre A, Canuto A. Stressful life events and neuroticism as predictors of late-life versus early-life depression. Psychogeriatrics. 2013; 13(4):221–228.
28. MacQueen G, Frodl T. The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? Mol Psychiatry. 2011; 16:252–264.
29. Qiao H, Li MX, Xu C, Chen HB, An SC, Ma XM. Dendritic spines in depression: what we learned from animal models. Neural Plast. 2016; 2016:8056370.
30. Chevalier G, Siopi E, Guenin-Mace L, Pascal M, Laval T, Rifflet A, Boneca IG, Demangel C, Colsch B, Pruvost A, Chu-Van E, Messager A, Leulier F, Lepousez G, Eberl G, Lledo PM. Effect of gut microbiota on depressive-like behaviors in mice is mediated by the endocannabinoid system. Nature Communications 2020; 11(1):1-15
31. Shehu A, Magaji MG, Yau J, Mahmud B, Ahmed A. Antidepressant effect of methanol stem bark extract of Adansonia digitata L. (Malvaceae) in mice. Trop J Nat Prod Res.2018;2(2):87-91
32. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, Jafari P, Akbari H, Taghizadeh M, Memarzadeh MR, Asemi Z, Esmaillzadeh A. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. Nutrition. 2016; 32(3):315-320.
33. Steenbergen L, Sellaro R, van Hemert S, Bosch JA, Colzato LS. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. Brain Behav Immun. 2015; 48:258-264.
34. Gacias M, Gaspari S, Santos PM, Tamburini S, Andrade M, Zhang F, Shen N, Tolstikov V, Kiebish MA, Dupree JL, Zachariou V, Clemente JC, Casaccia P. Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. eLife 2016; 5:e13442.
35. Wang H, Lee IS, Braun C, Enck P. Effect of probiotics on central nervous system functions in animals and humans: a systematic review. J Neurogastroenterol. Motil. 2016; 22:589–605.
36. Kazemi A, Noorbala AA, Azam K, Eskandari MH, Djafarian K. Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: a randomized clinical trial. Clin. Nutr. 2018. Doi: 10.1016/j.clnu.2018.04.010 [Epub ahead of print].
37. Vermeulen E, Brouwer IA, Stronks K, Bandinelli S, Ferrucci L, Visser M, Nicolaou M. Inflammatory dietary patterns and

depressive symptoms in Italian older adults. Brain Behav. Immunity 2018; 67:290–298.
38. Pfau ML, Menard C, Russo SJ. Inflammatory mediators in mood disorders: therapeutic opportunities. Annual Review of Pharmacology Toxicology. 2018; 58:411–428
39. Huang L, Lv X, Ze X, Ma Z, Zhang X, He R, Fan J, Zhang M, Sun B, Wang F, Liu H. Combined probiotics attenuate chronic unpredictable mild stress-induced depressive-like and anxiety-like behaviors in rats. Front. Psychiatry. 2022; 13:990465. Downloaded using Doi: 10.3389/fpsyt.2022.990465
40. Li N, Wang Q, Wang Y, Sun A, Lin Y, Jin Y and Li X. Oral Probiotics Ameliorate the Behavioral Deficits Induced by Chronic Mild Stress in Mice via the Gut Microbiota-Inflammation Axis. Front Behav Neurosci. 2018; 12:266.
41. Consonni A, Cordiglieri C, Rinaldi E, Marolda R, Ravanelli I, Guidesi E, Elli M, Mantegazza R, Baggi F. Administration of bifidobacterium and lactobacillus strains modulates experimental myasthenia gravis and experimental encephalomyelitis in Lewis rats. Oncotarget 2018; 9:22269–22287.
42. Chong HX, Yusoff NAA, Hor YY, Lew LC, Jaafar MH, Choi SB, Yusoff MSB, Wahid N, Abdullah MFIL, Zakaria N, Ong KL, Park YH, Liong MT. Lactobacillus plantarum DR7 alleviates stress and anxiety in adults: a randomised, double-blind, placebo-controlled study. Benef Microbes.2019; 10(4):355-373.
43. Gao L, CaiY, Wang H, Wang G, Zhang Q, Yan X. Probing prefrontal cortex hemodynamic alterations during facial emotion recognition for major depression disorder through functional near-infrared spectroscopy. J Neural Eng. 2019;16(2):026026
44. Campbell S, Marriott M, Nahmias C, MacQueen GM. Lower hippocampal volume in patients suffering from depression: a meta-analysis. Am J Psychiatry 2004; 161(4):598-607.
45. Michael DR, Davies TS, Loxley KE, Allen MD, Good MA, Hughes TR, Plummer SF. In vitro neuroprotective activities of two distinct probiotic consortia. Benef. Microbes 2019; 10(4):437–447
46. Bonfili L, Cecarini V, Berardi S, Scarpona S, Suchodolski JS, Nasuti C, Fiorini D, Boarelli MC, Rossi G, Eleuteri AM. Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. Sc. Rep. 2017; 7(1):2426.