TITLE PAGE

TITLE

EFFECTS OF PROBIOTICS SUPPLEMENTATION ON DEMENTIA AND COGNITIVE IMPAIRMENT: A SYSTEMATIC REVIEW AND META-ANALYSIS OF PRECLINICAL AND CLINICAL STUDIES

RUN TITLE

PROBIOTICS SUPPLEMENTATION ON DEMENTIA AND COGNITIVE IMPAIRMENT

AUTHORS

Cristofer Ruiz-Gonzalez[1], Pablo Roman[1,2,3*], Lola Rueda-Ruzafa[4], Miguel Rodriguez-Arrastia[5,6], Diana Cardona [1,2]

[1] Department of Nursing Science, Physiotherapy and Medicine, University of Almería, Ctra. Sacramento s/n, La Cañada, 04120, Almería, Spain

[2] Health Research Center. University of Almería, Spain

[3] Health Sciences Research Group (CTS-451), University of Almería, Spain

[4] Laboratory of Neuroscience, CINBIO, University of Vigo, Campus Lagoas-Marcosende, 36310, Vigo, Spain

[5] Faculty of Health Sciences, Pre-Department of Nursing, Jaume I University, 12071, Castello de la Plana, Spain

[6] Research Group CYS, Faculty of Health Sciences, Jaume I University, 12071, Castello de la Plana, Spain

CORRESPONDING AUTHOR

Pablo Roman Lopez

Edificio de Ciencias de la Salud, Universidad de Almería

Ctra. Sacramento s/n, 04120 La Cañada de San Urbano, Almería (Spain)

Telephone: +34950214563

Email: pablo.roman@ual.es

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ABSTRACT

Background: Dementia is a chronic syndrome characterized by cognitive and behavioral symptoms, which may include short-term memory impairment and problems related to orientation, language, attention and perception. Although cognitive impairment (CI) is increasingly considered the main geriatric condition predisposing to dementia, its early management could still promote symptomatic relief and delay disease progression. Recently, probiotics treatment has been studied as a potential new therapeutic approach to attenuate dementia-related decline and mild cognitive impairment (MCI). Therefore, we conducted a systematic review and meta-analysis to review and analyse the available evidence on the effect of probiotics on MCI and dementia.

Methods: A systematic search and meta-analysis were performed on Cochrane Library, ProQuest, Web of Science, PubMed-Medline, The Cumulative Index to Nursing and Allied Health Literature (CINAHL), Scopus, ScienceDirect and Open Grey. Search terms included diagnoses of interest (dementia and MCI) and the intervention of interest (probiotic, *lactobacillus* and *bifidobacterium*). Original articles reporting the use of probiotics supplementation for the treatment of dementia and MCI were screened and studied independently by two researchers. After that, a random and fixed effects model was used at the meta-analysis stage of the results to determine its effect size.

Results: A total of 16 articles (10 preclinical and 6 clinical) that met the inclusion criteria for the systematic review, and 15 articles (10 preclinical and 5 clinical) for meta-analysis were finally included. In humans, the administration of probiotics improved general cognitive function after the treatment period. Similarly, an improvement in memory and spatial/non-spatial learning was identified in the probiotic group of animals compared to the control group. On the other hand, the results showed an increase in the levels of the brain-derived neurotrophic factor, an improvement in the inflammatory profile and regulation of cellular biomarkers after probiotics administration.

Conclusion: Probiotics supplementation could be an adequate therapeutic strategy both in dementia and CI based on clinical and preclinical evidence. However, it is therefore important to translate preclinical data into clinical data where the evidence is more limited.

KEYWORDS: probiotics, dementia, cognitive impairment, mild cognitive impairment, cognitive decline, microbiota, systematic review and meta-analysis.

1. INTRODUCTION

Dementia is a chronic syndrome that affects the cognitive function of individuals, interfering with basic activities of daily living and social performance (Lee, Kim, Lee, Kim, & Kim, 2019). Approximately 47 million people worldwide suffer from dementia and, according to demographic estimates, this number would grow to 130 million people by 2050 if the prevalence of dementia remains constant today (Prince, Wimo, Guerchet, Ali & Wu, 2015).

Dementia typically begins to become visible around the seventh or eighth decade of life and worsens over time (Livingston et al., 2017). The most commonly detected initial symptoms are impaired short-term memory, followed by other symptoms such as withdrawal from hobbies or social activities, anxiety, depression and difficulties with orientation, language, attention and perception (Arvanitakis, Shah, & Bennett, 2019). Dementia is often preceded by a state called mild cognitive impairment (MCI), characterized by relatively intact daily function despite objective evidence of cognitive decline (Sanford, 2017). The transition of MCI to dementia is estimated to occur in 9.6% of cases in specialist clinical settings and 4.9% in community studies (Mitchell & Shiri-Feshki, 2009), whereas early interventions may foster symptomatic relief and delay disease progression (Tangalos, 2018).

Dementia can be classified into four main types: Alzheimer's disease (AD), Vascular Dementia (VD), Frontotemporal Dementia, and Dementia with Lewy bodies (Raz, Knoefel, & Bhaskar, 2016). AD is the most prevalent type of dementia, accounting for approximately 50 to 60% of all cases, followed by VD at 20% (Lee et al., 2019). Currently, the etiology of AD is unknown, albeit there are different theories postulated that would contribute to the development of the pathology. In this sense, the amyloidogenic hypothess is one of the most widely accepted hypotheses due to the presence of extracellular amyloid-beta (Aβ) deposits. However, multiple studies have not been able to reach their primary clinical outcomes in this line, which has led to significant concerns about this assumption (Hardy et al., 2014; Lopez et al., 2018; Sciacca, Tempra, Scollo, Milardi, & La Rosa, 2018). Genetic studies have revealed the implication of four genes: amyloid precursor protein (APP), presenilin 1 (PS1), presenilin 2 (PS2) and apolipoprotein E (ApoE) (Parihar & Hemnani, 2004). APP is metabolized by β- and γ-secretases. Fragments of Aβ are added and deposited forming toxic and insoluble

aggregates known as amyloid plaques (AP) (Tiwari, Atluri, Kaushik, Yndart, & Madhavan, 2019). The presence of neurofibrillary tangles (NFT) at the intracellular level is another characteristic alteration in AD. NFT are composed of helical filaments of abnormally hyperphosphorylated Tau protein. Physiologically, Tau acts by stabilizing the microtubules and thus promoting axonal transport. Therefore, its disordered accumulation leads to neuronal death (Lowe et al., 2018). Complementarily, a deterioration of brain-derived neurotrophic factor (BDNF) signaling has been shown in early stages of the disease associated with synaptic loss and cognitive impairment (CI) in patients and animal models of AD (Holsinger, Schnarr, Henry, Castelo, & Fahnestock, 2000; Peng et al., 2009). Furthermore, AD implicates an alteration in cholinergic system, although serotonin and noradrenergic systems dysfunction has also been described.

Pharmacological treatment may help improve thinking ability or change mood or behavior, but the benefits are limited (Dyer, Harrison, Laver, Whitehead, & Crotty, 2018). The gold treatment is cholinesterase inhibitors, which provide limited short-term cognitive benefits, whilst their clinical value remains a matter of controversy, considering the risk of side effects (Tricco et al., 2013; Hill et al., 2017). Indeed, the regulation of Amyloid-β protein precursor (AβPP) phosphorylation by small molecules is a novel therapeutic intervention for AD (Chen et al., 2019) . However, several studies have shown a reduction in Aβ load in the brain, but with the lack of beneficial effects on cognitive outcome measures, as well as impaired brain function (van der Kant, Goldstein, & Ossenkoppele, 2020). In addition, non-pharmacological strategies such as cognitive stimulation, music therapy, virtual reality, animal therapy or hyperbaric oxygen therapies are also frequently used (García-Soldevilla et al., 2019; Harch & Fogarty, 2018; You et al., 2019).

In recent years, several studies have shown a relationship between mental disorders such as AD, Parkinson's or depression and gut microbiota (GM) alterations (Aizawa et al., 2016; Keshavarzian et al., 2015; Vogt et al., 2017; Wallace & Milev, 2017). This relationship is based on the role of the gut-brain axis (GBA) as a bidirectional communication pathway between the gastrointestinal tract and the brain (Cryan et al., 2019; Dinan & Cryan, 2017). In this sense, probiotics, prebiotics, symbiotics, faecal transplantation, among other strategies, have shown a modulating capacity in GM. Even so, the term psychobiotic has also been used, as a probiotic administered in adequate quantities confers health benefits to patients with psychiatric disorders (Cheng, Liu, Wu,

Wang, & Tsai, 2019; Parmar, 2016; Sarkar et al., 2016). On this wise, anxiety and memory, functions that are altered in patients with dementia and CI are among the functions improved by the administration of probiotics (Jang, Lee, & Kim, 2019; Parashar & Udayabanu, 2017; Sharma & Singh, 2016). Therefore, the aim of this study is to review and analyse the available evidence on the effect of probiotics on MCI and dementia, as probiotics could attenuate or improve the CI associated with dementia or prevent cognitive decline.

2. MATERIALS AND METHODS

A systematic review and meta-analysis of clinical trials and preclinical studies published to date were carried out in March 2020. For this, the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) (Moher et al., 2009) and CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies) (Vesterinen et al., 2014) recommendations were followed, and the Cochrane Manual of Systematic Reviews was also considered (Higgins & Green, 2011). The protocol for this review was registered in PROSPERO (CRD42020188895). The structured Patient-Intervention-Outcomes question (PIO) (Stone, 2002) was used as follows: Are probiotics able to attenuate or improve the cognitive decline associated with dementia or MCI?

2.1. Data sources / information sources

A total of eight electronic databases were used for the search: Cochrane Library, ProQuest, Web of Science, PubMed-Medline, The Cumulative Index to Nursing and Allied Health Literature (CINAHL), Scopus, ScienceDirect and Open Grey. In addition, this search strategy was complemented by a snowball strategy.

In order to identify potential studies in electronic databases, using the PIO question as reference, the search strategy was used through a combination of natural and structured language, by using the Medical Subject Heading (MeSH) thesaurus (Table 1). After a preliminary search, it was agreed that outcome keywords for the final searches should be excluded as the available research was found to bee too limited.

INSERT TABLE 1 AROUND HERE

2.2. Study eligibility criteria

Studies included in this review were considered eligible if they met the following inclusion criteria:

- Randomised controlled trials (RCTs) or preclinical studies.
- Interventions which included the administration of probiotics, independently of their form of presentation.
- Studies on patients or animal models of dementia or CI.

Reviews, safety studies, non-randomized trials, conference publications, book chapters, trials with inadequate descriptions of intervention, and studies with only a theoretical approach to cognitive disorders were excluded. Studies were not excluded by date or language criteria.

2.3. Study selection and methodologic quality

The process of selection and analysis of the studies were carried out independently by two researchers (CR, LR). In case of discrepancy, another researcher (PR) was consulted. Likewise, the evaluation of the methodological quality of the full text articles was carried out in pairs, following the same procedure described. The following instruments were used for the evaluation process, according to the type of article:

- COCHRANE COLLABORATION Checklist for Clinical Trials (Higgins et al., 2011). This instrument consists of 7 items: random sequence generation, allocation concealment, blinding of participants and staff, blinding of evaluators of results, incomplete outcome data, selective reporting of results and other biases (Higgins et al., 2011). Each of these will be scored as 'criteria for a low risk of bias assessment' (+, 'criteria for a high risk of bias assessment' (-) or 'criteria for an unclear risk of bias assessment' (?)

- Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE'S) tool for preclinical testing (Hooijmans et al., 2014). This instrument consists of 10 evaluation items (sequence generation, baseline characteristics, allocation concealment, random housing, blinding of the investigator, random outcome assessment, blinding of outcome evaluators, incomplete outcome data, selective

outcome reporting and other sources of bias) (Hooijmans et al., 2014). Similarly, each section will be given a rating of "high" (-), "low" (+) or "unclear" (?) risk of bias.

The primary outcomes obtained in the selected articles describe the changes in the general cognitive function (memory, attention, language, orientation and learning), as well as the modifications observed in the BDNF levels of patients and animal models of AD and MCI. Moreover, secondary outcomes related to the histological and biochemical characteristics of the participants were also observed, such as synaptic plasticity, oxidative stress markers, cellular apoptosis or enzymatic levels.

2.4. Data extraction

The data from the selected studies included the following information: (1) reference; (2) country; (3) participants; (4) probiotic strain used; (5) duration of intervention; (6) variables analysed; (7) data collection tool and (8) main results.

In the situation that the results were only available in graphical format, data were extracted using the WebPlotDigitizer graph scanning software (Rohatgi, 2017). This software has previously been used in other reviews and meta-analyses and has been shown to be a valid method for extracting the data from studies (Guyot, Ades, Ouwens, & Welton, 2012; Tsafnat et al., 2014).

2.5. Statistical analyses

Meta-analysis of preclinical and clinical studies was performed with Review Manager (RevMan) software version 5.3 (The Cochrane Collaboration, 2014, Nordic Cochrane Center, Copenhagen, Denmark).

2.5.1. Effect size

The effect size was calculated from differences in means (MD) and standard deviation (SD) between intervention and control groups. For each variable analysed, the following tests were included:

- General cognitive function: Mini-Mental State Examination (MMSE), Repeatable Battery for the Assessment of Neurophychological Status (RBANS) and Test Your Memory (TYM).

- Spatial and non-spatial memory: Morris Water Maze test (MWM), Y- Maze test (Y-MT) and Passive Avoidance Test (PAT).
- BDNF: Values obtained through Western Blot, ELISA or Reverse Transcription PCR methods.

The studies included in the meta-analysis had to meet the following characteristics: (I) controlled clinical trials with random allocation to the probiotic group or placebo; (II) provide the mean, SD or standard error (SE) of the data obtained in each of the groups analysed. One article was excluded from the meta-analysis because it did not meet the second criteria and its data reported made meta-analysis impossible.

2.5.2. Heterogenity and publication bias

Depending on the degree of heterogeneity obtained, either a random-effects model or a fixed-effects model was used,. When I^2 was $\geq 50\%$, indicating significant heterogeneity, a random-effects model was used for meta-analysis. When I^2 was $< 50\%$, indicating no heterogeneity, a fixed-effect model was used. The analysis was represented at the Forest Plot. A value of $p < .05$ was considered significant.

Sensitivity analysis was used to observe the extent to which our results and conclusions were affected by changes in the data or the aim of the analysis. If the conclusions remained unchanged when the sensitivity analysis was applied, those conclusions were considered to be sound. In order to do this, each of the studies was excluded independently to identify those studies that significantly influenced the findings.

3. RESULTS

Out of a total of 1985 eligible articles listed in the initial search, 16 articles (10 preclinical and 6 clinical) that met the inclusion criteria for the systematic review, and 15 articles (10 preclinical and 5 clinical) for meta-analysis were finally included. Figure 1 shows the process for identifying and selecting studies.

INSERT FIGURE 1 AROUND HERE

3.1. Included studies

Table 2 shows the main characteristics of the included preclinical studies. 5 (50%) of them used rats as research subjects (Hwan Oh, Nam & Choi, 2020; Jeong, Woo, Kim,

Han, & Kim, 2015; Nimgampalle & Yellamma, 2017; Rezaei Asl et al., 2019; Rezaeiasl et al., 2019), while the other 5 used mice (Abraham et al., 2019; Corpuz et al., 2018; Kobayashi et al., 2017; Liu et al., 2015; Ni et al., 2019). The probiotics used were of the genera *Lactobacillus*, *Bifidobacterium* and *Clostridium.*

Half of the investigations (50%) were focused on animals models of AD induced after intracerebroventricular administration of Aβ (Kobayashi et al., 2017; Rezaei Asl et al., 2019; Rezaeiasl et al., 2019), after intraperitoneal administration of D-Galactose (Nimgampalle & Yellamma, 2017) or with the use of APP/PS1 transgenic mice (Abraham et al., 2019). One study (10%) used mice with induced VD after unilateral occlusion of the right common carotid artery (rUCCAO) (Liu et al., 2015). In addition, four studies (40%) analysed the effect of probiotics in animals with CI associated with aging, using 14- and 10-month-old mice (Corpuz et al., 2018; Ni et al., 2019), as well as 12- and 18-month-old rats (Hwan Oh et al. 2020; Jeong et al. 2015)

INSERT TABLE 2 AROUND HERE

Table 3 shows the main characteristics of the clinical studies included. In relation to the design of the clinical studies, all of them identified their research as a RCT (Agahi et al., 2018; Akbari et al., 2016; Hwang et al., 2019; Kobayashi, Kuhara, Oki, & Xiao, 2019; Tamtaji et al., 2019; Xiao et al., 2020). In half of the included studies (50%), the sample consisted of patients diagnosed with AD (Agahi et al., 2018; Akbari et al., 2016; Tamtaji et al., 2019); the remaining studies focused on individuals with MCI (Hwang et al., 2019;. Kobayashi et al., 2019; Xiao et al., 2020). The age range for selection of the participants differed in each study, ranging from 50 to 100 years. The most commonly used probiotics belonged to the genera *Lactobacillus* and *Bifidobacterium*, with an average length of treatment of 12 weeks.

INSERT TABLE 3 AROUND HERE

3.2. Methodological quality: assessment of bias

Figure 2A presents the assessment of the methodological quality and potential risk of bias of the included preclinical studies, evaluated using the SYRCLE'S scale. With the exception of the study by Rezaei Asl and colleagues (2019), most preclinical trials showed a questionable risk of bias in aspects such as describing their randomization and blinding process. Figure 2B shows the overall score for each item. It can be seen that both

the blinding of the evaluators of the results, the randomization for the same and the masking of the assignment are items that represent ceratin doubts, since the procedure used for these are not clearly described. Similarly, the blinding process for the evaluation, the allocation process and the description of the characteristics of the reference group were those with the highest risk of bias.

INSERT FIGURE 2 AROUND HERE

In relation to the clinical trials, Figure 3A shows the assessment of the methodological quality and possible risk of bias of the included studies, evaluated using the Cochrane Collaboration scale. The studies by Tamtaji and collaborators (2019) and Xiao (2020), showed a low risk of bias; the majority of trials (66,7%) reflected a doubtful risk of bias (Agahi et al., 2018; Akbari et al., 2016; Hwang et al., 2019; Kobayashi et al., 2019). Figure 3B shows a graph with the overall score in each item. It can be seen that both the blinding of participants, researchers and evaluators and the assignment process are the items of greatest doubt, since the procedure used for these are not clearly described.

INSERT FIGURE 3 AROUND HERE

3.3. Dementia

3.3.1. Alzheimer's disease

A total of 5 preclinical studies evaluated the effect of probiotics on cognitive function in animal models of AD (Abraham et al., 2019; Kobayashi et al., 2017; Nimgampalle & Yellamma, 2017; Rezaei Asl et al., 2019; Rezaeiasl et al., 2019). The study conducted by Nimgampalle and colaborators (2017) analysed the result after 60 days of administration of *Lactobacillus plantarum* MTCC1325 in rat model of AD, observing a significant reduction ($p < .05$) in the escape latency compared to control group (CG), indicating an improvement in memory and spatial learning. In the same line, the investigations by Rezaeiasl and collaborators (2019) and Rezaei Asl (2019) showed a significant decrease ($p < .004$) in the escape latency in rats that consumed the same multi-species probiotic during 6 and 8 weeks respectively, although no significant differences were observed in the total distance traveled, the speed of movement and the total time spent on the hidden platform, compared to the CG (Rezaeiasl et al., 2019).

In relation to non-spatial and working memory, Kobayashi and collaborators (2017) showed that oral intake of *Bifidobacterium breve* A1 in mice significantly increased the latency time in PAT ($p < .05$) and the number of alternations in Y-MT ($p < .001$).

Likewise, the study by Abraham and collaborators (2019) evaluated the effect of physical exercise together with the administration of a multi-species probiotic (*Bifidobacterium longum* and *Lactobacillus acidophilus*) in mice model of AD, observing a significant decrease in the escape latency ($p < .05$) and an increase in the number of alternations ($p < .005$) compared to the physical exercise only group and to the CG.

At the biochemical and histological level, Nimgampalle & Yellamma (2017) showed a significant increase ($p < .05$) in ACh levels followed by a decrease in AChE in the hippocampus and cerebral cortex. Similarly, animal models that received the probiotic improved in synaptic plasticity and decreased in AP and MDA oxidative index (Abraham et al., 2019; Rezaei Asl et al., 2019; Rezaeiasl et al., 2019).

Despite the extensive results observed in preclinical research, clinical studies are still limited, with only 3 studies evaluating the role of probiotics on general cognitive function in individuals who are suffering AD (Agahi et al., 2018; Akbari et al., 2016; Tamtaji et al., 2019). In this sense, the study conducted by Akbari and collaborators (2016) showed that the administration of a multi-species probiotic for 12 weeks significantly minimized CI ($p < .001$), measured through the MMSE, compared to CG. Besides, the combination of a multi-species probiotic with selenium over a 12-week period significantly improved the MMSE score ($p < .001$), compared to the CG and the selenium-only group (Tamtaji et al., 2019). However, the research conducted by Agahi and collaborators (2018) did not find a significant improvement in cognitive function ($p = 0.82$), as measured by the TYM test, after 12 weeks of treatment with a multi-species probiotic.

3.3.2. Vascular Dementia

Only the article by Liu and collaborators (2015) explored the role of probiotics in VD. Their results showed a significant decrease in escape latency ($p < .05$) and increased time spent on the platform quadrant ($p < .001$) in mice treated with *Clostridium butyricum* for 6 weeks, suggesting an improvement in memory and spatial learning.

In relation to histological and biochemical characteristics, the probiotic succeeded in attenuating morphological changes in the cells of the hippocampus and significantly raising BDNF levels ($p < .001$). In addition, there was also evidence of an increase in the ratio pAkt/Akt and Bcl-2/Bax, which resulted in a reduction in cell apoptosis (Liu et al., 2015).

3.4. Cognitive Impairment

The effect of probiotics in CI was evaluated in four preclinical studies (Corpuz et al., 2018; Hwan Oh et al., 2020; Jeong et al., 2015; Ni et al., 2019). In relation to memory and learning, Corpuz and collaborators (2018) carried out a study to analyse the impact of the probiotic *Lactobacillus paracasei* K71 on the cognitive state of mice with accelerated senescence. Their results indicated an improvement in spatial and non-spatial memory compared to the CG and the *Lactobacillus* 327 group, characterized by a significant reduction in escape latency ($p < .05$), an increase in platform area time and in latency time ($p < .05$). In addition, Jeong and collaborators (2015) observed that administration of *Lactobacillus pentosus var. plantarum* C29 for 8 weeks in a group of CI rats showed a significant decrease in escape latency, increased time on the hidden platform and an increase in the number of alternations compared to CG.

In the same line, Ni and collaborators (2019) compared the efficacy of two different probiotics, *Lactobacillus casei* and *Bifidobacterium longum*, on memory and learning in elderly mice with CI. Their results showed a reduction in the escape latency in both treatment groups, being significantly lower ($p < .05$) in the mice treated with *Bifidobacterium longum*. The discrimination and recognition rate increased significantly ($p < .05$) after administering both probiotics. Aditionally, avoidance frequencies were increased in both groups ($p < .001$), indicating an improvement in learning ability (Ni et al., 2019).

The study by Hwan Oh and collaborators (2020) showed that the administration of the glycoprotein from *Capsosiphon fulvescens* together with the probiotic *Lactobacillus plantarum* potency improved spatial memory in CI rats. Thus, a significant reduction in the escape latency ($p < .005$) was observed, compared to the mono-treatment of the glycoprotein or the probiotic.

Neurophysiologically, Corpuz and collaborators (2018) and Jeong (2015) observed a significant increase ($p < .05$) of the biomarkers of neuronal plasticity (BDNF and p-CREB) in the hippocampus of animals with CI after administering the probiotic, but not significant differences were observed in the cerebral cortex. Antioxidant Nrf2 proteins levels and neurodegenerative factors (Sirt1, FoxO1, FoxO3, P27 and Wisp 1) were also increased ($p < .05$) after probiotics treatment in older animals (Hwan Oh et al., 2020; Ni et al., 2019).

On the other hand, three clinical trials have been developed to understand the effect of probiotics on the cognitive function of individuals with CI (Hwang et al., 2019; Kobayashi et al., 2019; Xiao et al., 2020). In this regard, research by Kobayashi et al. (2019) studied the effect of *Bifidobacterium Breve* A1 in older adults with MCI for 12 weeks. Their results showed an improvement in language, attention, orientation and calculation (RBANS), both in the CG and in the probiotic group (PG). Similarly, the total MMSE score did not differ between both groups, although a significant increase in site orientation ($p < .001$), calculation ($p < .05$) and language ($p < .001$) was observed in the PG. The effect of this probiotic was also evaluated in a recent study by Xiao et al. (2020), in which the administration of *Bifidobacterium Breve* A1 for 16 weeks significantly ($p < .0001$) improved the overall RBANS score in MCI patients, especially in immediate memory, visuospatial, and delayed memory domains.

The effect of the probiotic *Lactobacillus Plantarum* C-29 on adults with MCI after 12 weeks of treatment was analysed by Hwang and collaborators (2019). Their results reflected a significant improvement in the cognitive function of the PG compared to the CG ($p = .02$), especially in the attention domain ($p = .02$). Furthermore, this improvement was associated with higher serum BDNF levels after probiotic consumption ($p = .007$) (Hwang et al., 2019).

3.5. Meta-analysis results

A total of 15 articles were included in the meta-analysis (10 preclinical and 5 clinical). The meta-analysis was performed on the effect of probiotics on general cognitive function, on spatial and non-spatial memory, and on BDNF levels.

.3.5.1. Effect of probiotics on general cognitive function

A total of 5 clinical studies evaluated the effect of probiotics on general cognitive function (Agahi et al., 2018; Akbari et al., 2016; Kobayashi et al., 2019; Tamtaji et al., 2019; Xiao et al., 2020). Meta-analysis of MMSE, RBANS or TYM test scores show an overall significant effect ($p < .0001$) (Figure 4), with a high heterogeneity observed ($I^2 = 99\%$).

INSERT FIGURE 4 AROUND HERE

3.5.2. Effect of probiotics on spatial and non-spatial memory

A total of 10 preclinical studies evaluated the effect of probiotics on spatial and non-spatial memory (Abraham et al., 2019; Corpuz et al., 2018; Hwan Oh et al., 2020; Jeong

et al., 2015; Kobayashi et al., 2017; Liu et al., 2015; Ni et al., 2019; Nimgampalle & Yellamma, 2017; Rezaei Asl et al., 2019; Rezaeiasl et al., 2019). Figure 5 shows the meta-analysis on spatial memory (10 studies; n=179), which showed a moderate heterogeneity (I^2 = 40%) with a significant improvement attributable to the PG (Z= 12.07; p < .00001). In non-spatial memory (2 studies; n=40) the meta-analysis showed a moderate heterogeneity (I^2 = 33%) with a significant improvement in favor of the PG (Z= 2.50; p = .01) (Figure 6).

INSERT FIGURE 5 AND 6 AROUND HERE

3.5.3. Effect of probiotics on BDNF levels

A total of 4 preclinical studies examined the effect of probiotics on BDNF levels (Corpuz et al., 2018; Jeong et al., 2015; Liu et al., 2015; Ni et al., 2019). Figure 7 shows the meta-analysis (4 studies; n=58) with a high heterogeneity (I2 = 62%) and a significant increase in BDNF levels after probiotic administration (Z= 7.82; p < .00001).

INSERT FIGURE 7 AROUND HERE

In relation to the sensitivity analysis, the results revealed no significant changes in the total effects with respect to subgroups.

4. DISCUSSION

The aim of this systematic review and meta-analysis was to review and analyse the available evidence about the effect of probiotics supplementation on MCI and dementia. To our knowledge, this systematic review and meta-analysis is the first to summary data that includes preclinical and clinical evidence on how effective certain probiotics are in modulating cognitive function loss seen on dementia and CI patients.

We reviewed the effects of probiotics on multiple cognitive functions in both patients and animal models of dementia and CI. Results suggest an improvement in spatial and non-spatial memory and in cognitive status. In addition, probiotics supplementation improves the biochemical and histology measures related with them (BDNF and p-CREB, between others). According to the cognitive status, our systematic review and meta-analysis found an improvement both in PG and CG. This improvement was observed after administration of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, *Bifidobacterium Breve* A1, and *Bifidobacterium longum* over a time period of 2 weeks to 16 weeks, primarly in language, attention, orientation and calculation domains (Akbari et al., 2016; Hwang et al., 2019;

Tamtaji et al., 2019; Xiao et al., 2020). However, this improvement in cognitive status was not found in the study of Agahi and collaborators (2018) after the administration of a multi-probiotic. It is interesting to note that improvement was significant in trials assessed by MMSE, the most commonly used; given that only two trials used other tests: RBANS and TYM. In this sense, MMSE has been the most used screening instrument for decades, being accurate for the detection of AD (Pinto et al., 2019). Thus, more studies evaluating probiotics supplementation on AD or other dementia are needed to explore their effects using other triage tests, such as Montreal Cognitive Assessment (MOCA).

In relation to memory, our findings suggest a significant improvement attributed to probiotic intervention in spatial and non-spatial memory, both in preclinical and clinical evidence (Abraham et al., 2019; Akbari et al., 2016; Corpuz et al., 2018; Hwan Oh et al., 2020; Hwang et al., 2019; Jeong et al., 2015; Kobayashi et al., 2017; Liu et al., 2015; Ni et al., 2019; Nimgampalle & Yellamma, 2017; Rezaei Asl et al., 2019; Rezaeiasl et al., 2019; Tamtaji et al., 2019). This memory improvement after probiotic treatment was observed in both AD subjects and CI subjects following multi-probiotic administration.

Therefore, our meta-analysis provides evidence that probiotics could be potential candidates for the control or prevention of age-related dementia and age-related CI. However, specific underlying mechanisms of this CI should be elucidated. Several studies reviewed supported the cognitive improvement in AD or animal models with biochemical and histology measures. In fact, the administration of probiotics restore synaptic plasticity (Rezaei Asl et al., 2019; Rezaeiasl et al., 2019), prevent the accumulation of AP (Abraham et al., 2019; Rezaei Asl et al., 2019) and modulate the levels of BDNF and cell cycle biomarkers (Liu et al., 2015; Rezaei Asl et al., 2019) in animals models of AD. In the same line, an increased protein expression of BDNF and CREB phosphorylation in the hippocampus was observed after probiotic treatment in rodents with CI (Corpuz et al., 2018; Jeong et al., 2015). Additionally, after co-administration of glycoproteins from edible algae *Capsosiphon fulvescens* along with *L. plantarum* in aged rats showed an improvement in spatial memory involving BDNF signaling in the dorsal hippocampus, as well as a down-regulated relationship between eEF2 kinase and JNK (Hwan Oh et al., 2020). Moreover, a combination of *Lactobacillus casei* LC122 and *Bifidobacterium longum* BL986 increased the secretion of mucin and tight junction as Claudin 1 or zonula occludens-1 in colon, as well as increased the expression of BDNF in the hippocampus of aged mice (Ni et al., 2019).

Both preclinical and clinical results have shown a potential and important role of GM in the etiology or pathogenesis of dementia and CI through the GBA; however, the exact mechanisms of CI and dementia is not completely understood. In general, it has been suggested that problems in the composition of the GM may be a contributor for behavioral disorders. For example, the bacterial fecal composition was modified in transgenic mice for APP, increasing bacteria of the genus *Rikenellaceae*, while *Akkermansia* and *Allobaculum* were shown to decrease (Harach et al., 2017). Inducing dysbiosis to a Drosophila AD model resulted in increased brain neuronal loss, while locomotor activity and life expectancy decreased. Besides, the alteration of the intestinal composition aggravated the inflammation in the brain of these flies (Wu, Cao, Chang, & Juang, 2017).

There are various theories that would contribute to the development of the pathology, for example: bacterial metabolites and amyloids can trigger central nervous system inflammation and cerebrovascular degeneration; impaired GM flora inhibits the autophagy-mediated protein clearance process; and gut microbiomes can change the neurotransmitter levels in the brain through vagal afferent fibres (Bostancikliоğlu, 2019). Indeed, a recent systematic review has related the GM with the glutamate metabolism, suggesting that d-glutamate metabolized by the gut bacteria may influence the glutamate NMDAR and cognitive function in dementia patients (Chang, Lin, & Lane, 2020).

Some of the mechanism discussed in the literature implicate GM dysbiosis associated with AD, that could affect short-chain fatty acid (SCFA) metabolism. In this sense, SCFAs are capable of potently inhibiting Aβ aggregations, protecting against AD. Indeed that SCFAs could regulate microglia homeostasis (reviewed in Shen & Ji, 2018). Bacterial-produced amyloids differ from brain amyloid in their structure, but are able to enhance an immunity response and even *E.coli* infection increases neuronal alpha-synuclein deposition in both gut and brain in rats (Chapman et al., 2002; Chen et al., 2016). Aβ is recognized as an antimicrobial peptide participating in the innate immune response, which their seeding and propagation may occur at different levels of the GBA; including neuron-to-neuron or distal neuron spreading, direct blood-brain barrier crossing or via other cells as astrocytes, fibroblasts, microglia, and immune system cells (Kowalski & Mulak, 2019).

In addition, the systemic inflammation and disruption of physiological barriers could be related with the GM disruption, given that gut inflammation and dysbiosis are directly associated with gut barrier dysfunction and increased intestinal permeability that can

contribute to the process of neurodegeneration (Khan, Ikram, Park, Park, & Kim, 2020). Furthermore, in AD patients, neuroinflammation has been reported, expressed by activation of microglia, reactive astrocytes, and complement in the vicinity of AP. And in that way, the Aβ has a key and fundamental action (Kowalski & Mulak, 2019).

Another critical aspect in dementia's physiology is BDNF protein, which is essential for neuronal development, differentiation, survival, and neuronal plasticity, and their expression is crucial for preserving brain function in cognitive processes. Circulating BDNF levels decrease with aging, also related to reduced hippocampal volume and poorer memory performance (Erickson et al., 2010). A deterioration of BDNF signaling has been shown in the early stages of the disease associated with synaptic loss and CI in patients and animal models of AD (Holsinger et al., 2000; Peng et al., 2009). In that sense, our meta-analysis showed a significant increase of BDNF after probiotic administration (Corpuz et al., 2018; Jeong et al., 2015; Liu et al., 2015; Ni et al., 2019).

Probiotics administration is able to restore synaptic plasticity (Rezaei Asl et al., 2019; Rezaeiasl et al., 2019), preventing the accumulation of AP (Abraham et al., 2019; Rezaei Asl et al., 2019) and efficiently modulating the neurotrophin (BDNF) and cell cycle biomarker levels (Liu et al., 2015; Rezaei Asl et al., 2019). In the study of Azm et al. (2017), their results showed an improvement in the oxidative stress biomarkers and a reduction of plaques in rats that received a multi-species probiotic, as well as the suppression of microglia and pro-inflammatory cells activation compared to mice in the CG (Azm et al., 2017; Bonfili et al., 2017). Furthermore, several studies have observed an increase in BDNF levels and CREB phosphorylation in the hippocampus, indicating an improvement in cognitive function compared to the CG (Corpuz et al., 2018; Jeong et al., 2015). Similar results were obtained in the research by Hwan Oh et al. (2020), where the combination of *Lactobacillus plantarum* and Cf-hGP increased both BDNF and Nrf2 phosphorylation, and decreased JNK phosphorylation compared to rats of advanced age, providing protection against oxidative stress. In the same vein, Ni et al. (2019) demonstrated that the characteristic reduction of neurodegenerative and neurotrophic factors in CI can be reversed with the consumption of probiotics, protecting animals against these changes. However, the research by Chung et al. (2014) did not obtain differences in BDNF levels between CG and PG.

Likewise, among cognition benefits associated with the probiotic consumption, several research has evaluated the effects of probiotics on CI-related deseases (Ceccarelli et al.,

2017; Liang et al., 2015; Luo et al., 2014; Perez-Pardo et al., 2017; Roman et al., 2018; Rudzki et al., 2019; Savignac, Tramullas, Kiely, Dinan, & Cryan, 2015). For example, the improvement of impulsivity and decision-making on patients diagnosed by fibromyalgia, after the administration of a multi-probiotic formulation.

Although our aim was to explore probiotics effects, other interventions that modulate GM have also shown a cognitive improvement in AD patients. In this manner, improvement in spatial learning ability, oxidative stress, inflammation and neurotransmitter synthesis was observed after prebiotic administration in AD rats (Chen et al., 2017). Furthermore, cell apoptosis and AP deposition were reduced. Similar results were found in the study by Chunchai and collaborators (2018), where, the administration of a xylooligosaccharide formula also reduced microglial function, restored synaptic plasticity and attenuated cerebral mitochondrial dysfunction in rats with induced dementia. In the study of Romo-Araiza et al. (2018), co-administration of the probiotic *Enterococcus faecium* with agave inulin for five weeks showed an improvement in spatial learning and memory, inflammatory status, BDNF, and butyrate levels, providing greater protection against age-related CI. Similarly, Nagpal and colleagues (2019) demonstrated that a ketogenic Mediterranean diet improved cognitive status and restored GM in individuals with CI compared to the CG.

Moreover, symbiotics, the combination of both prebiotic and probiotic, improved memory, visuospatial and abstraction skills, executive function and language in patients with AD. Also, a decrease in inflammatory markers and oxidative stress, as in cellular apoptosis was observed after its administration (Ton et al., 2020). Another potentially treatmen therapeutic option is fecal microbiota transplantation, where an improvement in memory spatial was observed that was accompanied too by decreased phosphorylation of tau protein and the levels of Aβ40 and Aβ42, as well as reversing the changes of GM and SCFAs (Sun et al., 2019).

In this systematic review and meta-analysis, we have focused only on the treatment-related with the GM, however, another study proposed that GM could be used as diagnostic criteria for AD. For example, by means of new types of biomarkers such as neuroinflammatory indices and mRNA, or by identifying and validating specific bacterial taxa sensitively altered in AD patients, helping to predict or detect AD and enhance the detection accuracy. Nevertheless, GM-based diagnosis is still premature with the current data available (Shen & Ji, 2018).

However, this systematic review and meta-analysis is not free of limitations. Firstly, most of the existing research exploring the potential modulation effects of probiotics on cognitive function have been conducted in animal models. Indeed, comprehensive profiling of GM composition and functionality was carried out in only six studies analysed (Abraham et al., 2019; Hwang et al., 2019; Kobayashi et al., 2017; Liu et al., 2015; Ni et al., 2019; Rezaei Asl et al., 2019). It is worth noting that the studies reviewed used different strains of probiotics, the majority of which used multi-strain probiotics. In this sense, it is difficult to discern which strain can produce the effects and therefore, further research on the evaluation of individual strains is required to identify strains that may have positive effects on CI.

5. CONCLUSION

Based on the results of this systematic review and meta-analysis, probiotics supplementation could be an adequate therapeutic strategy both in dementia and CI based on clinical and preclinical evidence. The most widely used probiotic strains were from the genera *Lactobacillus* and *Bifidobacterium*, highlighting the species *L. acidophilus* and *B. bifidum* in the preclinical studies, and species *B. longum* in the clinical trials. Having said that, additional research is needed to conclude on the specificity of the strain on this population.

Several pathogenesis mechanisms suggest the relation between GM and these medical conditions, such of these are the central nervous system inflammation and cerebrovascular degeneration, inhibitions of the autophagy-mediated protein clearance process and changes in brain neurotransmitter levels. However, as of this writing, the results are limited. So then it is necessary to translate the preclinical data into clinical data, where the evidence is more scarce. In addition, clinical studies could explore the effects of other microbiota modulators, such as prebiotics, synbiotics, or fecal transplantation, given that these strategies are almost unexplored.

REFERENCES

Abraham, D., Feher, J., Scuderi, G. L., Szabo, D., Dobolyi, A., Cservenak, M., … Radak, Z. (2019). Exercise and probiotics attenuate the development of Alzheimer's disease in transgenic mice: Role of microbiome. *Experimental Gerontology*, *115*, 122-131. https://doi.org/10.1016/j.exger.2018.12.005

Agahi, A., Hamidi, G. A., Daneshvar, R., Hamdieh, M., Soheili, M., Alinaghipour, A., … Salami, M. (2018). Does severity of Alzheimer's disease contribute to its responsiveness to modifying gut microbiota? A double blind clinical trial. *Frontiers in Neurology*, *9*(AUG). https://doi.org/10.3389/fneur.2018.00662

Aizawa, E., Tsuji, H., Asahara, T., Takahashi, T., Teraishi, T., Yoshida, S., … Kunugi, H. (2016). Possible association of Bifidobacterium and Lactobacillus in the gut microbiota of patients with major depressive disorder. *Journal of Affective Disorders*, *202*, 254-257. https://doi.org/10.1016/j.jad.2016.05.038

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

Arvanitakis, Z., Shah, R. C., & Bennett, D. A. (2019). Diagnosis and Management of Dementia: Review. *JAMA - Journal of the American Medical Association*, *322*(16), 1589-1599. https://doi.org/10.1001/jama.2019.4782

Azm, S. A. N., Djazayeri, A., Safa, M., Azami, K., Ahmadvand, B., Sabbaghziarani, F., … Vafa, M. (2017). Lactobacillus and Bifidobacterium Ameliorate Memory and Learning Deficits and oxidative stress in Aβ (1-42) Injected Rats Journal: *Applied Physiology, Nutrition, and Metabolism*.

Bonfili, L., Cecarini, V., Berardi, S., Scarpona, S., Suchodolski, J. S., Nasuti, C., … Eleuteri, A. M. (2017). Microbiota modulation counteracts Alzheimer ' s disease progression influencing neuronal proteolysis and gut hormones plasma levels, (April), 1-21. https://doi.org/10.1038/s41598-017-02587-2

Bostanciklioğlu, M. (2019). The role of gut microbiota in pathogenesis of Alzheimer's disease. *Journal of Applied Microbiology*, *127*(4), 954-967.

https://doi.org/10.1111/jam.14264

Ceccarelli, G., Fratino, M., Selvaggi, C., Giustini, N., Serafino, S., Schietroma, I., … d'Ettorre, G. (2017). A pilot study on the effects of probiotic supplementation on neuropsychological performance and microRNA-29a-c levels in antiretroviral-treated HIV-1-infected patients. *Brain and Behavior*, *7*(8), 1-8. https://doi.org/10.1002/brb3.756

Chang, C., Lin, C., & Lane, H. (2020). D-glutamate and Gut Microbiota in Alzheimer's Disease. *International Journal of Molecular Sciences*, *21*(2676), 1-17.

Chapman, M. R., Robinson, K. S., Pinkner, J. S., Roth, R., Heuser, J., Hammar, M., … Hultgren, S. (2002). Role of Escherichia coli Curli Operons in Directing Amyloid Fiber Formation. *Science*, *295*(5556), 851-855. https://doi.org/10.1126/science.1067484.Role

Chen, D., Yang, X., Yang, J., Lai, G., Yong, T., Tang, X., … Wu, Q. (2017). Prebiotic effect of Fructooligosaccharides from Morinda officinalis on Alzheimer's disease in rodent models by targeting the microbiota-gut-brain axis. *Frontiers in Aging Neuroscience*, *9*(DEC). https://doi.org/10.3389/fnagi.2017.00403

Chen, C, Zeldich, E., Khodr, C., Camara, K., Tung, T. Y., Lauder, E. C., … Abraham, C. R. (2019). Small Molecule Amyloid-β Protein Precursor Processing Modulators Lower Amyloid-β Peptide Levels via cKit Signaling. *Journal of Alzheimer's Disease*, *67*(3), 1089-1106. https://doi.org/10.3233/JAD-180923

Chen, S. G., Stribinskis, V., Rane, M. J., Demuth, D. R., Gozal, E., Roberts, A. M., … Friedland, R. P. (2016). Exposure to the Functional Bacterial Amyloid Protein Curli Enhances Alpha-Synuclein Aggregation in Aged Fischer 344 Rats and Caenorhabditis elegans. *Scientific Reports*, *6*(October), 1-10. https://doi.org/10.1038/srep34477

Cheng, L., Liu, Y., Wu, C., Wang, S., & Tsai, Y. (2019). Psychobiotics in mental health , neurodegenerative and neurodevelopmental disorders. *Journal of Food and Drug Analysis*, *27*(3), 632-648. https://doi.org/10.1016/j.jfda.2019.01.002

Chunchai, T., Thunapong, W., Yasom, S., Wanchai, K., Eaimworawuthikul, S., Metzler, G., … Chattipakorn, S. C. (2018). Decreased Microglial Activation Through Gut-brain Axis by Prebiotics, Probiotics, or Synbiotics Effectively

Restored Cognitive Function in Obese-insulin Resistant Rats. *Journal of Neuroinflammation*, *15*(1). https://doi.org/10.1186/s12974-018-1055-2

Chung, Y.-C., Jin, H.-M., Cui, Y., Kim, D. S., Jung, J. M., Park, J.-I., … Chae, S.-W. (2014). Fermented milk of Lactobacillus helveticus IDCC3801 improves cognitive functioning during cognitive fatigue tests in healthy older adults. *Journal of Functional Foods*, *10*, 465-474. https://doi.org/10.1016/j.jff.2014.07.007

Corpuz, H. M., Ichikawa, S., Arimura, M., Mihara, T., Kumagai, T., Mitani, T., … Katayama, S. (2018). Long-term diet supplementation with lactobacillus paracasei K71 prevents age-related cognitive decline in senescence-accelerated mouse prone 8. *Nutrients*, *10*(6). https://doi.org/10.3390/nu10060762

Cryan, J. F., O'riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., … Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews*, *99*(4), 1877-2013. https://doi.org/10.1152/physrev.00018.2018

Dinan, T. G., & Cryan, J. F. (2017). Brain-Gut-Microbiota Axis and Mental Health. *Psychosomatic Medicine*, *79*(8), 920-926. https://doi.org/10.1097/PSY.0000000000000519

Dyer, S. M., Harrison, S. L., Laver, K., Whitehead, C., & Crotty, M. (2018). An overview of systematic reviews of pharmacological and non-pharmacological interventions for the treatment of behavioral and psychological symptoms of dementia. *International Psychogeriatrics*, *30*(3), 295-309. https://doi.org/10.1017/S1041610217002344

Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Heo, S., Mclaren, M., … Kramer, A. F. (2010). Brain-Derived Neurotrophic Factor Is Associated with Age-Related Decline in Hippocampal Volume, *i*(15), 5368-5375. https://doi.org/10.1523/JNEUROSCI.6251-09.2010

García-Soldevilla, M. A., Enjuanes García, A., Barragán Martínez, D., Ayuso Peralta, L., Rojo Sebastián, A., & Tejeiro Martínez, J. (2019). Therapeutic aspects in dementias. *Medicine (Spain)*, *12*(74), 4357-4366. https://doi.org/10.1016/j.med.2019.03.014

Guyot, P., Ades, A., Ouwens, M. J., & Welton, N. J. (2012). Enhanced secondary analysis of survival data: reconstructing the data from published Kaplan-Meier

survival curves. *BMC Medical Research Methodology*, *12*(1), 9. https://doi.org/10.1186/1471-2288-12-9

Harach, T., Marungruang, N., Duthilleul, N., Cheatham, V., Mc Coy, K. D., Frisoni, G., … Bolmont, T. (2017). Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. *Scientific Reports*, *7*(February). https://doi.org/10.1038/srep41802

Harch, P. G., & Fogarty, E. F. (2018). Hyperbaric oxygen therapy for Alzheimer's dementia with positron emission tomography imaging: A case report. *Medical Gas Research*, *8*(4), 181-184. https://doi.org/10.4103/2045-9912.248271

Hardy, J., Bogdanovic, N., Winblad, B., Portelius, E., Andreasen, N., Cedazo-Minguez, A., & Zetterberg, H. (2014). Pathways to Alzheimer's disease. *Journal of Internal Medicine*, *275*(3), 296-303. https://doi.org/10.1111/joim.12192

Higgins, J. P., & Green, S. (2011). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. *The Cochrane Collaboration*.

Higgins, J. P., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., … Sterne, J. A. C. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ (Online)*, *343*(7829), 1-9. https://doi.org/10.1136/bmj.d5928

Hill, N. T. M., Mowszowski, L., Naismith, S. L., Chadwick, V. L., Valenzuela, M., & Lampit, A. (2017). Computerized cognitive training in older adults with mild cognitive impairment or dementia: A systematic review and meta-analysis. *American Journal of Psychiatry*, *174*(4), 329-340. https://doi.org/10.1176/appi.ajp.2016.16030360

Holsinger, R. M. D., Schnarr, J., Henry, P., Castelo, V. T., & Fahnestock, M. (2000). Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction : decreased levels in Alzheimer ' s disease, 347-354.

Hooijmans, C. R., Rovers, M. M., De Vries, R. B. M., Leenaars, M., Ritskes-Hoitinga, M., & Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Medical Research Methodology*, *14*(1), 1-9. https://doi.org/10.1186/1471-2288-14-43

Hwan Oh, J., Nam, T.-J., & Choi, Y. H. (2020). Capsosiphon fulvescens Glycoproteins Enhance Probiotics-Induced Cognitive Improvement in Aged Rats. *Nutrients*, *12*(3), 837. https://doi.org/10.3390/nu12030837

Hwang, Y. H., Park, S., Paik, J. W., Chae, S. W., Kim, D. H., Jeong, D. G., … Chung, Y. C. (2019). Efficacy and safety of lactobacillus plantarum C29-fermented soybean (DW2009) in individuals with mild cognitive impairment: A 12-week, multi-center, randomized, double-blind, placebo-controlled clinical trial. *Nutrients*, *11*(2). https://doi.org/10.3390/nu11020305

Jang, H.-M., Lee, K.-E., & Kim, D.-H. (2019). The Preventive and Curative Effects of Lactobacillus reuteri NK33 and Bifidobacterium adolescentis NK98 on Immobilization Stress-Induced Anxiety/Depression and Colitis in Mice. *Nutrients*, *11*(4), 819. https://doi.org/10.3390/nu11040819

Jeong, J. J., Woo, J. Y., Kim, K. A., Han, M. J., & Kim, D. H. (2015). Lactobacillus pentosus var. plantarum C29 ameliorates age-dependent memory impairment in Fischer 344 rats. *Letters in Applied Microbiology*, *60*(4), 307-314. https://doi.org/10.1111/lam.12393

Keshavarzian, A., Green, S. J., Engen, P. A., Voigt, R. M., Naqib, A., Forsyth, C. B., … Shannon, K. M. (2015). Colonic bacterial composition in Parkinson's disease. *Movement Disorders*, *30*(10), 1351-1360. https://doi.org/10.1002/mds.26307

Khan, M. S., Ikram, M., Park, J. S., Park, T. J., & Kim, M. O. (2020). Gut Microbiota, Its Role in Induction of Alzheimer's Disease Pathology, and Possible Therapeutic Interventions: Special Focus on Anthocyanins Muhammad. *Cells*, *9*(853), 1-21.

Kobayashi, Y., Kuhara, T., Oki, M., & Xiao, J. Z. (2019). Effects of bifidobacterium breve a1 on the cognitive function of older adults with memory complaints: A randomised, double-blind, placebo-controlled trial. *Beneficial Microbes*, *10*(5), 511-520. https://doi.org/10.3920/BM2018.0170

Kobayashi, Y., Sugahara, H., Shimada, K., Mitsuyama, E., Kuhara, T., Yasuoka, A., … Xiao, J. Z. (2017). Therapeutic potential of Bifidobacterium breve strain A1 for preventing cognitive impairment in Alzheimer's disease. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-13368-2

Kobayashi, Y., Sugahara, H., Shimada, K., Mitsuyama, E., Kuhara, T., Yasuoka, A., …

Xiao, J. Z. (2017). Therapeutic potential of Bifidobacterium breve strain A1 for preventing cognitive impairment in Alzheimer's disease. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-13368-2

Kowalski, K., & Mulak, A. (2019). Brain-gut-microbiota axis in Alzheimer's disease. *Journal of Neurogastroenterology and Motility*, *25*(1), 48-60. https://doi.org/10.5056/jnm18087

Lee, H. Y., Kim, D. W., Lee, W., Kim, H. Y., & Kim, Y. S. (2019). Preventive approach for overcoming dementia. *Archives of Pharmacal Research*, *42*(8), 647-657. https://doi.org/10.1007/s12272-019-01168-3

Liang, S., Wang, T., Hu, X., Luo, J., Li, W., Wu, X., … Jin, F. (2015). Administration of Lactobacillus helveticus NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. *Neuroscience*, *310*(September), 561-577. https://doi.org/10.1016/j.neuroscience.2015.09.033

Liu, J., Sun, J., Wang, F., Yu, X., Ling, Z., Li, H., … Xu, J. (2015). Neuroprotective Effects of Clostridium butyricum against Vascular Dementia in Mice via Metabolic Butyrate. *BioMed Research International*, *2015*. https://doi.org/10.1155/2015/412946

Livingston, G., Sommerlad, A., Orgeta, V., Costafreda, S. G., Huntley, J., Ames, D., … Mukadam, N. (2017, diciembre 16). Dementia prevention, intervention, and care. *The Lancet*. Lancet Publishing Group. https://doi.org/10.1016/S0140-6736(17)31363-6

Lopez, O. L., Becker, J. T., Chang, Y. F., Klunk, W. E., Mathis, C., Price, J., … Kuller, L. H. (2018). Amyloid deposition and brain structure as long-term predictors of MCI, dementia, and mortality. *Neurology*, *90*(21), E1920-E1928. https://doi.org/10.1212/WNL.0000000000005549

Lowe, V. J., Wiste, H. J., Senjem, M. L., Weigand, S. D., Therneau, T. M., Boeve, B. F., … Jack, C. R. (2018). Widespread brain tau and its association with ageing , Braak stage and Alzheimer ' s dementia, (2017), 271-287. https://doi.org/10.1093/brain/awx320

Luo, J., Wang, T., Liang, S., Hu, X., Li, W., & Jin, F. (2014). Ingestion of Lactobacillus strain reduces anxiety and improves cognitive function in the hyperammonemia

rat. *Science China Life Sciences*, *57*(3), 327-335. https://doi.org/10.1007/s11427-014-4615-4

Mitchell, A. J., & Shiri-Feshki, M. (2009). Rate of progression of mild cognitive impairment to dementia - Meta-analysis of 41 robust inception cohort studies. *Acta Psychiatrica Scandinavica*, *119*(4), 252-265. https://doi.org/10.1111/j.1600-0447.2008.01326.x

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. D., Group, T. P., Oxman, A., … Hopewell, S. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Medicine*, *6*(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097

Nagpal, R., Neth, B. J., Wang, S., Craft, S., & Yadav, H. (2019). Modified Mediterranean-ketogenic diet modulates gut microbiome and short-chain fatty acids in association with Alzheimer's disease markers in subjects with mild cognitive impairment. *EBioMedicine*, *47*, 529-542. https://doi.org/10.1016/j.ebiom.2019.08.032

Ni, Y., Yang, X., Zheng, L., Wang, Z., Wu, L., Jiang, J., … Fu, Z. (2019). Lactobacillus and Bifidobacterium Improves Physiological Function and Cognitive Ability in Aged Mice by the Regulation of Gut Microbiota. *Molecular Nutrition and Food Research*, *63*(22), 1-14. https://doi.org/10.1002/mnfr.201900603

Nimgampalle, M., & Yellamma, K. (2017). Anti-Alzheimer properties of probiotic, Lactobacillus plantarum MTCC 1325 in Alzheimer's disease induced albino rats. *Journal of Clinical and Diagnostic Research*, *11*(8), KC01-KC05. https://doi.org/10.7860/JCDR/2017/26106.10428

Parashar, A., & Udayabanu, M. (2017). Gut microbiota: Implications in Parkinson's disease. *Parkinsonism and Related Disorders*, *38*, 1-7. https://doi.org/10.1016/j.parkreldis.2017.02.002

Parihar, M. S., & Hemnani, T. (2004). Alzheimer's disease pathogenesis and therapeutic interventions. *Journal of Clinical Neuroscience*, *11*(5), 456-467. https://doi.org/10.1016/j.jocn.2003.12.007

Parmar, A. (2016). Gut – brain axis , psychobiotics , and mental health. *Asian Journal of Psychiatry*, *22*, 84-85. https://doi.org/10.1016/j.ajp.2016.05.004

Peng, S., Garzon, D. J., Marchese, M., Klein, W., Ginsberg, S. D., Francis, B. M., … Fahnestock, M. (2009). Decreased Brain-Derived Neurotrophic Factor Depends on Amyloid Aggregation State in Transgenic Mouse Models of Alzheimer ' s Disease, *29*(29), 9321-9329. https://doi.org/10.1523/JNEUROSCI.4736-08.2009

Perez-Pardo, P., de Jong, E. M., Broersen, L. M., Wijk, N. van, Attali, A., Garssen, J., & Kraneveld, A. D. (2017). Promising effects of neurorestorative diets on motor, cognitive, and gastrointestinal dysfunction after symptom development in a mouse model of Parkinson's disease. *Frontiers in Aging Neuroscience*, *9*(MAR). https://doi.org/10.3389/fnagi.2017.00057

Pinto, T. C. C., Machado, L., Bulgacov, T. M., Rodrigues-Júnior, A. L., Costa, M. L. G., Ximenes, R. C. C., & Sougey, E. B. (2019). Is the Montreal Cognitive Assessment (MoCA) screening superior to the Mini-Mental State Examination (MMSE) in the detection of mild cognitive impairment (MCI) and Alzheimer's Disease (AD) in the elderly? *International Psychogeriatrics*, *31*(4), 491-504. https://doi.org/10.1017/S1041610218001370

Prince M, Wimo A, Guerchet M, Ali GC, Wu YT, P. M. (2015). *World Alzheimer Report 2015 The global impact of dementia an analysis of prevalence, incidence, cost and trends.* Available from https://www.alz.co.uk/research/WorldAlzheimerReport2015.pdf

Raz, L., Knoefel, J., & Bhaskar, K. (2016). The neuropathology and cerebrovascular mechanisms of dementia. *Journal of Cerebral Blood Flow and Metabolism*, *36*(1), 172-186. https://doi.org/10.1038/jcbfm.2015.164

Rezaei Asl, Z., Sepehri, G., & Salami, M. (2019). Probiotic treatment improves the impaired spatial cognitive performance and restores synaptic plasticity in an animal model of Alzheimer's disease. *Behavioural Brain Research*, *376*. https://doi.org/10.1016/j.bbr.2019.112183

Rezaeiasl, Z., Salami, M., & Sepehri, G. (2019). The Effects of Probiotic Lactobacillus and Bifidobacterium Strains on Memory and Learning Behavior, Long-Term Potentiation (LTP), and Some Biochemical Parameters in β-Amyloid-Induced Rat's Model of Alzheimer's Disease. *Preventive nutrition and food science*, *24*(3), 265-273. https://doi.org/10.3746/pnf.2019.24.3.265

Rohatgi, A. (2017). WebPlotDigitizer, (https://apps.automeris.io/wpd/).

Roman, P., Estévez, A. F., Miras, A., Sánchez-Labraca, N., Cañadas, F., Vivas, A. B., & Cardona, D. (2018). A Pilot Randomized Controlled Trial to Explore Cognitive and Emotional Effects of Probiotics in Fibromyalgia. *Scientific Reports*, *8*(1), 10965. https://doi.org/10.1038/s41598-018-29388-5

Romo-Araiza, A., Gutiérrez-Salmeán, G., Galván, E. J., Hernández-Frausto, M., Herrera-López, G., Romo-Parra, H., … Ibarra, A. (2018). Probiotics and Prebiotics as a Therapeutic Strategy to Improve Memory in a Model of Middle-Aged Rats. *Frontiers in Aging Neuroscience*, *10*(December), 1-15. https://doi.org/10.3389/fnagi.2018.00416

Rudzki, L., Ostrowska, L., Pawlak, D., Małus, A., Pawlak, K., Waszkiewicz, N., & Szulc, A. (2019). Probiotic Lactobacillus Plantarum 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: A double-blind, randomized, placebo controlled study. *Psychoneuroendocrinology*, *100*(October 2018), 213-222. https://doi.org/10.1016/j.psyneuen.2018.10.010

Sanford, A. M. (2017). Mild Cognitive Impairment. *Clinics in Geriatric Medicine*. https://doi.org/10.1016/j.cger.2017.02.005

Sarkar, A., Lehto, S. M., Harty, S., Dinan, T. G., Cryan, J. F., & Burnet, P. W. J. (2016). Psychobiotics and the Manipulation of Bacteria – Gut – Brain Signals. *Trends in Neurosciences*, *39*(11), 763-781. https://doi.org/10.1016/j.tins.2016.09.002

Savignac, H. M., Tramullas, M., Kiely, B., Dinan, T. G., & Cryan, J. F. (2015). Bifidobacteria modulate cognitive processes in an anxious mouse strain. *Behavioural Brain Research*, *287*, 59-72. https://doi.org/10.1016/j.bbr.2015.02.044

Sciacca, M. F. M., Tempra, C., Scollo, F., Milardi, D., & La Rosa, C. (2018). Amyloid growth and membrane damage: Current themes and emerging perspectives from theory and experiments on Aβ and hIAPP. *Biochimica et Biophysica Acta - Biomembranes*, *1860*(9), 1625-1638. https://doi.org/10.1016/j.bbamem.2018.02.022

Sharma, B. C., & Singh, J. (2016). Probiotics in management of hepatic encephalopathy. *Metabolic Brain Disease*, *31*(6), 1295-1301. https://doi.org/10.1007/s11011-016-9826-x

Shen, L., & Ji, H. (2018). Associations Between Gut Microbiota and Alzheimer's Disease: Current Evidences and Future Therapeutic and Diagnostic Perspectives. *Journal of Alzheimer's Disease*, *68*(1), 25-31. https://doi.org/10.3233/JAD-181143

Stone, P. W. (2002). Popping the (PICO) question in research and evidence-based practice. *Applied nursing research : ANR*, *15*(3), 197-198.

Sun, J., Xu, J., Ling, Y., Wang, F., Gong, T., Yang, C., … Liu, J. (2019). Fecal microbiota transplantation alleviated Alzheimer's disease-like pathogenesis in APP/PS1 transgenic mice. *Translational Psychiatry*, *9*(1). https://doi.org/10.1038/s41398-019-0525-3

Tamtaji, O. R., Heidari-soureshjani, R., Mirhosseini, N., Kouchaki, E., Bahmani, F., Aghadavod, E., … Asemi, Z. (2019). Probiotic and selenium co-supplementation, and the effects on clinical, metabolic and genetic status in Alzheimer's disease: A randomized, double-blind, controlled trial. *Clinical Nutrition*, *38*(6), 2569-2575. https://doi.org/10.1016/j.clnu.2018.11.034

Tangalos, E. G. (2018). Mild Cognitive Impairment in Geriatrics. *Clinics in Geriatric Medicine*. https://doi.org/10.1016/j.cger.2018.06.005

Tiwari, S., Atluri, V., Kaushik, A., Yndart, A., & Madhavan, N. (2019). Alzheimer ' s disease : pathogenesis , diagnostics , and therapeutics, 5541-5554.

Ton, A. M. M., Campagnaro, B. P., Alves, G. A., Aires, R., Côco, L. Z., Arpini, C. M., … Vasquez, E. C. (2020). Oxidative Stress and Dementia in Alzheimer's Patients: Effects of Synbiotic Supplementation. *Oxidative Medicine and Cellular Longevity*, *2020*. https://doi.org/10.1155/2020/2638703

Tricco, A. C., Soobiah, C., Berliner, S., Ho, J. M., Ng, C. H., Ashoor, H. M., … Straus, S. E. (2013). Efficacy and safety of cognitive enhancers for patients with mild cognitive impairment: a systematic review and meta-analysis, *185*(16), 1393-1402.

Tsafnat, G., Glasziou, P., Choong, M. K., Dunn, A., Galgani, F., & Coiera, E. (2014). Systematic review automation technologies. *Systematic Reviews*, *3*(1), 74. https://doi.org/10.1186/2046-4053-3-74

van der Kant, R., Goldstein, L. S. B., & Ossenkoppele, R. (2020). Amyloid-β-independent regulators of tau pathology in Alzheimer disease. *Nature Reviews*

Neuroscience, *21*(1), 21-35. https://doi.org/10.1038/s41583-019-0240-3

Vesterinen, H. M., Sena, E. S., Egan, K. J., Hirst, T. C., Churolov, L., Currie, G. L., … Macleod, M. R. (2014). Meta-analysis of data from animal studies: A practical guide. *Journal of Neuroscience Methods*, *221*, 92-102. https://doi.org/10.1016/j.jneumeth.2013.09.010

Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzzi, A. P., Johnson, S. C., … Rey, F. E. (2017). Gut microbiome alterations in Alzheimer's disease. *Scientific Reports*, *7*(1), 1-11. https://doi.org/10.1038/s41598-017-13601-y

Wallace, C. J. K., & Milev, R. (2017). The effects of probiotics on depressive symptoms in humans: A systematic review. *Annals of General Psychiatry*, *16*(1), 1-10. https://doi.org/10.1186/s12991-017-0138-2

Wu, S. C., Cao, Z. S., Chang, K. M., & Juang, J. L. (2017). Intestinal microbial dysbiosis aggravates the progression of Alzheimer's disease in Drosophila. *Nature Communications*, *8*(1), 1-8. https://doi.org/10.1038/s41467-017-00040-6

Xiao, J., Katsumata, N., Bernier, F., Ohno, K., Yamauchi, Y., Odamaki, T., … Kaneko, T. (2020). Probiotic Bifidobacterium breve in Improving Cognitive Functions of Older Adults with Suspected Mild Cognitive Impairment: A Randomized, Double-Blind, Placebo-Controlled Trial. *Journal of Alzheimer's Disease*, 1-9. https://doi.org/10.3233/JAD-200488

You, Q., Li, L., Xiong, S. Q., Yan, Y. F., Li, D., Yan, N. N., … Liu, Y. P. (2019). Meta-analysis on the efficacy and safety of hyperbaric oxygen as adjunctive therapy for vascular dementia. *Frontiers in Aging Neuroscience*, *11*(APR). https://doi.org/10.3389/fnagi.2019.00086

FIGURE 1. Flow diagram (study selection methodology) for the systematic literature review process.

FIGURE 2. (A) Methodological quality of the preclinical trials included (n=10). **(B)** Graph of the methodological quality of the preclinical trials inlcuded**.**

FIGURE 3. (A) Methodological quality of the clinical trials included (n=6). **(B)** Graph of the methodological quality of the clinical trials included.

FIGURE 4. Forest plot displaying the effect of probiotics treatment on general cognitive function.

FIGURE 5. Forest plot displaying the effect of probiotics treatment on spatial memory.

FIGURE 6. Forest plot displaying the effect of probiotics treatment on non-spatial memory.

FIGURE 7. Forest plot displaying the effect of probiotics treatment on BDNF levels.

Table 1. Search strategies used in each database.

DATABASE	SEARCH STRATEGY
PubMed-Medline	(((probiotics[MeSH Terms]) OR lactobacillus[Title/Abstract]) OR bifidobacterium[Title/Abstract])) AND (((((((((gut[Title/Abstract]) OR gut[MeSH Terms]) OR microbiota[Title/Abstract]) OR microbiota [MeSH Terms]) OR microbiome[Title/Abstract]) OR gastrointestinal microbiome[MeSH Terms]) OR fecal microbiota[Title/Abstract])) AND (((((((alzheimer disease[MeSH Terms]) OR alzheimer's disease[Title/Abstract]) OR alzheimer's dementia[Title/Abstract]) OR cognitive frailty[Title/Abstract]) OR dementia[MeSH Terms]) OR cognitive[Title/Abstract]) OR mild cognitive impairment[Title/Abstract])
Scopus	probiotics OR lactobacillus OR bifidobacterium AND gut OR gut OR microbiota OR microbiota OR microbiome OR "gastrointestinal microbiome" OR "fecal microbiota" AND "alzheimer disease" OR "alzheimer's disease" OR "alzheimer's dementia" OR "cognitive frailty" OR dementia OR cognitive OR "mild cognitive impairment"
Web of Science **Cochrane Library** **ProQuest** **CINAHL** **Science Direct**	probiotics OR lactobacillus OR bifidobacterium AND gut OR gut OR microbiota OR microbiota OR microbiome OR gastrointestinal microbiome OR fecal microbiota AND alzheimer disease OR alzheimer's disease OR alzheimer's dementia OR cognitive frailty OR dementia OR cognitive OR mild cognitive impairment
Open Grey	Probiotics AND microbiota

Table 2. Characteristics of preclinical studies (n=10).

Reference	Country	Animals	Probiotic	Intervention characteristics	Variables	Tools	Results
Hwan Oh et al. (2020)	Korea	**CI rats** Male Sprague Dawley rats C.G. P.G. Cf-hGP G. prob + Cf-Hgp G. Anti BDNF + prob + Cf-hGp G.	*Lactobacillus plantarum* KCTC 3108 + *Capsosiphon fulvescens glicoprotina* (Cf-hGP)	4 weeks 15 mg/kg/day of Cf-hGP 10^9 CFU/rat/day of *L.plantarum* Oral adm.	Memory and spatial learning Protein concentration	MWM Western Blot	**Prob+Cf-Hgp G.** ↓ Latency to search the platform vs. the rats treated with only Cf-hGP or LP $p < .05$ ↑ Crossing frequency on platform vs. the rats treated with only Cf-hGP or LP $p < .05$ ↑ BDNF $p < .05$, Nrf2 $p < .05$ ↓ Grp 78 $p < .05$, JNK $p < .05$
Rezaeiasl et al. (2019)	Iran	**Rat model of AD** 40 male Sprague-Dawley rats	*Lactobacillus acidophilus* *Bifidobacterium bifidum* *Bifidobacterium longum*	6 weeks 500 mg 15 x 10^9 CFU Oral adm.	Memory and spatial learning PPF synaptic plasticity (pEPSPs)	MWM LTP induction	↓ Escape latency in Alz+ P.G. vs. Alz G. $p < .001$ No significant effect on total distance travelled $p < 0.228$ Reversal of the decline of fEPSPs observed in the Alz G. $p < .001$ Reversal of the increase in the PPF ratio observed in the Alz G. $p < .001$

Table 2. Continued.

Reference	Country	Animals	Probiotic	Intervention characteristics	Variables	Tool	Results
Rezaei et al. (2019)	Iran	**Rat model of AD** 45 male Wister rats C.G. (n=10) Alz G. (n=8) Alz + P.G. (n=8) Sham G. (n=10) Pro + Con G. (n= 9)	*Lactobacillus acidophilus* *Bifidobacterium bifidum* *Bifidobacterium longum*	8 weeks 500 mg 15 x 10^9 CFU Intragastric adm.	Memory and spatial learning PPF synaptic plasticity (pEPSPs) TAC MDA	MWM LTP induction Blood test	↓ Time to find the platform in Alz+P.G. vs. Alz G. Better sailing performance compared to C. and Sham G. $p < .05$ Restoration of synaptic plasticity No amyloid plaques on Alz+Pro G. $p < .001$ ↓ MDA ↑ TAC $p < .01$
Abraham et al. (2019)	Hungary	**Mouse model of AD** 32 male APP/PSI mice CG (n=8) Exercise G. (n=8) P.G. (n=8) Ex + Pro G. (n=8)	*Bifidobacterium longum* SGB05 *Lactobacillus acidophilus* SGL11	20 weeks 120 mg/day 5 times/week Oral adm.	Memory and spatial learning Working Memory Amyloid Plaques	MWM Y-MT Brain Section	↓ Time to locate the target Platform in the Ex+P.G. vs CG $p < .05$ ↑ Number of alternations in the Ex+P.G. vs. CG, ExG. and P.G. ↓ Number of amyloid plaques in all treated groups, especially in the Ex G.

Table 2. Continued.

Reference	Country	Animals	Probiotic	Intervention characteristics	Variables	Tool	Results
Corpuz et al. (2018)	Japan	**CI mice** 32 female SAMP 28 mice CG (n=12) L.327 G. (n=12) L.K71 G. (n= 12)	*Lactobacillus paracasei K-71*	43 weeks AIN-93M diet with 0.05% of probiotic Oral adm.	Memory and spatial learning Associative Memory Working Memory BDNF mRNA BDNF	BMT PAT Y-MT PCR Western Blot	↓ Escape latency to search the EB in the L.327 and L.K71 G. compared to the CG ↑ Time in the hole 0º in the LK71 G. vs. the CG. $p < .05$ ↑ Latency to enter the electric room in the LK71 G. vs. the CG. $p < .05$ No significant differences in alternating time ↑ BDNF mRNA, BDNF, BDNF staining, CREB/pCREB
Kobayashi et al. (2017)	Japan	**Mouse model of AD** Male ddY Mice CG= sodium acetate P.G. Donepezil G.	*Bifidobacterium Breve* A1	1 x 10^9 organisms in 0,2 ml Oral adm.	Working Memory Associative Memory	Y-MT PAT	↑ Number of alternations in the P.G vs. the CG $p < .01$ ↑ Latency time in the P.G vs. the CG $p < .05$
Nimgampalle et al. (2017)	India	**Rat model of AD** 48 male Wister rats CG = saline solution Alz G. = IP D-Galactose Alz + P.G. P.G.	*Lactobacillus plantarum* MTCC1325	60 days 10 ml/Kg 12 x 10^8 CFU/ml Oral adm.	Memory and spatial learning Nuclear chromatin ACh AChE	MWM Congo-Red Stain Kit Metcal and Ellman Method	↓ Escape latency in the Alz+P.G. vs. the CG $p < .05$ Healthy neurons with hyperchromatic nuclear chromatin in the Alz+P.G. ↑ ACh, ↓ AChE $p < .05$

Table 2. Continued.

Reference	Country	Animals	Probiotic	Intervention characteristics	Variables	Tool	Results
Jeong et al. (2015)	Korea	**CI rats** Male Fischer 344 rats YR= young control rats AR= aged control rats ARC= aged rats treated with LC-29 ARR= aged rats treated with rapamycin	*Lactobacillus pentosus var. Plantarum* C29	8 weeks 2 x 10^9 CFU/rat/day 6 days/week Oral adm.	Memory and spatial learning Working Memory Protein concentration	MWM Y-MT ELISA	↓ Escape latency in ARC vs. YR G. $p < .05$ ↑ Time spent on the platform in ARC vs. YR G. $p < .05$ ↑ Number of alternations in ARC vs. YR G. $p < .05$ ↑ DCX, BDNF, CREB phosphorylation $p < .05$
Liu et al. (2015)	China	**Mouse model of VD** 69 male ICR mice CG (n=12) VD G. (n=12) 3 VD + Pro G. (n=15 in each)	*Clostridium butyricum* WZMC1016 (CGMCC 9831)	6 weeks 1 x 10^6 CFU 1 x 10^7 CFU 1 x 10^8 CFU Intragastric adm.	Memory and spatial learning Neural characteristics BDNF p-Akt/Akt Bcl-2/Bax	MWM Electron microscope Western Blot	In the PG compared to the CG: ↑ Total distance travelled, Activity time, Time in the target Platform ↓ Rest time, Escape latency ↓ Neural malformations, Neuronal apoptosis ↑ BDNF, p-Akt/Akt, Bcl-2/Bax

Table 2. Continued.

Reference	Country	Animals	Probiotic	Intervention characteristics	Variables	Tool	Results
Ni et al. (2019)	China	**CI mice** 42 male C57BL/6 mice Young CG (n=10) Old CG (n=12) *Lactobacillus* G. (n=12) *Bifidobacterium* G. (n=12)	*Lactobacillus casei* LC122 *Bifidobacterium longum* BL986	12 weeks 0.2 ml 2 x 10^9 CFU Oral adm.	Memory and spatial learning Memory and non spatial learning Learning ability Ageing related proteins	MWM NORT ASAT RT-PCR	In the PG compared to CG, especially in the *Bifidobacterium* G: ↓ Latency time ↓ Entry time to the target platform ↑ Discrimination and Recognition Index in the PG compared to CG, especially in the *Lactobacillus* G. $p < .05$ ↑ Active avoidance frequency in both *Lactobacillus* and *Bifidobacterium* G. compared to the CG. $p < .05$ ↑ Sirt1, FoxO1, FoxO3, P27, Wisp1, BDNF, Gfra1y Ngf

ACh= Acetylcholine; **AChE**= Acetylcholinesterase ; **aCSF**= artificial cerebro-spinal fluid; **AD=** Alzheimer Disease; **Adm**= administration; **AIN**= American Institute of Nutrition; **Alz G**.= Alzheimer Group **AR**= aged rats; **ARC**= aged rats treated with C29; **ARR**= aged rats treates with rapamycin; **ASAT**= Active Shuttle Avoidance Test; **Aβ**= β-Amyloid; **Bcl-2/Bax**= antiapoptotic/proapoptotic protein; **BDNF**= Brain-derived Neurotrophic Factor; **BMT**= Barnes Maze Test; **Cf-hGP**= *Capsosiphon fulvescens* glycoproteins; **CFU**= colony-forming unit; **CG**= Control Group; **CI=** Cognitive Impairment; **CREB**= cAMP response element-binding; **DCX**= age-reduced doublecortin; **EB**= Escape Box; **ELISA**= Enzyme-Linked ImmunoSorbent Assay; **Ex+ Pro G**.= Exercise + probiotic group; **fEPSPs**= field excitatory postsynaptic potentials; **FoxO1** y **FoxO3**= Forkhead Box O1 y O2; **Gfra1**= GDNF family receptor alpha-1; **Grp78**= Glucose-regulated protein 78; **G.Sham**: falsa intervención; **ICV**= intracerebreventricular; **JNK**= c-Jun N-terminal kinase; **L. K71 G**.= Lactobacillus paracasei K71 group; **L.327 G**.= Lactobacillus casei L327 group; **LTP**= long-term potentiation; **MDA**= malondealdehyde; **mRNA**= Messenger ARN; **MWM**= Morris Water Maze test; **Ngf**= nerve growth factor; **NORT**= New Object Recognition Test; **Nrf2**= nuclear factor erythroid 2-related factor 2; **p- Akt/Akt**= Akt protein phosphorylation; **PAT**= Passive Avoidance Test; **PCR**= Polymerase Chain Reaction; **PPF**= paired-pulse facilitation**; PG**.= Probiotic group; **RT-PCR**= Reverse transcription polymerase chain reaction; **Sirt1**= Sirtuin 1; **TAC**= total anti-oxidant capacity; **VD=** Vascular Demetia; **VS**= versus; **Wisp1**= WNT1-inducible-signaling pathway protein 1; **Y-MY**= Y-Maze Test.

Table 3. Characteristics of clinical studies (n=6)

Reference	Country	Participants (PG/CG)	Probiotic	Intervention characteristics	Variables	Tools	Results
Xiao et al. (2020)	Japan	**MCI subjects** PG= 40 CG= 39	*Bifidobacterium Breve* A1 Capsules	16 weeks 2x 10^{10} CFU	Immediate memory Visuospatial memory Language Attention Delayed memory	RBANS JMCIS	**PG** ↑ RBANS score ↑ JMCIS score **CG** ↑ RBANS score ↓ JMCIS score $p < .0001$ $p = .052$
Tamtaji et al. (2019)	Iran	**AD patients** Se G. = 26 Se+P G. = 27 C.G. = 30	*Lactobacillus acidophilus* *Lactobacillus casei* *Bifidubacterium bifidum* Capsules	12 weeks 2 x 10^{9} CFU	Cognitive function	MMSE	**Se+ P G.** ↑ MMSE **CG** ↓ MMSE **Se G** ↑ MMSE $p < .001$
Hwang et al. (2019)	Korea	**MCI subjects** 50/50	Mixture of fermented soybean and *Lactobacillus plantarum* C-29 Capsules	2 weeks 1 x 10^{10} CFU	Attention Working memory Verbal memory Neurotrophins	ACPT DST VLT ELISA	**PG** ↑ Attention $p = .02$ ↑ Working memory $p < .05$ ↑ Verbal memory ↑ Combined cognitive function ↑ BDNF $p = .007$ **CG** No changes in BDNF levels $p = 0.15$
Kobayashi et al. (2019)	Japan	**MCI subjects** 59/58	*Bifidobacterium Breve* A1 Capsules	12 weeks 2 x 10^{10} CFU	Memory, visuospatial capacity, language and attention Cognitive function	RBANS MMSE	**PG** ↑ RBANS score (+5.27) ↑ Language, Attention $p < .001$ ↑ MMSE score $p < .001$ (+1.63) **CG** ↑ RBANS score (+4.65) $p = 0.87$ ↑ Language, Attention $p < .05$ ↑ MMSE score $p < .001$ (+1.76) $p = 0.62$

Table 3. Continued

Reference	Country	Participants (PG/CG)	Probiotic	Intervention characteristics	Variables	Tools	Results		
Agahi et al. (2018)	Iran	**AD patients** 25/23	*Lactobacillus fermentum, plantarum and acidophilus Bifidobacterium lactis, bifidum and longum* Capsules	12 weeks 3×10^9 CFU	Orientation, recognition, calculation, verbal fluency, similarity, naming, memory, visuospatial and copying skills	TYM	**PG** ↑ TYM score (14.64/17.42)	**CG** ↑ TYM score (14.35/17.47)	$p = 0.82$
Akbari et al. (2016)	Iran	**AD patients** 30/30	*Lactobacillus acidophilus, casei and fermentum Bifidobacterium bififidum* Milk	12 weeks 200ml/day 2×10^9 CFU/g for each	Cognitive function: attention, orientation, memory, recall, calculation, language and drawing	MMSE	**PG** ↑ MMSE score (+27.90% ± 8.07)	**CG** ↓ MMSE score (-5.03% ± 3.00)	$p < .001$

ACPT= Auditory continuous performance; **AD=** Alzheimer Disease; **Adm**= Administration; **BDNF**= Brain-derived Neurotrophic Factor; **CFU**= colony-forming unit; **CG**= Control Group; **DSST**= Digit Symbol Substitution Test; **DST**= Digit Span Test; **ELISA**= Enzyme-Linked InmunoSorbent Assay; **JMCIS**= Japanese version of the MCI Screen; **MCI=** Mild Cognitive Impairment; **MMSE**= Mini-Mental State Examination; **PG**= Probiotic Group; **POMS2**= Profile of Mood States 2nd Edition; **RBANS=** Repeatable Battery for the Assessment of Neurophychoogical Status; **SeG**= Selenium Group; **Se+P G**= Selenium + Probiotic Group; **TYM**= Test Your Memory; **VLT**= Verbal learning test.