The effect of probiotics on cognitive function across the human lifespan: a systematic review

The effect of probiotics on cognitive function across the human lifespan: A systematic review

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Abstract

Recently the scientific community has seen a growing interest in the role of the gut-brain axis and, in particular, how probiotic supplementation may influence neural function and behaviour via manipulation of the gut microbiota. The purpose of this review was to systematically review the current literature exploring the effect of probiotic intervention on cognitive function. PsychINFO, Web of Science, PubMed and Google Scholar were searched for human trials. Studies selected for inclusion administered a probiotic intervention and included at least one behavioural measure of cognitive performance. A total of 30 experimental papers were included, exploring the effect of probiotics across a variety of ages, populations and cognitive domains. The evidence suggests there may be potential for probiotics to enhance cognitive function or attenuate cognitive decline, particularly in clinically relevant adult populations for whom cognitive dysfunction may be present. However, the limited number of studies and the quality of the existing research makes it challenging to interpret the data. Further research is clearly warranted. PROSPERO: CRD42020164820.

Key words

Probiotics, cognition, cognitive function, gut-brain axis

1. Introduction

The gut microbiota (GM) plays a critical role in determining overall host health (Jandhyala et al., 2015) and is shaped by a number of factors across the lifespan, including mode of delivery, host genetics, age, diet and stress (Long-Smith et al., 2020). Residing in the human gastrointestinal tract, the GM is the vast community of microorganisms including bacteria, eukarya and archaea. Although the previously well-cited prediction that microbes outnumber human cells by 10:1 has recently been revised in favour of a figure closer to 1:1, estimates still suggest there are 100 times more genes in the gut microbiome than the human genome (Gilbert et al., 2018), highlighting the diversity of these organisms.

Although not a novel concept (Read et al., 1966; Quigley, 2017), research has converged over the last two decades to establish a bidirectional connection between the brain and the gut, often referred to as the microbiota-gut-brain axis following emerging evidence for a role of the GM and derived metabolites in altering neural function and behaviour (Heijtz et al., 2011; Cryan and Dinan, 2012). It is increasingly clear that this top-down, bottom-up exchange between enteric microbiota and the brain represents a fragile, symbiotic relationship that contributes to both host health and disorders of the body and the brain. This communication framework is thought to be served by a number of neuronal, endocrine and immunological pathways which are well summarised elsewhere and will not be repeated in this review (Farmer et al., 2014; Bauer et al., 2016; Long-smith et al., 2020), although precise mechanisms still remain somewhat elusive.

In particular, there is increasing evidence for an association of the gut microbiome with psychiatric and cognitive dysfunction. Studies of germ-free mice and antibiotic-induced dysbiosis have shown altered production of metabolites crucial to cognitive processes such as brain-derived neurotrophic

factor (BDNF), gamma-Aminobutyric acid (GABA), N-methyl-D-aspartate (NMDA) receptors and tryptophan (Bercik et al., 2011; Desbonnet et al., 2015; Fröhlich et al., 2016; Soto et al., 2018), in addition to alterations in cognitive function, anxious and social behaviours (Zhu et al., 2020). Interestingly, these behavioural phenotypes are reproduced in mice following faecal transplants of intestinal microbiota (Collins et al., 2013), providing evidence for a direct role of the gut microbiota in modulating neural function. Similarly, studies of conditions characterised in part by cognitive impairment such as Parkinson's Disease (PD), Alzheimer's Disease (AD), Schizophrenia and Major Depressive Disorder (MDD) have also implicated altered GM composition as a contributing factor to the onset of and disease progression (Rogers et al., 2016; Ticinesi et al., 2018; Dutta et al., 2019; Kowalski and Mulak, 2019). As such, the gut microbiota may provide a valuable target for modulation of cognitive health in both clinical and non-clinical populations (Sun et al., 2020).

As defined by the World Health Organisation, probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Joint, 2020). Oral consumption of probiotics can directly alter the GM by increasing the diversity and number of beneficial microbes, potentially leading to changes in microbiota-derived metabolite production, reduction in inflammation, alterations to HPA axis function and changes to gut-barrier integrity (Lebeer et al., 2018; Plaza-Diaz et al., 2019). Therefore, by exploiting the gut-brain axis, probiotics present an opportunity for modulation of the CNS and as such have been explored as therapeutic adjuncts to target a number of CNS related conditions (Wang et al., 2016; Dutta et al., 2019; Genedi et al., 2019; Liu et al., 2019; Ng et al., 2019; Smith and Wissel, 2019).

Increasingly, probiotics are being investigated for their potential to reduce cognitive deficits and to enhance cognition in the absence of clinical impairment. Studies in rodents have consistently reported positive effects of both single and multi-strain probiotics on spatial and non-spatial memory (Wang et al., 2016). Reversal of cognitive deficits have also been reported in animal models of diabetes (Davari et al., 2013), anxiety (Savignac et al., 2015) and Parkinson's (Castelli et al., 2020), to name a few. Experimental trials in humans, largely published within the last decade, have also explored this

potential benefit across a variety of clinical and non-clinical populations. A preliminary search for reviews of these experimental trials, across a range of resources including Google Scholar, JBI COnNECT+, Prospero and Cochrane Library, finds a small number of existing reviews. The literature in ageing populations experiencing Mild Cognitive Impairment (MCI) and AD was recently reviewed in a meta-analysis by Den et al (2020), who concluded that the preliminary evidence was promising for enhancing cognition in both MCI and AD. Conversely, a review into the impact of early probiotic intervention on subsequent neurocognitive development in infants and children up to age 13 found the evidence to be less compelling, with only one study reporting positive results in the form of a reduced risk of developing Attention Deficit Hyperactive Disorder (ADHD) or Autism Spectrum Disorder (ASD) (Rianda et al., 2019). The efficacy of probiotics for improved cognition was recently reviewed in a meta-analysis from Lv and colleagues (2020), who included 11 animal and 7 human trials in healthy and cognitively impaired populations across a range of ages. The overall effect of probiotic intervention on cognition was found to be non-significant in both animal and human studies when supplementing healthy populations. In populations with cognitive impairment, however, interventions in animals had a large effect size regardless of whether a single or multi-strain intervention was used, while the effect in human studies was small and showed greater efficacy following single strain interventions rather than multi-strain. Interestingly, the results appear to show a 'capping effect' of the length of intervention, where significant effects were only reported in studies of <12 weeks. Most recently, Marx and colleagues (2020) concluded, following a meta-analysis including 7 human trials, that the evidence was not sufficient to support the use of probiotic supplementation for cognitive outcomes, suggesting that a greater number of well-designed, adequately powered studies are needed.

Although a number of studies have now considered cognitive outcomes following probiotic intervention, heterogeneity within the methodologies employed makes navigating this literature and interpreting the results challenging. Where previous reviews have focused on the effects of supplementation within specific populations or age groups, and therefore only ever included a small number of human trials, this review aims to collate the full extent of the current human literature. This is important as interest in the field begins to grow, not only to consider the populations for whom

probiotics may provide a beneficial tool in the improvement of cognitive function, but to begin to discuss in what contexts an intervention might be successful with regards to probiotic strain(s), the length of supplementation and the cognitive domain(s) beneficially effected by probiotic treatment. Additionally, this review provides a unique opportunity to look at the overall quality of the existing literature and identify where future studies might improve upon this to further our understanding of how probiotics could enhance cognition.

As such, the aim of this review is to systematically review a broad range of experimental trials in human subjects to address the question of whether probiotic supplementation may improve cognitive function, and for whom this approach may be beneficial.

2. Method

Methods for conducting this review were pre-specified in a registered protocol on PROSPERO (registration number CRD42020164820).

Experimental human trials, recruiting participants of any age, gender or ethnicity, were eligible for inclusion if they supplemented participants with at least one live probiotic strain. With a view to including as many studies as possible, no restrictions were placed on type, quantity or length of probiotic intervention, and studies using probiotic supplements in conjunction with other interventions were also included. To that end, studies without a comparator, such as a placebo control group, were also included. To be eligible for inclusion, studies were also required to include at least one cognitive outcome measuring performance in a cognitive domain such as memory, executive function or attention. Studies that did not include a behavioural measure on a cognitive task were excluded. As such, studies solely measuring cognitive reactivity or cognitive control via use of questionnaires were not included, as these were not deemed standardised behavioural measures of cognitive performance. Studies using resting state functional Magnetic Resonance Imaging (fMRI) with no cognitive task were also excluded.

A search of the databases PsychINFO, Web of Science, PubMed and Google Scholar was performed between December 2019 and January 2020 to identify formally published experimental trials in humans published in the English language. Reference lists of relevant studies, including review papers, were also checked, and Scholar was used primarily for this purpose. As this review focused on formally published papers, grey literature databases were not searched. Each database was systematically searched using the following terms: probiotic*** AND** gut **AND** brain **AND** axis, probiotic*** AND** clinical **AND** trial, probiotic*** AND** cognit*****, probiotic*** AND** neuro*****, probiotic*** AND** brain, probiotic* **AND** (memory **OR** learning **OR** attention), Lactobacill* **AND** cognit*, Lactobacill* **AND** (memory **OR** learning **OR** attention), Bifidobacteri* **AND** cognit*, Bifidobacteri* **AND** cognit*, Bifidobacteri* **AND** (memory **OR** learning **OR** attention) (see supplementary data 1 for example of full search strategy). In PubMed and PsychINFO, each search was run through 'all fields', including title, abstract, keywords and Medical Subject Headings (MeSH), using the advanced search feature. For Web of Science, each term was searched using 'topic' search fields, which includes title, abstract, author keywords and keywords plus. No other filters or descriptors were used except for in PubMed, where searches were restricted to 'clinical' and 'human' due to the larger volume of animal and *in vitro* papers available.

Study selection was initially performed by JE, and excluded papers were independently verified by DL. Initially, papers were excluded based on the title if it was evident that the research fell outside of the inclusion criteria specified- e.g. animal studies. All studies of potential interest were then shortlisted before reading the full publications to decipher eligibility for inclusion. Where database searches flagged up relevant conference abstracts or study protocols, authors were contacted to enquire whether this data had since been published (Owen et al., 2014; Noorwali et al., 2017; Bloemendaal et al., 2019; Rieger et al., 2019). This was not the case for any of the research studies in question and therefore these were not included in this review.

Studies selected for inclusion were assessed for overall quality of methodology and the potential risk of bias using the Evidence Analysis Manual Quality Criteria Checklist (QCC) from the Academy of

Nutrition and Dietetics (2016). Studies were assessed independently by JE and DL and disagreements were resolved with a third party. Potential areas of bias included selection and randomisation procedures, use of blinding, and funding. As one of the aims of this review was to explore the quality of the existing literature and highlight current limitations, all eligible papers were included regardless of methodological quality.

Data extraction was conducted independently by JE and DL following the Evidence Analysis Manual Data Extraction Template from the Academy of Nutrition and Dietetics (2016). This allowed systematic extraction of key information regarding design, sample characteristics, intervention/ exposure/ compliance, outcome measures and reported results. For the purpose of this review, only data relevant to cognitive outcomes was extracted for analysis, although some papers also explored physical, psychological and biochemical outcome measures.

With regards to data synthesis, extracted data were handled in tabular form in order to aid comparison of study characteristics and guide the grouping of studies for narrative synthesis. Due to the heterogeneity in key study characteristics, namely population, intervention and cognitive outcome, statistical synthesis of study findings was not performed.

3. Results

Initial searches flagged a total of 7871 citations, which, after initially screening out 7441 papers based on titles and abstracts and a further 305 papers following more in-depth review, resulted in a total of 30 studies that met the inclusion criteria described (see figure 1).

3.1. Study characteristics

Selected papers included Randomised Control Trials (RCTs), single-arm Pilot Studies, a Non-Randomised Control Trial and one Non-Randomised Cross-over Trial published between 2007 and

2019 in a total of 19 countries. Of the 30 papers included, only 6 explicitly report the age range of participants (Benton et al., 2007; Tillisch et al., 2013; Ceccarelli et al., 2017a; Kelly et al., 2017; Ohsawa et al., 2018; Wallis et al., 2018) and many are unclear as to whether they are reporting mean or median and standard deviation or standard error (Malaguarnera et al 2010; Tillisch et al., 2013; Bajaj et al., 2014; Chung et al., 2014; Lunia et al., 2014; Agahi et al., 2018; Slykerman et al., 2018; Lew et al., 2019) of the sample. Based on the mean ages reported, these papers collectively included individuals from 27-weeks gestation to 82 years, although this may not reflect the full range of ages studied. Five papers studied infants and children (Chou et al., 2010; Firmansyah et al., 2011; Akar et al., 2017; Jacobs et al., 2017; Slykerman et al., 2018), 17 focused on a general adult population and 8 specifically on ageing adults (Chung et al., 2014; Akbari et al., 2016; Agahi et al., 2018; Inoue et al., 2018; Hwang et al., 2019; Kobayashi et al., 2019a; Kobayashi et al., 2019b; Tamtaji et al., 2019). Across these age groups there were a number of clinical populations targeted for probiotic intervention, including very low birth weight (VLBW) preterm infants (Chou et al., 2010; Akar et al., 2017; Jacobs et al., 2017), Human Immunodeficiency Virus-1 (HIV-1) (Ceccarelli et al., 2017a; Ceccarelli et al., 2017b), Cirrhosis (Malaguarnera et al., 2010; Bajaj et al., 2014; Lunia et al., 2014; Román et al., 2019), Fibromyalgia (Roman et al., 2018), Major Depressive Disorder (MDD) (Rudzki et al., 2019), Chronic Fatigue Syndrome (CFS) (Wallis et al., 2018), Mild Cognitive Impairment (MCI) (Hwang et al., 2019; Kobayashi et al., 2019a; Kobayashi et al., 2019b) and Alzheimer's Disease (AD) (Akbari et al., 2016; Agahi et al., 2018; Tamtaji et al., 2019) with a further 12 studies carried out in 'healthy' individuals. As such, outcome measures were often clinically relevant to the population studied, with only 24 papers stating a primary focus on cognition.

The majority of studies assessed cognitive outcomes at baseline and post-intervention, with the exception those studying infants and one other (Lew et al., 2019). Data were reported across a number of cognitive domains, as defined by Lezak and colleagues (2012), using a combination of 41 different composite and individual task measures (see table 1). Choice of measure(s) was often guided by age of the population, such as frequent use of the Bayley Scales of Infant Development for studies in

infants and the Mini Mental State Examination for those in ageing adults, or by medical condition, where cognitive ability was measured using assessment tools rather than standard cognitive tasks.

Cognitive outcomes were assessed following a variety of probiotic interventions. Most papers provided details of the exact probiotic strain(s) administered, while 8 only described the specie(s) (Chou et al, 2010; Lunia et al., 2014; Akbari et al., 2016; Akar et al., 2017; Agahi et al., 2018; Roman et al., 2018; Wallis et al., 2018; Tamtaji et al., 2019) and 1 just the genus (Malaguarnera et al., 2010). Fourteen studies utilised a single strain intervention, 16 a multi-strain intervention and 6 administered the probiotic supplement in conjunction with an additional treatment for a combined intervention. These included medicines (Wallis et al., 2018; Rudzki et al., 2019), exercise, and other dietary supplements (Malaguarnera et al., 2010; Firmansyah et al., 2011; Hwang et al., 2019; Tamtaji et al., 2019). Key study information regarding population, intervention used, and significant cognitive findings are summarised in table 2.

Using the QCC, the quality of all studies was assessed as 'neutral', with a small number demonstrating a stronger methodology and bordering a positive rating (Firmansyah et al., 2011; Ohsawa et al., 2018; Roman et al., 2018; Papalini et al., 2019; Rudzki et al., 2019) (supplementary data 2). Generally, the risk of bias across studies from sources of funding and use of blinding was low, but subject selection and randomisation procedures presented a higher risk for bias. Implications of this are discussed below in section 4.7.

3.2. Infants and children

Three RCTs used a prospective follow-up to assess the impact of early probiotic intervention on neurodevelopment in VLBW preterm infants (gestational age $\leq$ 32 weeks or birth weight $\leq$ 1500g). In each case neurodevelopment was assessed using the Bayley Scales of Infant Development (BSID) II (Chou et al., 2010; Akar et al., 2017;), or III (Jacobs et al., 2017), with one study also using the Wechsler Preschool and Primary Scale of Intelligence III as an alternative for children who were

followed up over the age of 42 months (Jacobs et al., 2017). Infants were supplemented with a mixture of *Lactobacillus reuteri* (Akar et al., 2017), *Lactobacillus acidophilus* and *Bifidobacterium infantis* (Chou et al., 2010), or *B. infantis, Streptococcus thermophilus* and *Bifidobacterium lactis* (Jacobs et al., 2017) from when first able to feed until discharged from hospital. All three studies reported no significant effects on neurodevelopment. Similarly, studies in full-term infants reported no positive effect of intervention on cognitive development, either when supplemented from a gestational age of 37 weeks until 2 years with *Lactobacillus rhamnosus* and *B. animalis* subsp. *Lactis* (Slykerman et al., 2018), or with a combined supplement of *Bifidobacterium longum* and *Lactobacillus rhamonosus*, prebiotics inulin and fructo-oligosaccharide and long-chain polyunsaturated fatty acids AA and DHA from 12 months until 24 months of age (Firmansyah et al., 2011). Cognitive outcomes were assessed at 11 years and 24 months, respectively.

3.3. Young and middle-aged adults

Hepatic Encephalopathy (HE) is a severe complication of cirrhosis resulting in brain dysfunction due to a build-up of toxins in the blood stream. A number of papers explored how probiotic intervention may reduce the incidence of HE in cirrhosis patients. One study in patients evidencing HE found a positive effect of a combined *Bifidobacterium* and fructo-oligosaccharide supplement on tasks measuring visuospatial awareness, processing speed and psychomotor and executive functions (Malaguarnera et al., 2010). This improvement in performance was evident after 30 days of intervention and similar to that which was reported in the comparison group taking lactulose (a common treatment in HE). A further 3 studies focused on cirrhosis patients with no evidence of overt HE. An improvement in PHES score (a composite assessment of cognitive impairment common in HE) was reported in 2 studies following multi-strain interventions for 12 weeks (Lunia et al., 2014; Román et al., 2019), while the other reported no significant effect of 8 weeks of *L. rhamnosus* GG on a selection of tasks from the PHES (Bajaj et al., 2014).

The cognitive functioning of individuals with HIV-1 was also a target for probiotic intervention, with the authors producing an initial pilot study (Ceccarelli et al., 2017a) followed by a larger placebo-controlled trial (Ceccarelli et al., 2017b). In both studies, HIV-1 infected adults were supplemented with the same multi-strain probiotic (*Lactobacillus plantarum, S. thermophilus, Bifidobacterium breve, Lactobacillus paracasei, Lactobacillus delbrueckii* subsp. *bulgaricus, L. acidophilus, B. longum* and *B. infantis*) for six months before change in cognition was assessed using a large battery of standardised tests covering memory, executive functions and fluid intelligence. In both studies, significant improvements relative to baseline and controls were reported in immediate and delayed memory, visuospatial working memory and verbal fluency, with additional improvement in executive function and psychomotor speed reported in the latter trial. It should be noted that, in the controlled trial, assignment to condition was not random but based on cerebral spinal fluid (CSF) neopterin levels at baseline, with only those who demonstrated higher levels assigned to take the probiotic treatment, presumably because higher levels of neopterin were correlated with lower cognitive performance at baseline, therefore providing greater potential for improvement. As a result, only 9 subjects were studied for change in cognitive function following probiotic treatment, compared to 26 control subjects.

Probiotic interventions may also positively affect cognitive status in adults with other clinical conditions associated with altered gut microbiota composition. One pilot study explored this potential in individuals with Fibromyalgia (Roman et al., 2018). Following 8 weeks of *L. rhamnosus* GG, *Lactobacillus casei, L. acidophilus,* and *Bifidobacterium bifidus,* those who received probiotic treatment displayed a significantly reduced number of impulsive choices in a reward based decision-making task. In another study combining antibiotic (erythromycin) and probiotic treatment (*L. rhamnosus, B. lactis, B. breve* and *B. longum*) over the course of four weeks (Wallis et al., 2018), moderate treatment effects were observed for attention, processing speed, cognitive flexibility, story memory and verbal fluency in subjects with CFS. However, this was a single-arm pilot study, making it difficult to attribute these effects specifically to the intervention. Finally, one study explored the use of *L. plantarum* in combination with selective serotonin reuptake inhibitor treatment (SSRI) for MDD

(Rudzki et al., 2019). After 8 weeks of supplementation, those taking the combined treatment as opposed to just SSRIs demonstrated improved visual search and short-term memory function, but no effect on other executive functions including inhibition and verbal fluency.

A number of studies also focused on the potential for improved cognition in clinically healthy adults. One study assessed cognition following 12 weeks of *L. plantarum* intervention in moderately stressed adults, pre-determined by the Cohen's Perceived Stress Scale, using a cognitive battery assessing memory, processing speed and social emotional cognition (Lew et al., 2019). Probiotic intervention was associated with significantly faster emotional processing in women and greater verbal memory in men compared to a placebo, although no baseline data was recorded and analysing by gender resulted in smaller samples than the authors' calculations suggested necessary for sufficient statistical power, particularly in male subjects. Similar findings were reported in a study employing emotional decision and recognition tasks during fMRI, where authors reported both a significant increase in response accuracy and significantly less decision change to unpleasant stimuli following a multi-strain intervention compared to those of the control treatment (Bagga et al., 2018). Similarly, a decrease in recruitment of affective, viscerosensory, and somatosensory regions was reported during an emotional decision task following supplementation with a multi-strain fermented milk (*Bifidobacterium animalis* subsp *lactis, S. thermophilus, L. bulgaricus* & *L. lactis*) in healthy adults when compared to a no-intervention group. However, a positive effect on emotional processing was not consistently reported across studies. One study utilising a range of tasks measuring attention, memory, learning and emotional processing only reported an improvement in visual memory and learning following four weeks of *B. longum*, and such improvements were also seen in the control group (Allen et al., 2016). Additionally, a study in female subjects found no effect of a four-week multi-strain intervention (*B. bifidum, B. lactis, L. acidophilus, L. brevis, L. casei, Lactobacillus salivarius & Lactococcus lactis*) on tasks focused on emotional processing or executive function, but did find that the probiotic intervention provided a 'buffer' of sorts against the negative effects of an acute physiological stressor on working memory (Papalini et al., 2019). One study, recruiting healthy middle-aged adults with self-reported forgetfulness, also found using a standardised composite measure of cognitive function

that total cognitive score, attention and delayed recall abilities significantly improved following 8 weeks of *Lactobacillus helveticus* fermented milk product. Attention scores were also significantly greater in the active group compared with the placebo group post-intervention. On the other hand, two studies in healthy adults that supplemented with *L. casei* and *L. rhamnosus* for 3 and 8 weeks, respectively, reported no significant effect of probiotic intervention on any of the cognitive domains assessed including memory, verbal fluency, attention, motor speed, learning, executive function, information processing and emotional cognition (Benton et al., 2007; Kelly et al., 2017).

3.4. Ageing adults

Three studies explored the efficacy of probiotic interventions for improving cognitive outcomes in ageing adults with MCI. Two of these were published in succession as an initial single-arm pilot study (Kobayashi et al., 2019a) followed by a larger placebo-controlled trial (Kobayashi et al., 2019b). Both studies explored the effects of *B. breve* over 24 weeks and 12 weeks, respectively, and used the MMSE to assess cognitive status and a digit symbol substitution task. The latter trial also included a larger task battery comprising of 11 other sub-tests to assess multiple facets of memory, language and executive function. In the pilot study, MMSE composite score significantly improved after 24 weeks of supplementation. In the latter trial, MMSE composite score significantly improved after 12 weeks, but this was true of both the active and placebo group. The probiotic group evidenced an improvement in delayed recall memory in both the MMSE and cognitive battery, but only in those with lower MMSE scores at baseline. Similarly, improvements in language and attention sub-tests were seen only in those with lower baseline scores, although once again the same improvements were also reported in the placebo group taking matched placebo capsules. The third study assessed change in the composite z score of three tasks measuring memory and attention following 12 weeks of *L. plantarum* and fermented soybean powder, finding a significant improvement in composite score driven by improvement in sustained attention (Hwang et al., 2019).

A further 3 studies using a similar dose of probiotic species explored the effects of 12-week probiotic supplementation in those with diagnosed AD. Using the MMSE as a sole measure of cognitive status, two studies found a significant improvement in total score following supplementation with *L. acidophilus, L. casei, Lactobacillus fermentum and Bifidobacterium bifidum* (Akbari et al., 2016), and with *L. acidophilus, B. bifidum* and *B. longum* administered in combination with selenium (Tamtaji et al., 2019). The third study utilised an alternative measure to the MMSE known as 'Test Your Memory' in order to assess the potential efficacy of a multi-strain intervention (*L. fermentum, L. plantarum, B. lactis, L. acidophilus, B. bifidum* & *B. longum*) but found no significant effect on cognition (Agahi et al., 2018).

In addition to those with age-related disorders, 2 studies utilised probiotic interventions in generally healthy ageing adults. One study aimed to explore the efficacy of a 12-week intervention with *L. helveticus* in improving performance, particularly during cognitive fatigue (Chung et al., 2014). Cognitive measures of information processing, executive function and sustained attention were administered consecutively and repeated four times to induce cognitive fatigue, while additional tasks assessed aspects of memory. Subjects on probiotic treatment showed significantly improved information processing and higher accuracy in a task of executive function compared with placebo-matched control subjects. The second combined 12 weeks of multi-strain (*B. longum, B. infantis* & *B. breve*) probiotic supplementation with moderate resistance training to explore the impact on cognitive function using a standardised battery of cognitive assessments covering memory, attention, language, executive function and visuospatial processing (Inoue et al., 2018). Both the active and control group (just resistance training) demonstrated a significant increase in composite score with no difference between groups, suggesting a significant effect of resistance training only.

4. Discussion

Overall, the evidence in this review provides some support for the use of probiotics to enhance cognition, with 21/30 of the included studies reporting an improvement in at least one cognitive

measure. This figure is somewhat skewed by the inclusion of studies in children, as those exploring early supplementation in infants consistently reported no effect on subsequent neurocognitive development up to 11 years of age, regardless of whether supplementing VLBW and premature infants or those who reached full-term. It may be that that the development during this period is too rapid to see any effect of a probiotic intervention. Additionally, studying infants brings with it a greater number of challenges. Most looked to supplement infants from when first able to feed until discharged from hospital; factors which are unique to the individual and therefore resulted in heterogeneity in supplementation length within studies. Due to personal circumstances or preferences, the vehicle for administration of the probiotic was also inconsistent for a number of these studies, with some parents using breast milk (a natural prebiotic), others formula, and some studies not disclosing the method of administration, making the nutrient content of the intervention itself a potential confounder (Deoni et al., 2018).

If we exclude those studies in children, 20/24 studies report a positive effect of probiotic cognition on cognition. The evidence suggests that probiotics may provide a useful therapeutic adjunct to those with a variety of conditions leading to impaired cognitive functioning. In young and middle-aged adults, improved cognition was reported in those with HIV-1 (Ceccarelli et al., 2017a; Ceccarelli et al., 2017b), MDD (Rudzki et al., 2019), Fibromyalgia (Roman et al., 2018) and CFS (Wallis et al., 2018), although it is important to note that these effects were explored in singular studies (with the exception of Ceceralli and colleagues who ran a follow up to their pilot study in HIV-1 patients), some of which being open-label and not randomised control trials. Reports of improved cognition were more consistent in studies exploring supplementation in cirrhosis patients, with three of four randomised control trials reporting improvement in PHES composite score (Lunia et al., 2014; Román et al., 2019) and similar sub-tests (Malaguarnera et al., 2010). While the aforementioned methodological issues need to be taken into consideration, the existing evidence in these clinical populations is positive and suggests a need for further study.

In older clinical populations, improved cognition was consistently reported in those with MCI. Interestingly, findings from the pilot study showing a significant improvement in MMSE score were only replicated in an RCT in subjects who had a lower score (poorer performance) at baseline (Kobayashi et al., 2019b), suggesting that disease progression influences the efficacy of the intervention. Two studies using the MMSE to assess cognition in those with AD both reported improvement following probiotic intervention compared to placebo or alternative therapy (Akbari et al., 2016; Tamtaji et al., 2019), while a third study using the TYM reported no significant effect (Agahi et al., 2018). All three studies used a 12-week intervention and similar multi-strain supplements. Again, lack of detail regarding exact strains, comprehensive demographic data and a 'probiotic only' group (Tamtaji et al., 2019) make it more challenging to integrate findings across studies. While the preliminary evidence is positive, more trials are needed to make informed conclusions. In particular, the clinical field would benefit from RCTs longer than 12 weeks to follow the progression of these conditions, and to explore more thoroughly whether probiotics are more effective during earlier stages of AD and MCI, or whether subjects respond better when cognitive impairment is more severe.

The evidence for enhancing cognitive function in 'healthy' subjects is more parsimonious. A total of 6 studies report a positive effect of probiotic intervention on cognitive function, with a further two reporting no effects on cognition. However, more sophisticated analyses highlight that these findings are not so clear-cut. Two studies report a positive effect on emotional cognition (Bagga et al., 2018; Lew et al., 2019) – the latter only finding so in women. An earlier fMRI study appears to support these findings, being the first to demonstrate modulation of cortical activity across a widely distributed brain network during an emotional decision task following supplementation with a probiotic fermented milk (Tillisch et al., 2013). However, the descriptive results for task performance were not provided here, making it difficult to infer the effect of the probiotic intervention on cognitive performance itself. Unfortunately, this study also used an all-female sample, providing no further opportunity to assess whether the effect could be more pertinent in females than in males. While there is some indication that affective cognition may be a domain for improvement through probiotic

supplementation, improved performance was not consistently reported (Allen et al., 2016; Kelly et al., 2017; Papalini et al., 2019). One study also reports significant improvement in attention and memory domains in the probiotic group, with attention scores being significantly greater than that of the placebo group following intervention. However, some improvements were also seen in learning and recall subtests in the placebo group, which the authors recognise may represent a learning effect across test visits (Ohsawa et al., 2018).

Despite seeing no improvement in cognitive performance across the task battery, Papalini and colleagues did find probiotic supplementation to be associated with maintenance of working memory performance under conditions of acute stress (induced by the socially evaluated cold-pressor test (SECPT)) where it was otherwise hindered, suggesting a buffering effect against the negative impact of stress on cognitive performance. Similar findings were reported by Allen and colleagues where total cortisol output following exposure to acute stress, again induced using the SECPT, was significantly lower following probiotic intervention, as were reported daily stress levels. Additionally, a greater improvement in conditional learning was observed in the latter following probiotic supplementation compared to the placebo. However, the stepwise improvement in learning appears to be consistent with practice effects and, given that the study employed a non-randomised design with no blinding, it is difficult to ascertain whether any of these results were affected by subject bias. Additionally, the authors included both an emotional recognition task and an emotional Stroop task but report no effect of intervention on either task, further adding to the inconsistency of findings in these heathy populations.

Looking specifically at studies using healthy ageing adults as participants, only two studies have explored the impact of probiotic supplementation on cognition to date, so conclusions are necessarily limited. One study reported improvements in executive function, working memory and sustained attention (Chung et al., 2014). The other reported an improvement in composite score of the Montreal Cognitive Assessment (MoCA), although, much like the MMSE, this is a brief screening tool for MCI and dementia and therefore may not be an appropriate measure for a healthy adult population.

Additionally, as probiotics were only administered in combination with resistance training, we can only assume that there is no additional effect of the probiotic supplement to that of the training, since the control subjects engaged solely in the resistance training programme demonstrated similar improvements.

Overall, the evidence in this review suggests that, for healthy young and middle-aged adults, there may be a protective effect against stress-induced declines in cognition and a potential to enhance cognitive function when processing emotional stimuli, but it is difficult to draw firm conclusions from the current literature and further well-controlled randomised trials are needed. Importantly, it should also be noted that no adverse effects on cognition were reported in any of the studies discussed here, including those in infants and children.

4.1. Single versus multi-strain supplements

Fourteen studies provided single strain supplements and 16 provided multi-strain supplements of between two and nine different strains. Of these, 9 papers report a positive effect on at least 1 cognitive measure following a single-strain intervention, and 11 report a beneficial effect following a combination of strains. Additionally, positive effects were reported across a range of both healthy and clinical populations, in younger, middle-aged and older adults. When comparing the efficacy of single- versus multi-strain interventions it is important to do so based on exact strains, taking into account the specific population being supplemented (McFarland, 2020). Unfortunately, there are too few studies incorporating the same strains into single and multi-strain supplements to draw such comparisons at present. As such, there doesn't appear to be any clear evidence for use of one supplement type over the other, regardless of age, population or cognitive domain being targeted. This is consistent with findings from a recent review which found that, in most cases, multi-strain interventions were no more effective than single strain interventions in relieving a range of medical conditions, despite speculation that multi-strain products would potentially cover a wider range of mechanisms of action or result in synergistic effects between the strains (McFarland, 2020).

503

504 4.2. Species/strains

505

506 Studies included species of *Lactobacillus* and *Bifidobacterium* as single-strain supplements, and
507 *Lactobacillus*, *Bifidobacterium*, *Lactococcus* and *Streptococcus* species in various combinations as
508 multi-strain supplements. The only species to be identified as a single-strain intervention in multiple,
509 separate studies was *L. plantarum* (Bagga et al., 2018; Hwang et al., 2019; Lew et al., 2019), although
510 it was used in combination with fermented soybean powder and SSRIs in two of these, and each study
511 used a different *L. plantarum* strain. Despite exploring the effects in very different populations, all
512 three report a significant positive effect of supplementation following a double-blind RCT,
513 particularly in the domain of sustained attention (Bagga et al., 2018; Hwang et al., 2019). *L.*
514 *plantarum* has demonstrated good survival and colonisation rates in the human GI tract compared to
515 other *lactobacilli* species (De Vries et al., 2006) and previous work has reported anti-inflammatory
516 properties, reducing the permeability of the intestinal barrier (White et al., 2006; Wang et al., 2018),
517 increasing SCFA levels (Wang et al., 2014) and restoring BDNF levels in cognitively impaired
518 participants (Jeong et al., 2015). A greater number of double-blind RCTs, preferably selecting strains
519 that have demonstrated relevant neuroactive potential and that include a group supplemented solely
520 with the probiotic strain, are needed to establish whether certain probiotic strains are more effective in
521 altering cognitive performance than others.

522

523 The variety of multi-strain supplements and lack of detail regarding exact strains that were included in
524 any intervention makes it challenging to explore whether there may be particular combinations of
525 strains that are consistently effective at improving cognitive performance. Competition between
526 strains is often quoted as a possible reason for reduced efficacy of multi-strain probiotic supplements,
527 although such literature does not yet exist in relation to cognitive outcomes (Joseph & Law., 2019).
528 However, even when strains are found to have inhibitory effects on each other in a mixed
529 environment, efficacy is not always reduced, and in some cases these combinations outperform the
530 strains individually (Chapman, Gibson & Rowland., 2012). While the complex nature of

host/probiotic interactions reduces the likelihood of a 'one size fits all' product, understanding more about the individual mechanisms of action and how stains may interact with, enhance or inhibit one another will be important for ensuring maximum efficacy of probiotic interventions for cognitive health.

4.3. Dose

Specified doses ranged from 7.5×10^6 - 1.8×10^{12} colony forming units (CFU) per day, with 3 studies not disclosing exact quantities (Malaguarnera et al., 2010; Bajaj et al., 2014; Chung et al., 2014). The evidence presented in this review suggests there is currently little consensus regarding an 'optimum' dose, with studies reporting positive effect across the full range of doses. While all trials reporting no significant effect of intervention on cognitive outcomes used a daily dose of below 10^{10} CFU, positive effects on cognition were reported following consumption of 10^9 CFU/day and lower. Additionally, trials reporting no significant effect of intervention did so across a range of clinical conditions, ages, single and multi-strain interventions.

4.4. Length of intervention period

Regarding length of intervention, the current literature comprises of studies ranging from 3 weeks to 6 months. A significant positive effect was consistently reported in studies between and including 4 weeks to 6 months. While other health benefits have been reported following 3 or fewer weeks of intervention (Nixon et al., 2012), it is perhaps the case that 3 weeks is too short to measure an effect of probiotic interventions on cognition. However, as there is only one study at this length it is not possible to draw any conclusions from this review.

4.5. Areas of cognition

Despite the number of studies that have now focused on change in cognitive performance following probiotic intervention, heterogeneity in cognitive tasks and common design issues such as randomisation procedures, lack of blinding and the potential for practice effects makes it inherently difficult to identify whether there are particular cognitive domains that are more sensitive to probiotic interventions than others. As described previously, there does appear to be some consistent findings regarding emotionally loaded cognitive tasks (Tillisch et al., 2013; Bagga et al., 2018; Lew et al., 2019), although further research is needed to explore this. A recent review (Long-smith et al., 2020) highlights the mounting support for the use of probiotics in the treatment of psychological disorders, with a number of studies reporting amelioration of affective symptoms and changes in mood. It is also well established that mood affects cognitive function, both in terms of valence and information processing (Forgas, 2017). In particular, studies have demonstrated a robust effect of mood on the processing of face stimuli, both in clinically depressed (Gilboa-Schechtman et al., 2002; Leppänen et al., 2004) and healthy subjects (Van Honk et al., 2003; Curby et al., 2012). This interplay between affect and cognition is perhaps one reason why these emotional decision and recognition tasks may be sensitive to the effects of probiotic intervention.

While not a direct effect on cognitive performance itself, the limited research currently available indicates that probiotics may provide a buffering effect against stress, meaning that cognitive performance is maintained where it would otherwise be negatively affected (Staar et al., 2008). Similar findings have previously been reported following supplementation with milk-based phospholipids, where reaction times in an attention switching task following the SECPT were improved post-intervention compared to pre-intervention performance. Studies in this review employing the SECPT to induce psychological and physiological stress have reported maintenance of working memory performance (Papalini et al., 2019) and lower cortisol output (Allen et al., 2016) following probiotic interventions compared to that of placebos. The effects of probiotics on stress and anxiety are well documented, with animal studies consistently reporting behavioural and biochemical alterations following supplementation, not only in models of physiological stress, but also in those of social and chronic stress (Zareie et al., 2006; Machos et al., 2016). Additionally, a recent human trial

found altered neural activity following supplementation during a game designed to induce social stress in adults (Wang et al., 2019). While further research is needed to ascertain the legitimacy of this buffering effect following probiotic intervention, future work may wish to establish whether the protective effects extend not only to other cognitive domains, but whether there is a potential to improve cognitive function in individuals facing chronic or perceived stress, as opposed to acute, physiological stress.

4.6. Possible mechanisms of action

The mechanisms through which probiotics may exert effects on the CNS are not well understood, with much of the current evidence originating from studies in animal models. Bacterial species may produce a number of neurotransmitters including GABA, dopamine, serotonin and norepinephrine (Barrett et al., 2012; Holzer and Farzi, 2014), as well as increasing the availability of precursors such as tryptophan (Yano et al., 2015) (see figure 2). Probiotics may also increase the availability of neuroactive compounds indirectly by stimulating metabolites that promote biosynthesis (Yano et al., 2015). A study in adult male mice demonstrated that chronic supplementation with *L. rhamnosus* was associated with altered expression of GABA receptors in the brain and consistent reductions in stress-related behaviour and corticosterone output (Bravo et al., 2011). Additionally, magnetic resonance spectroscopy (MRS) research in mice found that supplementation with *L. rhamnosus* led to a significant increase in functional metabolites in the brain, including glutamate, N-acetyl aspartate and GABA. These studies indicate that probiotic induced changes to the gut likely led to functional changes in the brain, providing some mechanistic insight into behavioural changes. However, exactly how changes in gut derived metabolites mediates altered neurochemistry is still unclear. For example, Bravo and colleagues (Bravo et al., 2011) found that these effects were not demonstrated in vagotomised mice, suggesting a role of the vagus nerve, while others found increased hippocampal brain-derived neurotropic factor (BDNF) expression and exploratory behaviour to be independent of

whether mice received a vagotomy or not (Bercik et al, 2011). This suggests the vagus nerve may only be a partial mediator in these gut-brain interactions.

In addition to altered neurotransmitter production, it is thought that probiotics may influence the production of other bacteria-derived metabolites, particularly short-chain fatty acids (SCFAs), which are thought to be implicated in gut-brain axis communication (Dalile et al., 2019; Silva et al., 2020). *In vitro* models have demonstrated an increase in SCFAs (particularly acetate, butyrate and propionate) as a result of probiotic bacteria (Nagpal et al., 2018; Sivieri et al., 2013). Additionally, Wang and colleagues (2018) conducted a human trial supplementing young, middle-aged and older adults with *L. plantarum* and found that faecal levels of acetate and propionate significantly increased in all three age groups, and slowly declined to near baseline levels once supplementation ceased.

Finally, probiotics may influence neural function via interactions with immunological pathways. Alterations to the gut microbiota is a key contributing factor to chronic, low-grade inflammation which is present in a number of clinical conditions (Z Alam et al., 2014; Bauer and Teixeira, 2019; Walker et al., 2019) and is thought to contribute to cognitive dysfunction across the lifespan (Marsland et al., 2015; Arnoriaga-Rodríguez and Fernández-Real, 2019; McGrattan et al., 2019). In particular, poor gut barrier integrity leads to a rise in systemic inflammation as a result of endotoxin being able to cross the lumen into the blood stream. Increased levels of plasma endotoxin have been shown to increase blood-brain-barrier permeability both directly and indirectly, leading to notions such as the endotoxin hypothesis of neurodegeneration (Brown, 2019). Probiotics have been associated with improved gut barrier integrity and reduced permeability (van Hemert et al., 2013), thought to occur as a result of increased mucin expression and tight-junction stability, protecting the epithelial barrier (Stoidis et al., 2011). As a result, probiotic intervention may reduce endotoxemia and therefore levels of inflammation.

In addition, probiotics may offer an opportunity to attenuate the damaging effects of pro-inflammatory cytokines on the gut barrier, both by reducing proinflammatory and increasing anti-

641 inflammatory responses. For example, in humans, chronic supplementation with *L. salivarius* has
642 been associated with a significant reduction in serum concentrations of inflammatory markers such as
643 high sensitivity C-reactive protein (hs-CRP), interleukin (IL) 6, IL-1b, and TNF- α (Rajkumar et al.,
644 2015). These findings were echoed in a recent review which discussed frequent reports of a
645 significant reduction in serum concentrations of proinflammatory markers, particularly tumour
646 necrosis factor alpha (TNF- α) and C-reactive protein, in addition to less frequent reports of increased
647 anti-inflammatory markers following probiotic intervention (Maia et al., 2019). However, the
648 mechanisms responsible for changes in inflammatory response are less clear. One suggestion is that
649 introduction of probiotic bacteria can alter the signalling for inflammatory cytokine activation. For
650 example, *in vitro* work has demonstrated that *L. rhamnosus* GG reduced the effects of pro-
651 inflammatory cytokines on epithelial barrier integrity, in part, through inhibition of NF-kB signalling
652 (Donato et al., 2010).
653
654 Of the studies included in this review, only a handful looked to explore potential mechanisms behind
655 change to cognition, all of which supplemented clinical or sub-clinical populations. Bajaj and
656 colleagues found following supplementation with *L. rhamnosus* GG that subjects with HE displayed a
657 significant decrease in endotoxemia and TNF- α in faecal microbiota analysis, in addition to various
658 changes to serum and urine metabolites including amino acids, secondary bile acid and vitamins
659 (Bajaj et al., 2014). However, it should be noted that these metabolic changes were found in the
660 absence of change to cognitive performance. Lew and colleagues describe similar findings following
661 *L. plantarum* intervention in mildly stressed adults, where better emotional cognition and recognition
662 memory were associated with a significant reduction in pro-inflammatory cytokines interferon gamma
663 (IFN- γ) and TNF-α (Lew et al., 2019). Two studies supplementing patients with AD with multi-strain
664 *Lactobacillus* and *Bifidobacterium* interventions reported similar changes in metabolic outcomes,
665 including reduced serum hs-CRP, triglycerides and a decrease in insulin resistance and increase in
666 insulin sensitivity (Akbari et al., 2016; Tamtaji et al., 2019). Additionally, Tamtaji and colleagues
667 report a downregulation in gene expression of TNF-α and a concurrent upregulation in genes
668 associated with maintenance of low cholesterol and energy homeostasis (low-density lipoprotein

receptor and peroxisome proliferator-activated receptor gamma, respectively). However, subjects here were supplemented with a combination of selenium and probiotic strains, and, while these effects were greater than in those just taking selenium, no probiotic alone group was included. In subjects with MCI, improvement in cognitive function, particularly sustained attention, following consumption of *L. plantarum* was associated with increased serum BDNF levels (Hwang et al., 2019) – an important protein for neural health and one that is heavily implicated in learning and memory processes (Cunha et al., 2010). Finally, combined supplementation of SSRIs and *L. plantarum* was found to be associated with a decrease in kynurenine concentration, which may affect cognition via a number of mechanisms (Rudzki et al., 2019). While it seems *L. plantarum* supplementation is associated with different metabolic changes in each study that it was used, it is important to note that each of these studies focused on different biochemical outcomes and therefore common mechanisms of action cannot be ruled out.

By altering the composition of the gut microbiota, probiotic interventions may affect neural function and thus cognition via one or a combination of mechanisms. The current literature provides some evidence for improved cognition in clinical populations via modulation of immunological pathways and reduction in systemic inflammation, but these effects are inherently linked to physiological changes in the clinical parameters of interest, and there is little understanding regarding potential mechanisms in healthy subjects. Further research is required to elucidate precise mechanisms and factors that may influence these, such as host age, health status and microbiota composition.

4.7. Limitations and considerations for future work

While this area of research is gaining traction, this review highlights a number of recurring limitations to study designs which impede our ability to integrate the studies and draw reliable inferences for the effect of probiotics on cognitive function. While many studies employed RCT designs, a number of these were not carried out double-blind to the intervention and a small number used alternative single-

arm or non-randomised designs. The QCC also highlighted a general lack of clarity regarding participant demographics across the studies in this review, with many not providing basic information such as an explicit age range and mean, or not indicating gender splits. Additionally, a number of studies did not include any form of power calculation to determine sample size, and those who did often did not reach this quota for all cognitive measures. Going forward it is important that studies are well powered, particularly as nutrition interventions do not typically have large effects and require a sensitive design (Flanagan et al., 2020).

As this review aimed to incorporate as many experimental trials as possible, this led to the inclusion of a number of studies that used probiotics in combination with additional treatments for cognitive impairment or a particular clinical condition. While important to explore combined effects, not all included a comparison group only taking the probiotic supplement. As such, it is difficult to extrapolate reliably the effect of the probiotic supplement relative to the other. It would be helpful in future studies wishing to explore combined effects to include a comparison group for each treatment component separately, in order to better understand both the individual and combined treatment effects.

One of the key limitations in the current literature is a lack of explicit detail regarding the probiotic interventions themselves, particularly in neglecting to specifically identify the strain(s). This is increasingly important as research suggests that effects are frequently strain specific (Savignac et al., 2014; Kekkonen et al., 2018). Additionally, despite investigating how alterations to the GM might affect cognitive function, few studies performed faecal analysis to assess microbiota composition post-intervention and none to date have collected pre-intervention samples. Assessing both pre- and post-intervention faecal community allows insights into how the intervention may have altered the composition of the resident microbiota. While this data is useful to have, current research actually suggests that probiotic interventions are unlikely to result in observable changes to the composition, particularly in healthy populations, both in terms of diversity and richness (Kristensen et al., 2016). Instead, it may be of greater insight to explore how probiotics help to stabilise and reinforce the

microbiota, as opposed to numerically changing the composition (Sanders, 2016). Additionally, faecal samples provide an opportunity to explore how probiotics may enhance neurotransmitter synthesis through changes to metabolite production, which may also be crucial to understanding the mechanisms behind change in cognitive function following supplementation. Due to the complex nature of the human gut, the same probiotic intervention will inherently affect different hosts in a multitude of different ways (Wieërs et al., 2020). For example, baseline microbiota composition and diet have been identified as factors that may influence the efficacy of a dietary supplement such as probiotics for the host (mobini et al., 2017; Volokh et al., 2019). As such, it may be of greater importance for future studies to collect baseline faecal samples to see for whom certain probiotics may be more effective. To this end it may be useful to collect information regarding habitual diet, too.

While the majority of studies in this review utilised standardised cognitive tasks with clear outcome measures, very few indicated whether parallel task versions had been used where appropriate in order to avoid practice effects. In addition, few, if any, provided subjects with sufficient practice in the cognitive tasks prior to beginning the experimental trial. Including such practice allows subjects to become comfortable with the task(s) and perform towards the ceiling of their natural capacity at baseline, therefore helping to remove practice as a confound for improved performance (Bell et al., 2018). Finally, factors such as time-of-day effects were rarely acknowledged. There is strong evidence for the existence of time-of-day effects in cognitive testing, where an individual's performance on a range of cognitive tasks can differ depending on the time of day that it is being tested (Schmidt et al., 2007). The same is true of meals, where exacerbated time-of-day effects known as post-prandial dips can be seen in cognitive performance following food intake, particularly after lunch (Craig, 1986; Rogers et al., 1994). Again, this phenomenon was rarely acknowledged in the current literature, with very few stating what time in the day cognitive performance was measured, whether participants were provided with a standardised meal prior to cognitive testing and whether time of testing remained consistent both within and between participants. These are therefore important considerations going forwards in order to strengthen the design of studies exploring probiotic effects on cognition.

With regards to limitations of the review itself, study selection was initially performed by one author (JE) and independently verified by another (DL). As the full process was not performed by two independent authors, it should be noted that this is a potential source of bias. Additionally, while this review acknowledges the importance of factors which undoubtably influence the efficacy of probiotic interventions, such as strain and dose, heterogeneity within the current literature makes it difficult to draw conclusions. As this field continues to grow it will be necessary to explore these factors further, likely by way of a meta-analysis, in order to better understand how these factors interact with age and clinical status to affect cognitive function.

5. Conclusions

In summary, the evidence thus far provides some support for enhancing cognition through probiotic intervention. Studies in infants and children find very little benefit of early probiotic supplementation to enhance subsequent neurocognitive development. However, studies in young and middle-aged adults do provide some support for supplementary probiotics, particularly in clinical populations where cognitive function may be negatively affected. Affective cognition and cognition under stress may be two aspects of cognitive function that are particularly sensitive to any effect of probiotics at this age. Similarly, studies in older adults provide some consistent evidence for a beneficial effect of probiotics, particularly on memory processes. However, this review has highlighted a number of consistent methodological issues within the current literature that make interpretation of data challenging. A greater number of well-controlled RCTs with a primary focus on cognitive performance and potential mechanisms of action are needed in order to clarify how effective probiotic interventions are for improving cognitive function, and which cognitive functions, within specific populations. Such research may then inform exciting opportunities for both clinical and individual practice for those who might see a benefit of supplemental probiotics on cognitive function.

Funding

This research is funded by the University of Reading and Winclove Probiotics as part of a matched funding studentship award.

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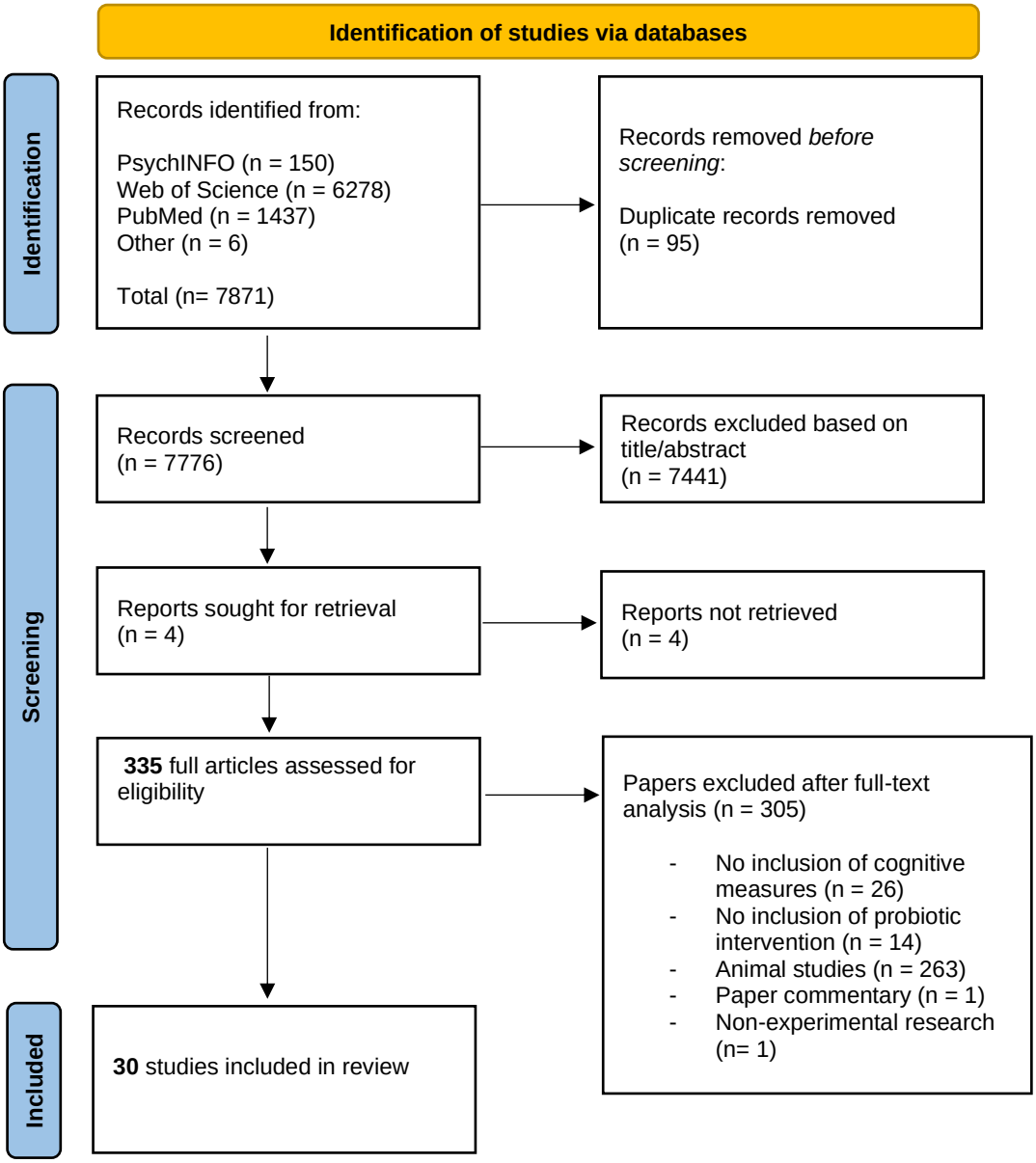

Figure 1- Flow diagram illustrating the identification of studies for inclusion.

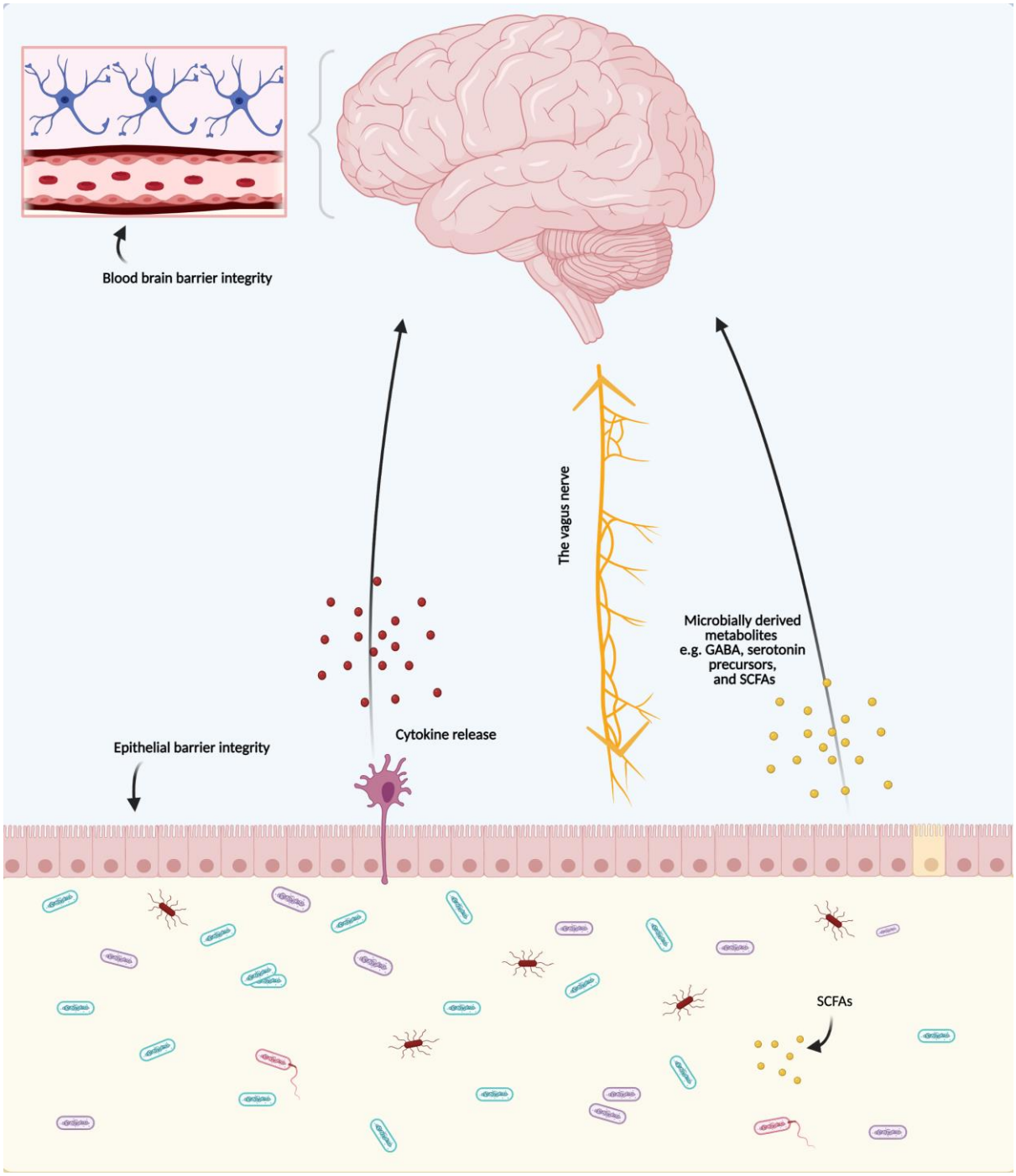

Figure 2 - Key microbiota-gut-brain communication pathways that may be modulated by probiotic interventions to affect cognitive health. These include synthesis of short-chain fatty acids (SCFAs), neuroactive compounds such as GABA and serotonin precursors, immune responses such as pro-inflammatory cytokine release and the integrity of both the gut epithelial barrier and the blood brain barrier (illustration made with BioRender.com).

Table 2 - Key characteristics of included experimental trials.

Citation	Design	Participants			Intervention		Cognitive measures	Significant cognitive outcomes
		No.	Age	Clinical population	Length	Probiotic strain(s)		
Agahi et al. (2018)	Double-blind RCT	48	m = 80 (assumed mean)	AD	12 weeks	*Lactobacillus fermentum*, *Lactobacillus plantarum*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Bifidobacterium longum* (3×10^9 CFU/day)	TYM	No effect of intervention on cognition
Akar et al. (2017)	RCT with prospective follow-up	249	m = 28 weeks gestation (assumed mean)	VLBW preterm infants	Supplemented from first feed until discharge Followed up at between 18-24 months	*Lactobacillus reuteri* (1 x 10^8 CFU/day)	BSID-II	No effect of intervention on cognitive development
Akbari et al. (2016)	Double-blind RCT	52	m = 79	AD	12 weeks	200 ml/day probiotic milk containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus fermentum* (2 $\times 10^9$ CFU/day)	MMSE	Significant improvement in MMSE score in the probiotic group following 12 weeks of supplementation compared to placebo
Allen et al. (2016)	Non-randomised crossover (no blinding)	27 (all male)	m = 25	N/A	4 weeks of placebo 4 weeks of probiotic + 2-week follow-up	*Bifidobacterium longum 1*714 (1×10^9 CFU/day)	PAL RVIP Emotional recognition task Emotional Stroop task	Significantly less errors in PAL following probiotics compared to baseline. Similar improvement seen following placebo

Bagga et al. (2018)	Double-blind RCT	45	m = 27	N/A	4 weeks	*Lactobacillus casei* W56, *Lactobacillus acidophilus* W22, *Lactobacillus paracasei* W20, *Bifidobacterium lactis* W51, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, *Bifidobacterium lactis* W52, *Lactobacillus plantarum* W62 and *Bifidobacterium bifidum* W23. ($7.5x10^6$ CFU/day)	Emotional decision task Emotional recognition task	Significantly less decision change for unpleasant stimuli following probiotics compared with placebo controls (improved emotional attention). Also, a significant increase in response accuracy to unpleasant stimuli in the recognition task
Bajaj et al. (2014)	Double-blind RCT	30	m = 57 (assumed mean)	Cirrhosis	8 weeks	*Lactobacillus* GG AT strain 53103	NCT-A NCT-B DST[a] BDT	No effect of intervention on cognition
Benton et al. (2007)	Double-blind RCT	124	48 - 79 m = 61	N/A	3 weeks	Yoghurt drink with *Lactobacillus casei* 6.5×10^9	WMS Logical memory Recall of capital cities Verbal fluency task	No effect of intervention on cognition
Ceccarelli et al. (2017a)	Single-arm pilot (no blinding)	10 (all male)	22 - 53 med = 42	HIV-1	6 months	*Lactobacillus plantarum* DSM 24730, *Streptococcus thermophilus* DSM 24731, *Bifidobacterium breve* DSM 24732, *Lactobacillus paracasei* DSM 24733, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 24734, *Lactobacillus acidophilus* DSM 24735, *Bifidobacterium longum* DSM 24736, and *Bifidobacterium infantis* DSM 24737 (1.8×10^{12} CFU/day)	ROCF RAVLT Verbal fluency CBTT VST TMT STEP PVF/SVF RCPM	Significant improvement from baseline in immediate and delayed recall of RAVLT and immediate and delayed copying in ROCF. Also, significant improvements in PVF, STEP and CBTT test scores.

Ceccarelli et al. (2017b)	Non-randomised control trial (no blinding)	35	IQR 38 - 54 med = 48	HIV-1	6 months	*Lactobacillus plantarum* DSM 24730, *Streptococcus thermophilus* DSM 24731, *Bifidobacterium breve* DSM 24732, *Lactobacillus paracasei* DSM 24733, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 24734, *Lactobacillus acidophilus* DSM 24735, *Bifidobacterium longum* DSM 24736, and *Bifidobacterium infantis* DSM 24737 (9×10^{11} CFU/day)	ROCF RAVLT Verbal fluency CBTT VST TMT STEP PVF/SVF RCPM	Significant improvement from baseline in immediate and delayed recall of RAVLT and immediate and delayed copying of ROCF in the probiotic group. Also, significant improvements in STEP, PVF, TMT-A and CBTT test scores.
Chou et al. (2010)	RCT with prospective follow-up	301	m = 28 weeks gestation (assumed mean)	VLBW preterm infants	Supplemented from 7 days old until discharge Followed up at 3 years CA	*Lactobacillus acidophilus* and *Bifidobacterium infantis* (2×10^9 CFU/day)	BSID-II	No effect of intervention on cognitive development
Chung et al. (2014)	Double-blind RCT	36	m = 65 (assumed mean)	N/A	12 weeks	fermented milk with *Lactobacillus helveticus* IDCC380	DST[b] Story recall test VLT RVIP Stroop task Serial 3/7s	Significant improvement from baseline in Stroop accuracy and serial 3/7s in probiotic group. Significantly higher accuracy following probiotics compared to placebo in RVIP and Stroop task.
Firmansyah et al. (2011)	Double-blind RCT	290	m =377 days	N/A	12 months	*Bifidobacterium longum* BL999, *Lactobacillus rhamnosus* LRR + inulin, fructo-oligosaccharides and Long-chain polyunsaturated fatty acids (~ 1.7×10^7 CFU/day)	BSID-III	No effect of intervention on cognitive development
Hwang et al. (2019)	Double-blind RCT	92	m = 68	MCI	12 weeks	*Lactobacillus plantarum* C29 (1.25×10^{10} CFU/day) + fermented soybean powder	VLT ACPT DST[b]	Significantly greater improvement in composite score following probiotics than placebo, which appears to be driven by improvement in ACPT
Inoue et al. (2018)	Double-blind RCT	38	m = 70	N/A	12 weeks	*Bifidobacterium longum* subsp. *longum* BB536, *Bifidobacterium longum* subsp. *infantis* M-63, *Bifidobacterium breve* M-16V and *Bifidobacterium breve* B-3 (1.25×10^{10} CFU/day) + resistance training	MoCA Modified Flanker task	Significant improvement in composite score of both groups

Jacobs et al. (2017)	Double-blind RCT	664	m = 27 weeks gestation	VLBW preterm infants	Supplemented from first feed until discharge Followed up at 2 - 5 years	*Bifidobacterium infantis* BB–02 96579, *Streptococcus thermophilus* TH–4 15957 and *Bifidobacterium lactis* BB-12 15954 ($1x10^9$ CFU/day)	BSID-III	No effect of intervention on cognitive development
Kelly et al. (2017)	Cross-over RCT (no blinding)	29 (all male)	20 - 33 m = 24	N/A	8 weeks	*Lactobacillus rhamnosus* ($1x10^9$ CFU/day)	MOT PAL AST RVIP Emotional recognition task Emotional Stroop task	No effect of intervention on cognition
Kobayashi et al. (2019a)	Open-label single-arm pilot	27	m = 82	MCI	6 months	*Bifidobacterium breve* A1 ($2x10^{10}$ CFU/day)	MMSE DSST (WAIS III)	Significant improvement in MMSE score following probiotic supplementation
Kobayashi et al. (2019b)	Double-blind RCT	117	m = 61	MCI	12 weeks	*Bifidobacterium breve* A1 ($2x10^{10}$ CFU/day)	RBANS MMSE	Significant improvement in delayed memory score (MMSE) in 'low scorers' at baseline. Also, significant improvement following both probiotic and placebo treatment in language and attention (RBANS) in 'low scorers' at baseline
Lew et al. (2019)	Double-blind RCT	103	m = 31 (assumed mean)	N/A	12 weeks	*Lactobacillus planturum* P8 (2×10^{10} CFU/day)	CBB	Significantly greater social emotional cognition in women and greater recognition memory in men following probiotic intervention compared to a placebo
Lunia et al. (2014)	RCT (no blinding)	160	m = 48 (assumed mean)	Cirrhosis	3 months	*Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus bulgaricus, and Streptococcus thermophilus* (3.3×10^{11} CFU/day)	PHES	Significant improvement in PHES score following probiotic intervention

Malaguarnera et al. (2010)	Double-blind RCT	125	m = 50 (assumed, for control group only)	Cirrhosis/mild HE	60 days	*Bifidobacterium* + fructo-oligosaccharides	TMT SDMT BDT	Significant improvement from baseline in all 3 tasks following probiotic intervention. Similar improvements seen in control group taking lactulose
Ohsawa et al. (2018)	Double-blind RCT	60	M = 58	N/A	8 weeks	Fermented milk with *Lactobacillus helveticus* CM4	RBANS	Significant improvement from baseline in total score, delayed recall and attention following the fermented milk. Difference between placebo and intervention group was significant post-intervention for attention.
Papalini et al. (2019)	Double-blind RCT	58 (all female)	m = 21	N/A	4 weeks	*Bifidobacterium bifidumW23, Bifidobacterium lactisW51, Bifidobacterium lactisW52,L. acidophilusW37,Lactobacillus brevisW63,Lactobacillus caseiW56,Lactobacillus salivariusW24, Lactococcus lactisW19 and Lactococcus lactisW58* (5×10^9 CFU/day)	Emotional face matching paradigm Emotional Stroop task Stroop task DST-backwards	Working memory performance maintained in DST under acute stress following probiotic but not placebo treatment. Probiotics associated with a 'buffering effect' against stress
Roman et al. (2018)	Double-blind RCT (pilot)	31	m = 52	Fibromyalgia	8 weeks	*Lactobacillus rhamnosus* GG® *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Bifdobacterium bifdus* (1.2×10^7CFU/day)	Two-choice task Iowa gambling task MMSE	Significantly reduced number of impulsive choices following probiotic treatment

Román et al. (2019)	Double-blind RCT	34	m = 64	Cirrhosis	12 weeks	*Streptococcus thermophilus* DSM 24731, *Bifidobacterium longum* DSM 24736, *Bifidobacterium infantis* DSM 24737, *Lactobacillus paracasei* (L. paracasei) DSM 24733, *Lactobacillus acidophilus* DSM 24735, *Lactobacillus delbrueckii* subsp *bulgaricus* DSM 24734, and *Lactobacillus plantarum* DSM 24730 *Bifidobacterium breve* DSM 24732 (9×10^{11} CFU/day)	PHES	Significant improvement in PHES score after probiotic treatment.
Rudzki et al. (2019)	Double-blind RCT	60	m= 39	MDD	8 weeks	SSRI + *Lactobacillus plantarum* 299v 10×10^9 CFU/day	APT Stroop task TMT AVLT RFFT	Significant improvement in work speed (APT) and total AVLT recall in probiotic group compared to placebo
Slykerman et al. (2018)	Single-blind RCT	342	no data	N/A	From 35 weeks gestation until six months if breastfeeding and their infants the same treatment from birth to two years.	*Lactobacillus rhamnosus* HN001 ($6x10^9$ CFU/day) or *Bifidobacterium animalis* subsp. *lactis* HN019 (9×10^9 CFU/day)	WISC -IV AST SWM OTS	No significant effect of either probiotic treatment on neurocognitive outcomes.
Tamtaji et al. (2018)	Double-blind RCT	79	m = 77	AD	12 weeks	*Lactobacillus acidophilus, Bifidobacterium bifidum* and *Bifidobacterium longum* + 200 mg of selenium (6×10^9 CFU/day)	MMSE	Significantly greater improvement in MMSE score in the probiotic + selenium group than selenium alone or control groups.
Tillisch et al. (2013)	Double-blind RCT	27 (all female)	18 - 53 (m = 30)	N/A	4 weeks	Fermented milk with *Bifidobacterium animalis* subsp *lactis (*I-2494), *Streptococcus thermophilus* (I-1630), *Lactobacillus bulgaricus* (I-1632 and I-1519) and *Lactococcus lactis* subsp *lactis* (I-1631) ($\sim 2.9 \times 10^{10}$ CFU/day)	Emotional decision task and matched control	FMPP associated with decreased activity in widely distributed brain network during emotional task, particularly in the somatosensory cortices and insula.

Wallis et al. (2018)	Open-label single-arm pilot	44	16 - 85 (m = 44)	CFS	6 weeks	Combined antibiotic and probiotic therapy on alternate weeks: Erythromycin (800 mg) during weeks 2 and 4 and *Lactobacillus rhamnosus* (2.5×10^{10} CFU/day), *Bifidobacterium lactis* (1.5×10^{10} CFU/day), *Bifidobacterium breve* (5×10^{6} CFU/day), *Bifidobacterium longum* (5×10^{6} CFU/day) weeks 3 and 5.	RVIP AST SWM PAL RAVLT Logical Memory (WMS-IV) COWAT	Large treatment effects suggested for attention, processing speed, cognitive flexibility, story memory and verbal fluency.[1]

TYM, Test Your Memory; BSDI, Bayley Scales of Infant Development; MMSE, Mini Mental State Examination; PAL, Paired Associated Learning; RVIP, Rapid Visual Information processing; NCT, Number Connection Test; DST[a], Digit Symbol Test; BDT, Block Design Test; WMS, Wechsler Memory Scale; ROCF, Rey-Osterrieth Complex Figure Test; (RA)VLT; (Rey Auditory) Verbal Learning Task; CBTT, Corsi Block-Tapping Test; VST, Visual Search Task; TMT, Trail Making Task; STEP, Time and Weight Estimation Test; PVF, Phonological Verbal Fluency; SVF, Semantic Verbal Fluency; RCPM, Ravens Coloured Progressive Matrices; DST[b]; Digit-Span Test; ACPT, Auditory Continuous Performance Test; MoCA, Montreal Cognitive Assessment; MOT, Motor Screening Test; AST, Attention Switching Task; DSST, Digit Symbol Substitution Task; WAIS, Wechsler Adult Intelligence Scale; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; CBB, CogState Brief Battery; PHES, Psychometric Hepatic Encephalopathy Score; SDMT, Symbol Digit Modalities Test; DST-backwards, DST[b]-backwards; APT, Attention and Perceptivity Test; RFFT, Ruff Figural Fluency Test; WISC, Wechsler Intelligence Scale for Children; SWM, Spatial Working Memory; OTS, One Touch Stockings; COWAT, Controlled Oral Word Association Task.

Table 1 - Number of studies reporting a significant positive effect of probiotic intervention versus no effect of probiotic intervention (effect/no effect) on cognitive tasks, and the respective cognitive function(s) targeted.

Cognitive function	**Tasks used**
Attention/vigilance (6/6)	Attention Switching task (1/2) Rapid Visual Information Processing (2/2) Digit Symbol Substitution Task/ Symbol Digit Modalities Test (1/2) Attention and Perceptivity Test (1/0) Auditory Continuous Performance Test (1/0)
Working memory (3/4)	Digit span (1/3) Serial 3/7s (1/0) Spatial Working Memory (1/1)
Immediate spatial memory (2/0)	Corsi-blocks (2/0)
Verbal memory (immediate) (6/5)	(Rey Auditory) Verbal Learning Task (4/2) Paired Associated Learning (1/2) Wechsler Memory Scale logical memory (1/1)
Verbal memory (delayed) (4/2)	(Rey Auditory) Verbal Learning Task (3/0) Weschler Memory Scale logical memory (1/1) Story recall (0/1)
Visuo-spatial memory (delayed) (2/0)	Rey-Osterrieth Complex Figure Test (2/0)
Episodic memory (0/1)	Capital city recall (0/1)
Psychomotor skill (2/4)	Trail Making Test A/B (2/2) Motor Screening Test (0/1) Number Connection Test A/B (0/1)
Executive function (8/16)	Stroop task (classic) (1/3) Controlled Oral Word Association Task (1/0)

	Block Design Test (1/1) Phonemic Verbal Fluency (2/0) Ruff Figural Fluency Test (0/1) Semantic Verbal Fluency (0/2) Stroop task (emotional) (0/2) Verbal Fluency Task (0/3) One Touch Stockings (CANTAB) (0/1) Flanker task (0/1) Iowa Gambling Task (0/1) Number Connection Test B (0/1) Two-choice task (1/0) Emotional decision task (2/0)
Affective processing (3/4)	Stroop task (emotional) (0/2) Emotional recognition task (1/2) Emotional decision task (2/0)
Composite measures (9/7)	Mini Mental State Examination (4/1) Montreal Cognitive Assessment (1/0) Repeatable Battery for the Assessment of Neuropsychological Status (2/0) CogState Brief Battery (1/0) Bayley Scales of Infant Development II/III (0/4) Psychometric Hepatic Encephalopathy Score (2/0) Test Your Memory (0/1) Wechsler Intelligence Scale for Children -IV (0/1)
Fluid intelligence (0/2)	Ravens Coloured Progressive Matrices (0/2)