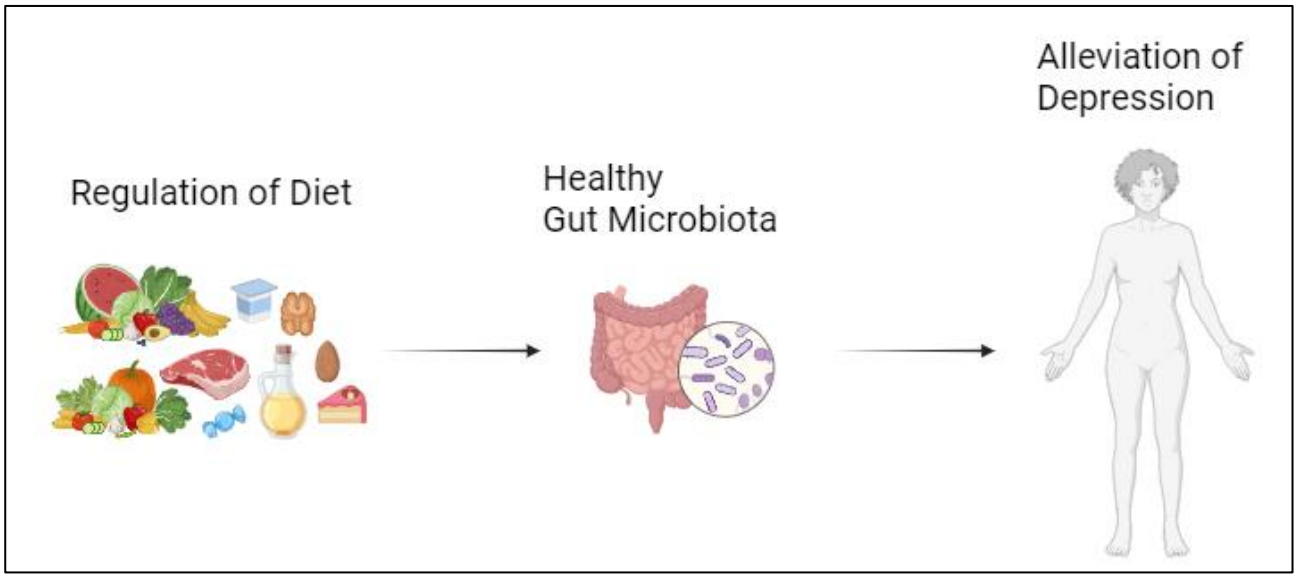
university of groningen / faculty of science and engineering

Exploring the Potential Benefits of Dietary Intervention on Depression through Modulation of the Gut Microbiota.

Unravelling the Interplay between Nutritional Strategies, Depression, and the Gut Microbiota.



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Cover Image: figure showing the hypothesis advanced in this thesis, namely that dietary intervention can regulate the gut microbiota composition resulting in amelioration of depressive symptoms. This figure was made with BioRender.

Summary

The following study aims at analysing dietary intervention as novel treatment approach for depression via modulation of the gut microbiota, since current pharmacological treatments lack efficacy and present various side effects. The current literature already supports the influence of diet on depression as well as the modulatory effects of diet on gut microbiota composition. Hence, dietary supplementations are proposed to exhibit positive effects on the gut microbiota composition resulting in alleviation and/or prevention of depression.

The current research first presents strong evidence for a causal correlation between the gut microbiota composition and depression in both human and animal studies. Indeed, dysbiosis is clearly associated with depressive symptoms and induced depression in animal model results in gut microbiota alterations. Secondly, the link between dietary modulation, gut microbiota composition, and incidence of depressive symptoms is illustrated. Specifically, probiotics, prebiotics, and certain foods (walnuts, almonds, and fermented beverage) are demonstrated to yield beneficial effects on gut health population and depression.

Conclusively, substantial evidence is presented to infer that dietary interventions seem effective treatments to ameliorate depressive symptoms via the regulation of the gut microbiota. Lastly, the potential mechanisms underlying the pathogenesis of depression involving the gut microbiota are hypothesised.

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Introduction

In the last few years, mental disorders have become increasingly more prevalent in the world population. Particularly, depression is the neuropsychiatric disorder with the highest incidence, followed by anxiety and bipolar disorder. People exhibiting depressive-like symptoms have been rising after the COVID-19 pandemic resulting in approximately 280 million depressive patients worldwide (*Mental Health and COVID-19: Early Evidence of the Pandemic's Impact*, 2022). The WHO defines mental disorders as a significant impairment in one's cognition, emotional regulation, or behaviour (*Mental Health and COVID-19: Early Evidence of the Pandemic's Impact*, 2022). As a results, individuals suffering from a mental disorder struggle in their everyday lives and very often require medical attention.

Depression is further described as a condition in which patients might experience depressed mood, loss of interest or pleasure, poor concentration, or sleeping disturbance (*Mental Health and COVID-19: Early Evidence of the Pandemic's Impact*, 2022). On a more clinical prospective, the Diagnostic and Statistical manual of Mental Disorders, Fifth Edition (DSM-V) characterises major depressive disorder (MDD) in humans by the appearance of at least five symptoms within two week-time (Tolentino & Schmidt, 2018). The primary and main symptoms are either depressed mood or anhedonia (or loss of pleasure) while the secondary symptoms range from psychomotor agitation to suicidality (Tolentino & Schmidt, 2018). Depending on the intensity, frequency, and number of symptoms, moderate depression disorder (MDD) and severe depression (SD) have been differentiated. Currently, the measurements of depression severity mostly rely on scoring scales such as the Hamilton Depression Rating Scale (HAMDS) and the Depression Anxiety Stress Scale (DASS) (Tolentino & Schmidt, 2018).

The pathophysiology of depression is complex and only partially understood, even though the Serotonin Theory of Depression, postulated in the 1994, is still considered highly relevant (Kerr, 1994). The Serotonin Theory, also confirmed by human postmortem studies, affirms that depressive disorders are marked by a reduction in serotonin transmission due to decreased serotonin (5-HT) availability and impaired activity of the serotonin receptors in the central nervous system (CNS) (Kerr, 1994, Saveanu & Nemeroff, 2012). In addition to 5-HT disbalances, low norepinephrine (NE) metabolites were recorded in MDD patients, suggesting that also NE alteration in the CNS influences depressive symptoms ("Saveanu & Nemeroff, 2012). Furthermore, depression is associated with alterations in neuroendocrine and immune pathways. Indeed, depressed patients often show both hypersecretion of cortisol, caused by hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis, and increased chronic inflammation, caused by overproduction of pro-inflammatory cytokines resulting in microglia activation (Saveanu & Nemeroff, 2012). Interestingly, all these pathways are modulated to a certain extent by the gut microbiota and enteric nervous system (ENS), the collection of neurons located in the human gastrointestinal (GI) tract (Nezami & Srinivasan, 2010).

Considering the high incidence of depression on the worldwide population, providing effective treatments appear to be essential. Current approaches mainly focus on pharmacological interventions selectively targeting the neurotransmitter disbalance found in depressive patients. For instance, selective serotonin reuptake inhibitors (SSRIs), like fluoxetine, and serotonin-norepinephrine reuptake inhibitors (SNRIs) count as the most common drug employed. However, a meta-analysis on the use of SSRIs showed that drug related treatments compared to placebo yielded

a response difference of only 10-15% (Leucht et al., 2012), proving their partial efficacy. Two major well-established limits of SSRIs are drug resistance over time and numerous side effects, defined as undesired health-damaging symptoms caused by drug intake (Chang et al., 2022b, Edinoff et al., 2021). Side effects of SSRIs range from headache and insomnia, to increased suicidality, observed mainly in children, and Serotonin Syndrome (Edinoff et al., 2021). As a result, recent treatments focus more on a combined approach providing both psychotherapy and pharmacological medications. Nonetheless, this approach was proved to be effective only on 74% of patients (Makris et al., 2020b). Therefore, more efficacious treatments for depression must be designed.

Recent studies focused on the relation between diet and depression as therapeutic strategy. A meta-analysis published in 2013 already showed that both high and moderate adherence to Mediterranean diet is associated with decreased depressive symptoms (Psaltopoulou et al., 2013b). A human randomised control trial on 85 MDD patients showed that Mediterranean diet implementation over 6 months resulted in lower scoring in the Depression Anxiety Stress Scale (DASS) and higher scoring in the Assessment of Quality of Life (AQoL) (Parletta et al., 2017). Consistently, another human trial confirmed that adoption of Mediterranean diet by MDD patients over 12-week treatment yielded improved scoring in both Montgomery Asberg Depression Rating Scale (MADRS) and Clinical Global Impression-Improvement (CGI-I) compared to control group (Jacka et al., 2017). Notably, the latter study established that decreased depressive symptoms were not reflected in cardiovascular disease (CVD) biomarkers, such as plasma fatty acids and fasting glucose (Jacka et al., 2017). Therefore, the gut microbiota was hypothesised to be responsible for the beneficial effects of diet on depression.

The association between diet and the gut microbiota has been recently under investigation. The gut microbiota is defined as the collection of all microorganisms located in the human gastrointestinal tract (Figure 1) (Thursby & Juge, 2017). The gut microbiota has several beneficial effects on the host such as maintenance of the intestinal mucus layer, production of short chain fatty acids (SCFAs) and neurotransmitters such as serotonin, and modulation of both the HPA axis and the inflammatory response (Thursby & Juge, 2017). Importantly, the gut microbiota composition and relative abundance are subject to changes over one's lifespan depending on various factors, ranging from environment to genetics (Thursby & Juge, 2017, Makris et al., 2020b). Although, a twin study demonstrated that the main influencing element determining the gut microbiota composition is diet (Vílchez-Vargas et al., 2022). In fact, the analysis of 10 human subjects following a plant-based or an animal-based diet confirmed a significantly altered gut microbial composition (David et al., 2013b).

Despite the high variability in the composition, healthy gut microbiota composition is characterised in contrast to gut microbiota dysbiosis, where dysbiosis is defined as a disbalance in the gut microbiota resulting in reduction of microbial diversity and beneficial microbes (Petersen & Round, 2014). Microbial composition is often measured as microbial diversity. Diversity accounts for bacterial richness, described as the total number of species in the studied sample, and evenness, defined as the relative differences in the abundance of species in the community (Young & Schmidt, 2009).

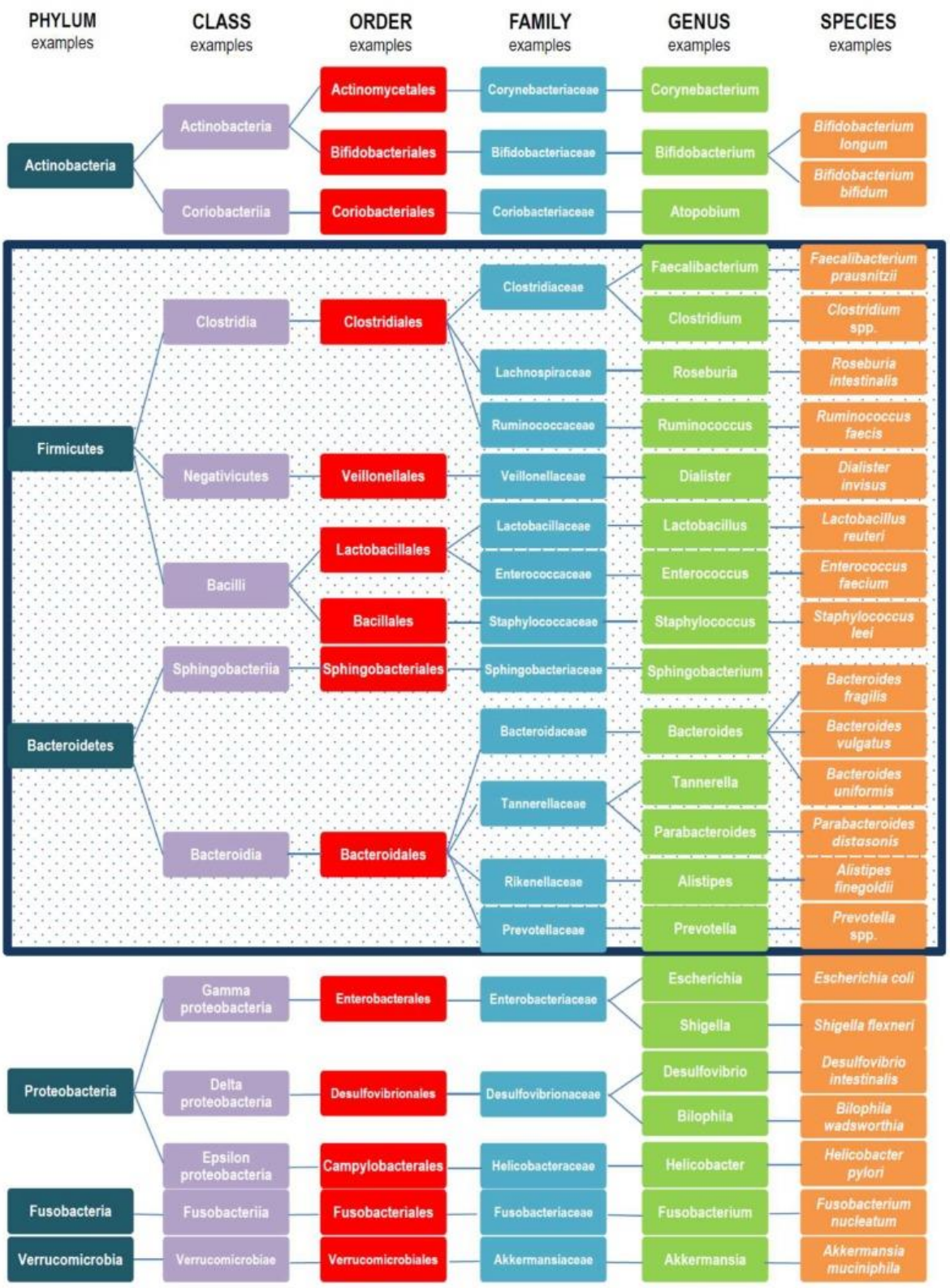

Figure 1. The major gut bacteria observed in healthy individuals and their taxonomic classification are shown. Firmicutes and Bacteroidetes (highlighted) count for around 90% of the entire gut microbial population, (Rinninella et al., 2019).

Human healthy gut microbiota composition presents Firmicutes and Bacteroidetes phyla, counting for approximately 90%, followed by Actinobacteria, Verrucomicrobia, and Proteobacteria phyla (Figure 2) (García-Mantrana et al., 2018). The order Bacteroidales counts for the majority of Bacteroidetes, while Clostridiales represents the main order of Firmicutes (Rinninella et al., 2019). Notably, Bifidobacterium genus composes the majority of the Actinobacteria phylum (Figure 1) (Rinninella et al., 2019). Lastly, *Faecalibacterium prausnitzii*, a species belonging to Firmicutes phylum, is highly associated with healthy gut microbiota compositio (Figure 1) (Parsaei et al., 2021).

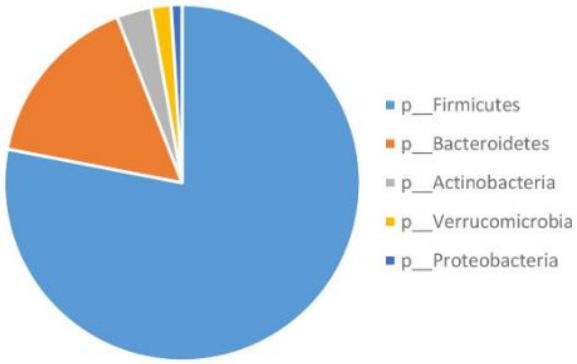

Figure 2. A pie chart representing the relative abundance of the main gut microbiota phyla in healthy individuals, (García-Mantrana et al., 2018).

Conversely, gut microbiota dysbiosis is, among others, caused by stress and results in all kinds of gut microbial disbalances. For instance, mice studies proved that exposure to induced stress results in decreased Bacteroides and increased Clostridium genera (Rinninella et al., 2019). Dysbiosis also originates following Western or high-sugar (HS) diet. An animal study performed on male mice documented higher presence of Bacteroidetes phylum and an overall decreased bacterial count after a 12-week high-fat (HF) diet treatment (De La Serre et al., 2010b). A further study confirmed that high sugar consumption in juvenile and adolescence rats alters the gut microbiota composition independently of obesity and caloric intake (Noble, Hsu, Jones, et al., 2017b, Noble et al., 2017). Consequently, exogenous factors such as stress and diet exercise a modulatory effect on the gut microbiota composition causing dysbiosis.

Interestingly, in recent years it has been discovered a complex communication between the central nervous system (CNS), the enteric nervous system (ENS), and the gut microbiota (Clapp et al., 2017). This connection is known as the microbiome-gut-brain (MGB) axis and it seems to be modulated via exchange of the hormone cortisol, neurotransmitters, such as serotonin, and immunological factors, both pro- and anti-inflammation (Makris et al., 2020b). Specifically, studies verified that dysbiosis has negative effects on the CNS due to increased cortisol secretion, decreased serotonin, and increased pro-inflammatory cytokines production (Clapp et al., 2017). Notably, these same factors associated with depressive disorder are partially modulated by the gut microbiota composition. Previous evidence showing a strong correlation between dysbiosis, and depression was observed in humans (Sonali et al., 2022). Hence, it is hypothesised that gut microbiota dysbiosis causally leads to depression, probably due to cortisol, serotonin, and pro-inflammatory disbalances reaching the CNS (Winter et al., 2018b).

To produce more effective treatments for depression, the modulation of the gut microbiota is investigated as a novel approach. Indeed, regulation of the gut microbiota richness and evenness via dietary interventions can potentially alleviate depressive symptoms. Thus, the current review aims at investigating dietary manipulations as potential novel treatment approaches for depression via modulatory effects exercised on the gut microbiota. The dietary interventions discussed are probiotics and prebiotics supplementation, and daily intake of certain foods such as nuts and fermented products. The current review first presents strong evidence for a connection between the gut microbiota modulation and amelioration of depression both in human and animal studies. Secondly, dietary interventions regulating the gut microbiota is proved to yield positive effects on depressive-like behaviours in animal studies. Moreover, studies comparing probiotics and prebiotics use to SSRIs are included, showing more effective outcomes of probiotics and prebiotics on gut microbiota composition and depression.

Main Body

Correlation between the gut microbiota and depression

In recent years, the interest toward the gut microbiota as a modulator of various biological processes involved in pathogenesis of diseases has progressively increased. Many studies have focused on the relation between the gut microbiota and depression. Both human and animal research attempted to explain if and how a link between the gut microbiota and major depressive disorders (MDD) exists.

Human studies demonstrated a strong relation between altered gut microbiota composition and depressive symptoms. Faecal and blood samples were collected from healthy control (HC) and MDD patients recruited from The Seventh People's Hospital of Hangzhou (Zhejiang, China) (Jiang et al., 2015). MDD patients were divided into active-MDD, or untreated MDD patients, and responding-MDD, or MDD patients having already a history of intervention. A-MDD group exhibited decreased Firmicutes and Actinobacteria phyla, and increased Bacteroidetes and Proteobacteria phyla compared to HC. Additionally, a rise in Clostridium and a drop in Bacteroides genera was observed in A-MDD compared to HC. Interestingly, Faecalibacterium genus was negatively associated with the severity of depression since it exhibited different abundances between A-MDD and R-MDD. Finally, inflammatory markers were measured to attest the inflammatory state among the three groups. The results showed no difference in cytokine levels (Jiang et al., 2015). This latter result seems to suggest that inflammation is not causal in depression, although many studies proved that chronic inflammation is associated with depressive symptoms (Lee & Giuliani, 2019).

An additional human study opted for a meta proteomics approach, focusing on bacterial proteins and phyla in MDD patients and healthy control (HC) (Chen et al., 2018). The outcome documented an altered bacterial protein expression in MDD compared to HC. On the phylum level, Firmicutes and Actinobacteria were reduced while Bacteroidetes and Proteobacteria were elevated in the MDD group (Chen et al., 2018). Following research confirmed that increased Bacteroidetes phylum was associated with depressive symptoms compared to healthy controls, in accordance with the previous studies (Mason et al., 2020). Notably, the genus Faecalibacterium, known to be associated with a

healthy gut microbiota composition, was confirmed to be correlated with alleviation of depressive symptoms (Mason et al., 2020). Overall, strong evidence was presented in support of a correlation between gut microbiota dysbiosis and depressive disorder in humans.

Researchers tried to assess a causal relation between the gut microbiota composition and the pathogenesis of depression. Hence, depressive-like behaviours were induced in mice models. To verify whether depression resulted in gut microbiota dysbiosis, Park et al. performed on female mice olfactory bulbectomy (OB), a technique designed to induce depression in mice (Harkin et al., 2003). The depressive-like behaviours were validated via tail suspension test (TST) and open field test (OFT), where mice showed prolonged immobility and reduced exploratory behaviour, respectively (Park et al., 2013). To compare gut microbiota composition between the OB and control group, bacterial profiling was performed from mice faecal samples. The results evidenced a gut microbial similarity of only 49.1% between the two groups and a significantly altered proportion among the major phyla (Figure 1) (Park et al., 2013). Hence, OB depressed mice were proven to exhibit gut microbiota dysbiosis in contrast to healthy composition in the control group.

Another animal study showed how gut microbiota dysbiosis results from induced stress, one of the exogenous factors correlated with depression. Meng et al. performed chronic variable stress (CVS) procedure on male mice, in contrast to healthy controls (HC), and faecal samples were collected to analyse the gut microbial composition (Meng et al., 2017). The results showed decreased Firmicutes and increased Bacteroidetes phyla in the CVS group, while the genus Clostridiales dropped in the intervention group compared to control (Meng et al., 2017). Moreover, alteration of sixteen faecal metabolites was associated with dysbiosis in CVS mice (Meng et al., 2017), showing that faecal metabolome disbalances result from gut microbiota dysbiosis.

Substantial evidence demonstrated that depressive-like behaviours in healthy mice were temporarily preceded by gut microbiota dysbiosis (Li et al., 2019). In this research, chronic unpredictable mild stress (CUMS) procedure was applied for eight weeks on male mice (Li et al., 2019, Harkin et al., 2003). Both behavioural and physiological tests (faecal and plasma samples) were performed several times over the entire duration of the treatment. The comparison between CUMS and healthy mice testified altered gut microbiota alpha-diversity in CUMS mice, where alpha-diversity is defined as the microbial intra-community diversity or microbial richness (Thukral, 2017, Li et al., 2019). Importantly, the genus Faecalibacterium decreased in CUMS group compared to control, as already observed in human studies. Thus, it was concluded that gut microbial dysbiosis was temporarily followed by the surge of depressive-like behaviours (Li et al., 2019), suggesting a causal role of dysbiosis on depression.

Overall, induced depression via olfactory bulbectomy or stress induced procedures on mice models resulted in gut microbiota dysbiosis in contrast to healthy control. Specifically, decreased Firmicutes and increased Bacteroidetes composition were consistently observed over the studies. Furthermore, it was shown that gut microbiota dysbiosis temporarily proceeds depressive-like behaviours, suggesting a causal relation between dysbiosis and depression.

Causality between gut microbiota dysbiosis and surge of depression was confirmed by Kelly et al., who performed faecal microbiota transplantation (FMT) from the gut microbiota belonging to both

MDD and healthy human patients into depleted gut microbiota mice (Kelly et al., 2016). Firstly, behavioural tests, namely sucrose preference test (SPT), elevated plus maze (EPM), and open field test (OFT) were performed to assess depression. Secondly, faecal and blood samples were analysed for bacterial composition and levels of inflammatory cytokines. The outcome from the behavioural tests indicated depressive-like behaviours in the MDD mice group, due to lower sucrose intake, decreased visit to the open arm in the EPM, and lower time spent in the centre of the field in the OFT. Following data established that gut microbiota diversity (richness and evenness) dropped in the MDD mice group compared to the healthy group, consistently with the data obtained from the human donors. Interestingly, the genus Bifidobacterium, a well-established beneficial bacterium, was decreased in the depressed group compared to control group. Lastly, cytokine levels did not significantly differ between groups (Kelly et al., 2016). These results prove the hypothesis that selective manipulation of the gut microbiota composition from MDD patients is causally linked to the surge of depressive-like behaviours in healthy mice.

A final compelling study performing FMT from MDD human patients and healthy controls on germ free (GF) male mice confirmed the previous results (Zheng et al., 2016). Behavioural tests performed on the second week from the procedure documented depressive-like behaviours in the MDD transplanted mice. In fact, increased immobility in the tail suspension test and decreased time spent in the open field in the open field test were observed. Faecal analysis confirmed that the gut microbiota composition found in human patients was likewise reflected in the mice groups, and MDD gut microbiota presented lower Bacteroidetes, increased Actinobacteria, and no significant difference in Firmicutes composition compared to the gut microbiota found in the healthy control group (Zheng et al., 2016).

Taken collectively, these studies substantiate the causal connection between the gut microbiota composition and the pathogenesis of depression. In fact, induced depressed mice models attested gut microbiota dysbiosis. At the same time, faecal microbiota transplantation (FMT) of gut microbiota from MDD human patients resulted in depressive-like behaviours in healthy mice models (Kelly et al., 2016, Zheng et al., 2016). However, contradictory data regarding the exact disbalances in the gut microbiota composition were observed in the presented studies. Overall, the causal connection between gut microbiota dysbiosis and surge of depression is confirmed and hence targeting the gut microbiota composition via dietary interventions may be an effective treatment to alleviate depression.

Dietary interventions resulting in alleviation of depression via modulation of the gut microbiota

Convincing evidence already established the strong correlation between diet and depression as well as the relation between diet and the gut microbiota. Moreover, the causal connection between depression and the gut microbiota was proved by the previous studies. Hence, dietary intervention is proposed as a potential treatment for depression due to its regulatory effect on the gut microbiota. Most of the studies investigated the role of probiotics and prebiotics, defined respectively as living microorganisms and gut microbiota-degraded nutrients that exhibit health benefits on the host (Hill et al., 2014, Davani-Davari et al., 2019). Nonetheless, some studies focused also on the use of

specific foods such as nuts and fermented products to modulate the gut microbiota composition and alleviate depression.

A randomised human trial on elderly MDD patients investigated the effects of probiotics administration for a period of 12 weeks (Kim et al., 2020). Microbial community was assessed via the 16rRNA sequencing. At the phylum level, no significant abundance difference was measured, although at the genus level, a statistically significant alteration in the gut microbial composition was observed between the intervention and the control group. Specifically, the relative abundance of Clostridiales genus dropped in the probiotic group. Depression levels were measured via the Geriatric Depression Scale (GDS-K) which attested a significant decrease in depression and stress scoring. In addition, brain-derived neurotrophic factor (BDNF), a neuroprotective neurotrophic factor found in the CNS (Bathina & Das, 2015), raised after the probiotic treatment and resulted in enhanced mental flexibility measured via mental flexibility test (Kim et al., 2020). Therefore, this trial demonstrated a positive correlation between probiotics administration and healthier gut microbiota composition, resulting in alleviation of depression. Notably, probiotics administration was also associated with increased BDNF which may be causally responsible for improved mental flexibility.

A mice study demonstrated the beneficiary effects of prebiotic fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) administration on the gut microbiota composition and depression (Burokas et al., 2017). Male mice were divided into four groups where 3-week administration of FOS only, GOS only, FOS in combination with GOS (FOS + GOS), and water control was conducted. After the treatment, caecal microbiota composition of the FOS + GOS and FOS only groups measured increased presence of Bifidobacterium, a genus associated with healthy gut microbiota. Behavioural tests confirmed that the FOS + GOS mimicked the healthy control behaviours, since more time in the open field in the open field test and a decreased immobility in the tail suspension test were measured (Burokas et al., 2017). Consistently with the previous study, increased BDNF was found in all the intervention groups, even though FOS + GOS groups showed the sharpest increase. Thus, prebiotic supplementations both improved healthy gut microbiota composition, by increasing Bifidobacterium abundance, and ameliorated depressive-like symptoms. Importantly, this research proved that treatment with a combination of prebiotics yielded the most effective outcome on gut microbiota composition, alleviation of depression, and BDNF levels.

Since Bifidobacterium genus was positively associated with decreased depressed symptomatology and improved gut microbiota composition, daily Bifidobacterium probiotics intake was further investigated. Firstly, Tian et al. investigated the efficacy of supplementation of *Bifidobacterium longum* strain CCFM687 in contrast to the SSRI fluoxetine on chronically stressed mice (Tian et al., 2019). The behavioural analysis performed after six weeks of treatment evidenced reduced immobility in the forced swimming test and in the tail suspension test in both the probiotic and the fluoxetine group, confirming the beneficiary effects of both treatments on the induced depressive-like behaviour. Although, the gut microbiota alpha-diversity in the probiotic group improved significantly in contrast to the fluoxetine group, even though the disbalances of Bacteroidetes and Firmicutes were irreversible in all intervention groups (Tian et al., 2019). Following, Tian et al. also attested the use of *Bifidobacterium breve* CCFM1025 as probiotic supplementation on CUMS male mice, in contrast to the use of SSRI fluoxetine (Tian et al., 2020). Behavioural tests (open filed test, tail suspension test, sucrose preference test) and faecal 16rRNA sequencing were performed after five weeks of CUMS procedure on probiotic, fluoxetine, and control group. Increased time spent in the open field, decreased immobility, and increased sucrose intake was measured in the

Bifidobacterium group compared to the fluoxetine group. Furthermore, gut microbiota composition in the probiotic group mimicked more evenly the microbial composition of the healthy control, showing comparable levels of Bacteroides/Firmicutes ratio and Bifidobacterium genus (Tian et al., 2020). Overall, the results showed a greater efficacy of the probiotic *Bifidobacterium* supplementations rather than fluoxetine, evidencing the enhanced benefits of probiotics on both gut microbiota health and depression, compared to SSRIs.

Interestingly, a randomised clinical trial investigated the effects of both probiotic and prebiotic supplementation (Kazemi et al., 2019). In the 2-month human trial, depression levels in probiotics and prebiotics intervention group, and a placebo group were measured via the Beck Depression Inventory (BDI). The outcome showed a significant decrease in BDI score in the probiotic group while the prebiotic intervention group did not exhibit any score improvements (Kazemi et al., 2019). Consequently, probiotic supplementation seemed to be more promising than prebiotics, even though limitations of the study were the lack of faecal microbiome analysis and the seasonal difference at which the trials were performed (Kazemi et al., 2019).

Aside from probiotics and prebiotics interventions, scientists also contemplated the use of specific foods to modulate the gut microbiota and hence alleviate depression. One recent study researched the effect of walnut consumption in a human randomised feeding trial (Herselman et al., 2022). In the 16-week trial, the intervention group was assigned a daily intake of 56g of walnut in contrast to the control group. Faecal samples, Depression Anxiety Stress Scale (DASS), and the Assessment of Quality of Life (AQoL) were assessed three times over the intervention period. At the end of the trial, the intervention group presented an increased alpha-diversity, as increased intra-community microbial species were measured. Importantly, increased Firmicutes and decreased Bacteroidetes phyla were observed in the walnut intervention group, mimicking the healthy gut microbiota composition. Moreover, lower scoring in the DASS and higher scoring in the AQoL were observed in comparison with the control group, suggesting decreased depressive symptoms and increased overall quality of life (Herselman et al., 2022). As a result, walnut intake showed healthier gut microbiota composition and decreased depressive symptoms in humans.

Another human trial investigated the use of almonds coupled with a decreased carbohydrate diet (a-LCD) on depressed type 2 diabetes patients in contrast to a low-fat diet (LFD) (Ren et al., 2020). For three months, LCD and a daily 56g portion of almond was implemented in the intervention group, in contrast to the LFD group. At the end of the trial, both an increase in alpha-diversity and a drop in Firmicutes phylum was measured in the a-LCD group compared to the LFD. Furthermore, self-report depression questionnaire evidenced a decrease in depression in the a-LDC group (Ren et al., 2020). Consequently, daily administration of almond paired with a low-carbohydrate diet was demonstrated to benefit both the gut microbiota composition and depressive symptoms. However, a control for assessing whether solely almond intake or the low-carbohydrate diet (LCD) were causal for the results was not included.

An alternative food of interest hypothesised to favour the gut microbiota composition and depression was fermented milk beverage kefir. Evidence already showed the modulatory effect of kefir on the gut microbiota in rodents (Hsu et al., 2018). Hence, a mice study explored whether kefir administration over three weeks on depressed male mice also resulted in alleviation of depressive-like behaviours and improved gut microbiota composition (Van De Wouw et al., 2020). The identified

groups were unfermented milk control, kefir Fr1, kefir UK4, two kefir presenting different microbial compositions, and undisturbed control. After the three-week treatment, both kefir intervention groups evidenced increased alpha-diversity and specifically kefir Fr1 presented a high abundance of Bifidobacterium genus (Van De Wouw et al., 2020). Additionally, behavioural tests testified that kefir UK4 group spent the most time sniffing in the female urine sniffing test, and kefir Fr1 increased saccharin preference test (Van De Wouw et al., 2020). These data demonstrated increased reward-seeking behaviour and decreased depressive-like behaviours in both kefir intervention groups in contrast to both the control groups (Van De Wouw et al., 2020). Overall, fermented beverage kefir was confirmed to have positive effects on both gut microbiota composition and depression-like behaviour in mice model.

The comprehensive analysis of all the studies presented affirm a strong connection between dietary implementations and alleviation of depression via regulation of the gut microbiota composition. Particularly, nuts and fermented beverage administration resulted in both healthier gut microbiota composition, by promoting its diversity (richness and evenness), and amelioration of depressive symptoms. Notably, mice studies comparing probiotics, prebiotics, and SSRIs showed healthier gut microbiota composition and enhanced alleviation of depressive symptoms by probiotics and prebiotics use. As a result, dietary interventions are confirmed to be an effective treatment approach for depression via restoring a healthy gut microbiota population.

Discussion

The present research aims at investigating whether dietary interventions result in alleviation and/or prevention of depression via the modulation of the gut microbiota to support the application of a novel and more effective treatment approach for depression. Since current treatments such as SSRIs tend to lack efficacy and present various side effects (Chang et al., 2022b), the current review suggests an intervention approach which mostly relies on habitual shifts, rather than solely on drug use.

Well-established evidence already indicated a positive relation between Mediterranean diet and alleviation of depressive symptoms in humans (Psaltopoulou et al., 2013b). The hypothesis that diet yields beneficial effects on depression via modulation of the gut microbiota was investigated in the present review. Indeed, recent research on both animals and humans revealed the strong association between diet and gut microbial diversity (richness and evenness) (De La Serre et al., 2010b, David et al., 2013b).

The studies reported present clear evidence for a causal relation between the gut microbiota and depression. In fact, faecal microbiota transplantation from MDD patients to gut microbiota depleted mice results in surge of depressive-like behaviours (Kelly et al., 2016). Additionally, gut microbiota composition of MDD donor patients resemble gut microbiota composition of transplanted GF mice which developed depression (Zheng et al., 2016). Notably, most of the animal studies used male mice, thus neglecting the potential hormonal modulations of depression in females.

Following studies demonstrated the intricate link between diet, gut microbiota, and depression. Specifically, dietary implementations of probiotics, prebiotics, and certain foods (e.g., almond, walnut, fermented beverage kefir) positively correlated with a healthy gut microbiota composition and alleviation of depressive symptoms in both animal and human studies. Therefore, mechanisms concerning the communication between the gut microbiota and the CNS must be responsible for the beneficial effects exercised by diet on depression.

The communicative routes connecting the gut microbiota and the CNS are complex and only partially uncovered. It is recognised that the gut microbiota, the enteric nervous system (ENS), and the CNS are connected via the microbiota-gut-brain (MGB) axis. Specifically, three pathways are recognised to modulate the MGB axis communication. The first mechanism operates via the exchange of neurotransmitters such as serotonin (5-HT), produced in the GI tract and carried to the CNS where serotonergic neurons are activated (Appleton, 2018). *Escherichia coli*, *Lactobacillus plantarum*, and *Streptococcus thermophilus* (Figure 1) are only few of the gut microbes known to produce serotonin in the gut (Strandwitz, 2018). Importantly, decreased activation of the serotonergic pathway in the CNS due to decreased 5-HT production is a well-established biomarker for depression (Figure 3) (Saveanu & Nemeroff, 2012).

The second communication route is via inflammation where pro- and anti-inflammatory cytokines are produced in the gut and travel to the CNS modulating inflammation (Clapp et al., 2017). For instance, intestinal Bifidobacterium was found responsible to enhance antibody synthesis and produce interleukin IL-10, a potent anti-inflammatory cytokine (Dong et al., 2010), whereas *Bacteroides fragilis* (Figure 1) is responsible for IL-6 production, a pro-inflammatory cytokine (Schirmer et al., 2016). Additionally, short chain fatty acids (SCFAs) like butyrate, mostly produced by Firmicutes phylum like *C. butyricum*, exercise a modulatory function on the immune response. Specifically, butyrate inhibits the release of pro-inflammatory cytokine, such as IL-6 (Zhu et al., 2021). Notably, chronic inflammation in the CNS is also a biomarker in depression and it is caused by hyperproduction of pro-inflammatory cytokines like IL-6 (Figure 3). However, some research reported normal levels of pro-inflammatory cytokines in depressed patients challenging the role of inflammation in the pathogenesis of depression (Saveanu & Nemeroff, 2012).

The third pathway is regulated via the hormone cortisol. In fact, its production by the HPA axis is stimulated by pro-inflammatory cytokines, such as IL-6, in response to stress. Observational studies show that chronic cortisol rise in the CNS is highly associated with depression (Figure 3) (Dziurkowska & Wesołowski, 2021), and decreased cortisol caused by modulation of the gut microbiota composition alleviated depressive symptoms in humans (Schmidt et al., 2014).

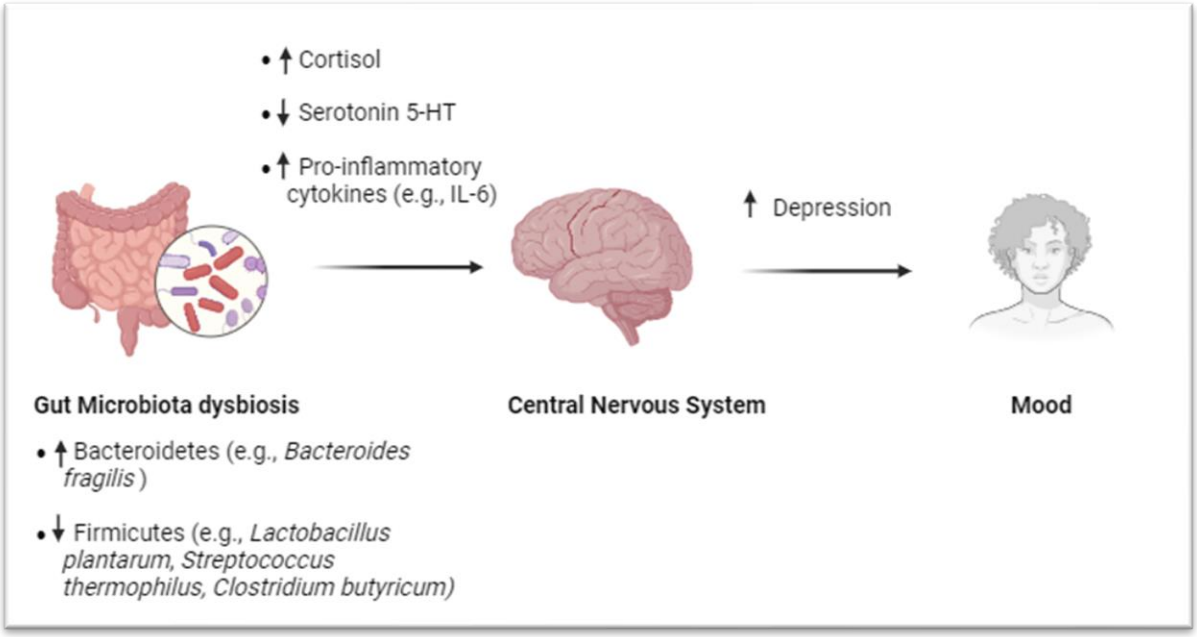

Figure 3. Schematic representation of the mechanisms involved in the pathogenesis of depression. The figure was creased with BioRender.

Based on the current knowledge, the pathogenesis of depression seems influenced via numerous biological pathways involved in the MGB axis, although further research is required to understand the exact mechanisms underlying the modulation of depression by the gut microbiota, as well as the role of inflammation and of SCFAs.

According to the current opinion in the field, a causal relationship between an altered gut microbiota and depression cannot be concluded yet. In fact, on the one hand, animal studies demonstrate how dysbiosis results in depressive-like behaviours in healthy mice (Zheng et al., 2016). On the other hand, it is proved how induced depression in mice model results in gut microbiota dysbiosis (Park et al., 2013). Consequently, it seems that depression can cause and is caused by an altered gut microbiota composition. However, based on the research presented in this review, gut microbiota dysbiosis was found to temporarily proceed the rise of depression. Accordingly, modulation of the gut microbiota composition via diet alleviated depressive symptoms, strongly suggesting how the gut microbiota is causally connected with depression. Even though gut microbiota modifications differed in various studies, decreased Firmicutes and increased Bacteroidetes phyla seemed to result in depression, which might be explained via increased production of pro-inflammatory cytokines by Bacteroidetes and decreased 5-HT production via Firmicutes (Figure 3). However, further examination needs to evaluate the exact alterations in the gut microbiota composition. Potential investigations might also clarify how the modulation of BDNF originates and how that relates to an improved mental flexibility.

Current knowledge allows to develop a novel treatment approach for depression. In fact, reported studies show that daily intake of probiotics or prebiotics is beneficial for both the gut microbiota composition and depressive symptoms. Consequently, probiotic, and prebiotic daily supplementation could be an effective and easy lifestyle adaptation which results in gut microbiota stabilisation and decreased depression. Furthermore, further studies should investigate whether these supplements

also exhibit preventative effects for depression. Interestingly, a combination of probiotics and diet modification might yield even more pronounced benefits on patients. In the future, research must include studies where the combination between probiotic supplementation and diet regulation are analysed in relation to depression and gut microbiota. A proposed study protocol focusing on probiotics and a gluten-free diet was already proposed in 2019 (Karakuła-Juchnowicz et al., 2019b). On the contrary, prebiotics intake presented contrasting results on depression modulation in the research. Consequently, probiotics seem to be more promising than prebiotics as treatment approach.

Attested side effects of probiotics were immunological, such as fever and auto-immune diseases, and impaired metabolic activities (Marteau & Shanahan, 2003). However, very low evidence reported these negative side effects (Marteau & Shanahan, 2003). Thus, probiotics seem a safe treatment for depression via regulation of the gut microbiota composition. Interestingly, in the future, designed palatable foods containing the necessary probiotics for depression amelioration could be made available to the public as preventative strategy.

Solely dietary interventions are also affirmed to be beneficial for both the gut microbiota and depression. Despite the scarce research literature on the topic, strong evidence suggests a positive effect on gut microbiota and depression by a healthy and balanced diet, such as the Mediterranenan one, in contrast to a Western or high-fat diet (Figure 4) (Parletta et al., 2017, Noble et al., 2017). Nonetheless, the Western diet is progressively becoming more wide-spread and generally preferred as considered more palatable due to the high sugar intake, especially in youngers. Consequently, increased rates of diseases related to a high-fat diet, such as cardiovascular disease (CVD) and depression, are observed in the worldwide population (Clemente-Suárez et al., 2023). In light of the current review, a Mediterranean diet, in contrast to a Western diet, protects from gut microbiota dysbiosis and hence depression. Thus, to enhance the preference for a healthy diet, increased exposure to healthy foods should be promoted from childhood. Indeed, it is proven that exposure to foods in the first years of life are crucial for determining food preference and hence dietary patters later in life (Cooke, 2007). Consequently, early life exposure and consumption of healthy foods, such as the ones contained in the Mediterranean diet, can exercise preventative effects on development of depression and dysbiosis later in life (Figure 4).

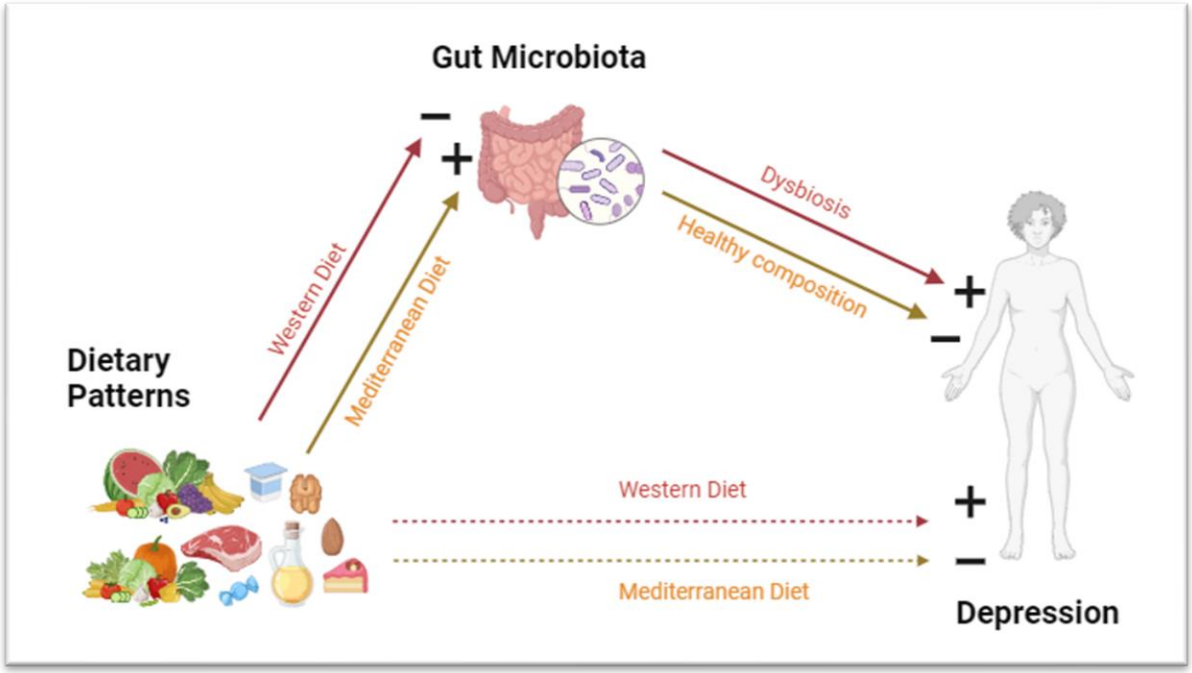

Figure 4. Relation between Mediterranean and Western Diet on both gut microbiota and depression. The figure was created with BioRender.

To achieve increased prevention, the world population requires reliable and precise information delivered from authoritative sources. The World Health Organisation (WHO) plays an essential role in sharing scientific knowledge worldwide. Thanks to the WHO's Comprehensive Mental Health Action Plan 2013-2030, the beneficial effects of healthy diet on the alleviation of depression and gut microbiota composition can be effectively communicated and may result in decreased depressive symptoms and increased prevention. Finally, dietary education from a young age can also lead to significant prevention against depression as well as dysbiosis, ensuring a healthier and improved quality of life worldwide.

Conclusion

Based on the gathered evidence, a causal relation between gut microbiota dysbiosis and surge of depression is established in mice studies. Specifically, several studies showed how a decrease in Firmicutes and an increase in Bacteroidetes phyla result in depressive-like behaviours. Additionally, Clostridiales genus is associated with unhealthy gut microbiota leading to depression, while Faecalibacterium and Bifidobacterium are predominant in healthy gut microbiota and depressive symptoms amelioration.

Following, dietary interventions, such as probiotics, prebiotics, and certain foods (nuts and fermented beverage), are proved to alleviate depression via modulation of the gut microbiota. Specifically, combinational probiotic supplementations seem the most promising intervention in alleviating

depression and stabilising the gut microbiota, also considering the rare side effects. Consequently, diet yields beneficial effects on gut microbiota composition resulting in amelioration of depressive symptoms. The current data suggest that dietary interventions can be applied as effective treatment approach for alleviating depression by maintaining a healthy gut microbiota and may be used preventatively.

Further research is suggested to specifically clarify the exact alterations in the gut microbiota composition observed in depressed patients and the mechanism underlying depression. Lastly, evidence showed increased BDNF following healthy gut microbiota composition due to probiotic intake, suggesting a novel field of research focusing on BDNF modulation and its positive effects on mental flexibility.

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