

**IMPACT OF PSYCHOTROPICS ON THE GUT MICROBIOTA AND POTENTIAL OF
PROBIOTICS TO ALLEVIATE RELATED DYSBIOSIS**

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Abstract

There is an increasing interest in how therapeutic drugs could alter the human gut microbiota composition and function. While some knowledge is accumulating on the antimicrobial impact of some psychotropics on isolated strains or the gut microbiota of animal models, information about other classes of psychotropics and representative species from the human gut is poorly investigated. The antimicrobial effect of psychotropic drugs is usually neglected as a confounding factor when investigating gut microbiome biomarkers, knowing that patients are generally put in long-term medication. The purpose of the present study was to investigate (*in vitro* and *ex-vivo*) the antimicrobial activity of some oral commonly prescribed psychotropics from different therapeutic classes on colonic microbiota diversity and metabolism and the potential capacity of probiotics to alleviate related dysbiosis. The findings of this study revealed an important *in vitro* inhibitory activity of psychotropic drugs, which were also expressed as drastic alterations in gut microbiota composition *ex-vivo*. Indeed, the relative abundances of Firmicutes and Actinobacteria were lowered while the Proteobacteria population was increased. Families of *Lachnospiraceae*, *Lactobacillaceae*, and *Erysipelotrichaceae* were also declined by psychotropics (aripiprazole) treatment. These microbial changes were translated into a decrease of the major SCFA (butyrate, acetate, and propionate) at the metabolic level. The addition of a probiotic combination (*Lactobacillus rhamnosus* and *Bifidobacterium longum*) concomitantly with a psychotropic (aripiprazole) had a protective effect by attenuating the decline of microbiota composition and increasing the concentrations of SCFA. These findings provide evidence that psychotropics, through their antimicrobial effect, have the potential to alter the human gut microbiota composition and metabolism, while probiotics can mitigate the related dysbiosis.

Keywords: Gut microbiota, psychotropics, antimicrobial activity, dysbiosis, probiotics.

Résumé

On s'intéresse de plus en plus à la manière dont les médicaments thérapeutiques pourraient modifier la composition et la fonction du microbiote intestinal humain. Alors que certaines connaissances s'accumulent sur l'impact antimicrobien de certains psychotropes sur des souches isolées ou sur le microbiote intestinal de modèles animaux, les informations sur d'autres classes de psychotropes et sur des espèces représentatives de l'intestin humain sont peu étudiées. L'effet antimicrobien des psychotropes est généralement négligé en tant que facteur confondant lors de l'étude des biomarqueurs du microbiome intestinal, sachant que les patients sont généralement mis sous médication à long terme. L'objectif de la présente étude était d'étudier l'activité antimicrobienne de certains psychotropes oraux couramment prescrits, appartenant à différentes classes thérapeutiques, sur la diversité et le métabolisme du microbiote colique et la capacité potentielle des probiotiques à soulager la dysbiose associée. Les résultats de cette étude ont révélé une importante activité inhibitrice *in vitro* des médicaments psychotropes, exprimée sous forme d'altérations de la composition du microbiote intestinal *ex vivo*. En effet, les abondances relatives des Firmicutes et Actinobacteria ont diminué alors que la proportion de Protéobactéries a augmenté. Les familles de *Lachnospiraceae*, *Lactobacillaceae* et *Erysipelotrichaceae* ont également été inhibées par le traitement psychotropique (aripiprazole). Au niveau métabolique, ces changements microbiens se sont traduits par une diminution des principaux acides gras à courte chaîne AGCC (butyrate, acétate et propionate). L'ajout d'une combinaison de probiotiques (*Lactobacillus rhamnosus* et *Bifidobacterium longum*) en même temps que le psychotrope (aripiprazole) a eu un effet protecteur en atténuant la diminution de la composition du microbiote et en augmentant les concentrations des AGCC. Ces résultats démontrent que les psychotropes, par leur effet antimicrobien, ont le potentiel de modifier la composition et le métabolisme du microbiote intestinal humain tandis que les probiotiques peuvent atténuer la dysbiose qui y est associée.

Mots-clés: Microbiote intestinal, psychotropes, activité antimicrobienne, dysbiose, probiotiques.

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General introduction

The human gastrointestinal tract harbors trillions of diverse microbial cells whose activities are believed to be involved in human health and disease. Such microbial populations reach their highest density in the colon and rectum, forming a complex microbial community referred to as “gut microbiota”. The gut microbiota can be considered as a “necessary virtual organ” or a “second genome” as it helps the host to complete a wide variety of physiological and biochemical functions through the synthesis of several metabolites, including nutrient production and absorption, metabolic homeostasis, regulation of the immune system and defense functions (Young, 2017). Although the composition of a “healthy” gut microbiota has not been defined globally, there is no doubt that balanced, functional gut microbiota contributes significantly to the host-microbiome symbiotic relationship (Lozupone et al., 2012).

Growing evidence has revealed that certain psychiatric and neurological diseases, such as autism, anxiety, depression, and neurodegeneration, are associated with dysbiotic gut microbiota (Dinan and Cryan, 2017; Sampson and Mazmanian, 2015; Sharon et al., 2016). Such alterations of gut microbiota in mental disorders may be either directly related to the disease pathogenesis but also to the chronic use of psychotropic medications, which could be undervalued confounding factors (Ait Chait et al., n.d.). Indeed, multiple studies investigating microbiome biomarkers have reported, albeit confusing, changes in microbiome abundance in mental diseases where the antimicrobial effect of psychotropic drugs was usually neglected as a confounding factor, knowing that patients with mental disorders are generally put in long-term medication. While some knowledge is accumulating on the antimicrobial impact of some psychotropics on isolated strains or the gut microbiota of animal models, information about other classes of psychotropics and representative species from the human gut is poorly investigated. The purpose of the present study was to investigate (i) the antimicrobial activity of some oral commonly prescribed psychotropics from different therapeutic classes on intestinal bacteria and probiotics *in vitro*; (ii) the impact of psychotropics on colonic microbiota diversity and metabolism *ex vivo*; (iii) growth and capacity of probiotics to alleviate antimicrobial effects of psychotropics on colonic microbiota in a simulated human colon.

Chapter 1

Nutritional and therapeutic approaches for protecting human gut microbiota from psychotropic treatments

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Abstract

Emerging evidence highlighted the essential role played by the microbiota-gut-brain axis in maintaining human homeostasis, including nutrition, immunity, and metabolism. Much recent work has linked the gut microbiota to many psychiatric and neurodegenerative disorders such as depression, schizophrenia, and Alzheimer's disease. Shared gut microbiota alterations or dysbiotic microbiota have been identified in these separate disorders relative to controls. Much attention has focused on the bidirectional interplay between the gut microbiota and the brain, establishing gut dysbiotic status as a critical factor in psychiatric disorders. Still, the antibiotic-like effect of psychotropic drugs, medications used for the treatment of these disorders, on gut microbiota is largely neglected. In this review, we summarize the current findings on the impact of psychotropics on gut microbiota and how their antimicrobial potency can trigger dysbiosis. We also discuss the potential therapeutic strategies, including probiotics, prebiotics, and fecal transplantation, to attenuate the dysbiosis related to psychotropics intake.

Keywords: Gut microbiota, gut-brain axis, antipsychotics, dysbiosis, probiotics, prebiotics.

1. Introduction

The human gastrointestinal tract is home to trillions of many and diverse microbial cells whose activities are believed to be involved in human health and disease. Such microbial populations reach their highest density in the colon and rectum, where they form a complex microbial community referred to as “gut microbiota”. The collective genome of the gut microbiota, commonly known as the “microbiome”, contains at least 100 times as many genes as our genome (Bermon et al., 2015). These microorganisms living in a complex ecosystem mainly dominated by bacteria are in continuous interactions with each other and their human host forming relationships ranging from symbiosis to parasitism (Ventura et al., 2009). The gut microbiota can be considered as a “virtual metabolic organ” or a “second genome” as it helps the host to complete a wide variety of physiological and biochemical functions through the synthesis of several metabolites, including nutrient production and absorption, metabolic homeostasis, regulation of the immune system and defense functions (Young, 2017). Therefore, the concept of microbiota should be considered as a metabolic interactome influenced by diet, gut and other environmental stimuli that affects host absorption of nutrients, immune defenses, and metabolism. Although the composition of “healthy” gut microbiota has not been defined globally, there is no doubt that balanced, functional gut microbiota contributes significantly to the host-microbiome symbiotic relationship (Lozupone et al., 2012).

Diet, drugs, age, and environment (Heiman & Greenway, 2016; Iizumi et al., 2017a; Odamaki et al., 2016) can affect the microbiota composition and function, which might lead to a “dysbiosis”, an altered microbial community that contributes to diseases (Wang et al., 2018). Indeed, dysbiosis has been linked to increased intestinal permeability/leaky gut (Tremaroli & Bäckhed, 2012) and is thought to be a key factor in the pathogenesis of several diseases such as metabolic disorders (i.e. diabetes, obesity, etc.) (Harsch & Konturek, 2018), inflammatory bowel disease (IBD) (Rajilic-Stojanovic et al., 2015), and cancer (Ahn et al., 2013). Gut microbiota is also closely correlated with brain functions, constituting the renowned microbiota-gut-brain axis (Sampson & Mazmanian, 2015). Growing evidence have revealed that certain psychiatric and neurological diseases, such as autism, anxiety, depression, and neurodegeneration, are associated with dysbiotic gut microbiota. (Dinan & Cryan, 2017; Sampson & Mazmanian, 2015; Sharon et al., 2016). Such alterations of gut microbiota in mental disorders may be either directly related to the disease pathogenesis or to the chronic use of psychotropic medications which could be undervalued

confounding factors (Ait Chait et al., 2020). Thus, these drugs were reported to exhibit antimicrobial properties, which can lead to deleterious effects on gut microbiota (Ait Chait et al., 2020; Cussotto et al., 2018; Flowers et al., 2017). These findings highlight the importance of considering medications as cofounders in gut microbiome studies. Without doing so, it will be even harder to distinguish whether any alteration in the gut microbiota is caused by the used medications or by the underlying disease.

Rebalancing the gut microbiota and alleviating dysbiosis can be achieved through different strategies, including diet and fecal microbiota transplants (FMT), which were shown efficient in restoring balanced microbiota in the context of several diseases (Kanwal et al., 2018; Ying Shi et al., 2017). Here, we review the link between the gut microbiome and psychiatric illnesses, and how the antimicrobial effects of antipsychotic medications may lead to potentially harmful effects on gut microbiota. This review also evaluates the potential of probiotics, prebiotics, and fecal microbiota transplantation as effective approaches to alleviate dysbiosis related to the intake of antipsychotic medications.

2. Human gut microbiota: Composition and Dysbiosis

The human gut hosts an estimated 10^8 - 10^{11} cells of ~ 1000 different species (Sender et al., 2016), that co-evolved with the host in a symbiotic and mutualistic relationship. The gut microbiota is mainly dominated by bacteria; the remainder includes archaea, eukarya as well as viruses (Gill et al., 2006). It is believed that the colonization of the gastrointestinal tract begins immediately after birth, although this notion was compromised by some studies reporting the presence of microorganisms in the placenta and meconium (Aagaard et al., 2014). A series of factors such as gestational age, mode of delivery (vaginal vs C-section), feeding regime, antibiotic treatment and prematurity contribute to shaping the infant gut microbial colonization, growth, composition, and diversity (C. J. Hill et al., 2017; Yasmin et al., 2017). The intestinal tract of the newborn is initially colonized by facultative anaerobes such as lactobacilli, enterobacteria and enterococci (Nagpal et al., 2017). Depletion of the available oxygen creates the necessary environment for establishment of strict anaerobes such *Bifidobacterium*, *Bacteroides*, *Clostridia* and *Parabacteroides* (C. J. Hill et al., 2017; Nagpal et al., 2017). At this stage, the diversity of the microbiota is low, variable and mainly dominated by the phyla Proteobacteria and Actinobacteria. As the neonate grows, the microbiota becomes more diverse to resemble that of an adult at age of three which is dominated

by the phyla Firmicutes and Bacteroidetes (accounting for 90% of the known gut microbiota), followed by Actinobacteria, Proteobacteria and Verrucomicrobia at less abundance (Yatsunenکو et al., 2012). The genera *Bifidobacterium*, *Lactobacillus*, *Akkermansia*, *Fecalibacterium*, *Eubacterium*, *Roseburia*, *Ruminococcus* and *Blautia* are some of the most essential groups in human gut microbiota that are involved in a good health outcome (O'Callaghan & Corr, 2019).

The evolution of human gut microbiota shows a continuous, dynamic adaptation forming a unique community to each individual, but it is also known to vary drastically during the whole course of life. In normal conditions, the human-microbiota ecosystem maintains a balanced symbiotic relationship and a homeostatic equilibrium, a status known as “eubiosis”. This latter is characterized by a preponderance of potentially beneficial species for human health while potentially pathogenic species, such as those belonging to the phylum Proteobacteria (*Enterobacteriaceae*) are present in a very low abundance (Iebba et al., 2016). This balance is mostly preserved (microbiota resilience), however, face to sudden changes, the composition, evenness and relative species abundance of microbiota can be altered (Rothschild et al., 2018). Indeed, various factors may have a deleterious effect on the gut microbiota including diet (Heiman & Greenway, 2016), age (Odamaki et al., 2016), genetics (J. Li et al., 2014), geographical location (Suzuki & Worobey, 2014), medications (antibiotic and non-antibiotic drugs) (Iizumi et al., 2017b; Maier et al., 2018) and hormonal changes (Koren et al., 2012). Such disturbances in the microbiome structure may lead to a shift to another microbiota status called “dysbiosis”. Although there is no consensus about its definition, dysbiosis has been considered as a compositional and functional change that is characterized by a significant reduction in the beneficial microorganisms and an increase in opportunistic or pathobiont microbes (Chan et al., 2013). In other words, the dysbiotic microbiota is characterized by reduction of beneficial microbes, expansion of pathobionts, and lower species diversity (Drago et al., 2019). Microbiota dysbiosis can destroy the gut barrier and increase intestinal permeability, promote the translocation of gut bacteria, bacterial overgrowth, and dysplasia of the immune system, which may lead to a “leaky gut” (Zhou et al., 2020). A vicious cycle can ensue, since the leaky gut worsens inflammation and dysbiotic status, ultimately leading to systemic inflammation and related diseases (Luissint et al., 2016). The chronic neuropsychiatric disorders such as autism and depression are among a long list of chronic disease linked to dysbiotic microbiota.

3. Gut microbiota dysbiosis in mental disorders

Nowadays, it is increasingly accepted that gut microbiota and brain are engaged in a continuous communication, influencing each other to coordinate functions in health and disease (Cryan et al., 2019; Rhee et al., 2009). The microbiota-gut-brain axis is a complex bi-directional system that communicate between each other through different routes including endocrine, immune and neurotransmitter systems, the vagus nerve and signaling metabolites such as short-chain fatty acids, branched chain amino acids, and peptidoglycans (Cryan et al., 2019). Accumulating evidences from animal and clinical studies support the co-morbidity between gut microbiota dysbiosis and psychiatric and neurological disorders. In case of Major Depressive Disorders (MDD), a number of human studies reported differences in fecal microbiota composition in MDD patients when compared with healthy subjects (Jiang et al., 2015b; Kelly et al., 2016b; Naseribafrouei et al., 2014; Zheng et al., 2016). Thus, in a cohort study of 46 concurrently depressed patients, an increase of α -bacterial diversity was reported in the active-MDD fecal microbiota compared to healthy group (Jiang et al., 2015b). The same study demonstrated that the level of Firmicutes was significantly reduced in MDD-patients, whereas the level of Bacteroidetes, Proteobacteria, and Actinobacteria were increased compared the healthy control group. At genus level, the MDD group had increased levels of Enterobacteriaceae and *Alistipes* along with the reduced levels of *Faecalibacterium* which was negatively correlated with severity of the depressive symptoms (Jiang et al., 2015b). Moreover, reduction in the abundance of the beneficial bacteria genus, *Bifidobacterium*, along with an increased abundance of potentially pathogenic *Desulfovibrio* and *Clostridia* genera were detected in children with Autism Spectrum Disorder (ASD) (Finegold, 2011; Kang et al., 2018). In addition, Evans and coauthors (Evans et al., 2017) observed a decrease in fractional representation of *Faecalibacterium* and an unclassified member from the *Ruminococcaceae* family, both from the phylum Firmicute, in bipolar disorder patients. It is important to highlight that most of the previous studies conducted to investigate the gut microbiota-brain axis have not adequately mentioned details regarding the antipsychotic medication used that can be a confounding factor in microbiome characterization, since they might exhibit a direct or indirect antimicrobial potential that might modulate the gut microbiota of patients. Thus, the following sections of this review will focus on the antimicrobial effects of psychotropic medications and how they can trigger microbiota dysbiosis.

4. Dysbiosis associated with the antimicrobial effects of psychotropic treatments

Psychotropic medications (antidepressants, antipsychotics, mood stabilizers, anxiolytics, etc.) are widely used for the treatment of a variety of psychiatric and neurodegenerative diseases (Kaye et al., 2018). Antidepressants are used to treat Major Depressive Disorders (MDD), social anxiety disorder, seasonal affective disorder and mild chronic depression, as well as other conditions (Delgado, 2004), while, antipsychotics are primarily effective to treat symptoms of schizophrenia but are often used to treat other psychiatric illnesses such as acute episodes of bipolar disorders (Kaye et al., 2018). Based on their mechanism of action, antidepressants can be divided into four classes: tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenaline reuptake inhibitors (SNRIs) and monoamine oxidase inhibitors (MAOIs). However, antipsychotic medications, are broadly classified as first generation or typical antipsychotics and second generation known as atypical antipsychotics (Kaye et al., 2018). Different metabolic side effects were associated with psychotropic treatments including weight gain, risk of cardiovascular disease and type 2 diabetes (Kahl et al., 2017; Sharma et al., 2014). Emerging evidences demonstrated another adverse effect of psychotropics which is related to their antimicrobial potency that can trigger dysbiosis in gut microbiota (Ait Chait et al., 2020; Cussotto et al., 2019; Flowers et al., 2017). Researchers' attention was drawn to the antimicrobial activity of psychotropics due to the need for new chemotherapeutics and as an alternative strategy to fight increasing antibiotic-resistant microbial infections. Thus, in this section, we will review different studies (*in vitro*, in animal and human) investigated the antimicrobial potential of these drugs as well as their consequences on gut microbiota (Figure 1.1).

4.1. Evidence from *in vitro* studies

Several *in vitro* studies have tested the antimicrobial effect of antidepressants and antipsychotic medications against various bacterial and fungal strains. The SSRIs class of antidepressants, including sertraline, paroxetine, and fluoxetine, were reported to exhibit potent antimicrobial effects against Gram-positive bacteria such as *Enterococcus* and *Staphylococcus* species (Muhammad Ayaz, Subhan, Ahmed, Khan, Ullah, Ullah, et al., 2015a; Kalayci et al., 2015; Munoz-Bellido et al., 2000). Additionally, SSRIs have been shown to inhibit potentially the growth of toxigenic *Enterobacteria*, such as *Citrobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Morganella morganii* and even *Acinetobacter* (Muhammad Ayaz, Subhan,

Ahmed, Khan, Ullah, Ullah, et al., 2015a; Kalayci et al., 2015). Moreover, the growth of anaerobe species found in human gut such as *Bacteroides fragilis*, *Prevotella* spp. could be inhibited by sertraline drug (Munoz-Bellido et al., 2000). Several studies highlighted the antifungal potential of SSRIs; fluoxetine, sertraline, and paroxetine against *Aspergillus* spp., *Candida parapsilosis*, and *Candida albicans* (Costa Silva et al., 2017a; Gu et al., 2016a). Several SSRIs have been reported to also have antimicrobial enhancer properties, which was confirmed by their synergistic effect when combined with antibiotic against antibiotic-resistant bacteria (i.e. combination of sertraline with ciprofloxacin or norfloxacin against *S. aureus* 6538) (Muhammad Ayaz, Subhan, Ahmed, Khan, Ullah, Sadiq, et al., 2015; Bohnert et al., 2011). This synergistic effect is confirmed by decreases in the minimum inhibitory concentrations of antibiotics when combined with antidepressants. For example, sertraline has been shown to affect bacterial transcription and increase the susceptibility of resistant *Escherichia coli* APEC_O2 to tetracycline *in vitro* (L. Li et al., 2017a). Nevertheless, high-dose treatments with sertraline as an adjuvant for treatment of antibiotic resistant *E. coli* infections was recently reported to exacerbate the pathological outcome of infection in chickens (Kromann et al., 2017a).

Amitriptyline, a tricyclic antidepressant (TCAs), exhibited significant inhibitory action at variable doses on *Staphylococcus* spp., *Bacillus* spp., and *Vibrio Cholerae*. It also inhibited fungi, notably *Cryptococcus* spp. and *Candida albicans* (Mandal et al., 2010). Promethazine and imipramine, belonging to the same class, have been demonstrated to prevent the growth of *E. coli* and *Yersinia enterocolitica* through interference with plasmid replication (Csiszar & Molnar, 1992). Another FDA-approved TCA drug, maprotiline, has shown a potential to reduce the severity of *Francisella* infection by decreasing virulence without being bactericidal (Dean & van Hoek, 2015a). Maprotiline and chlorpromazine TCA drugs have a strong antibiofilm activity against *Francisella* (Dean & van Hoek, 2015b). Besides its antibiofilm inhibitory activity in *Salmonella typhimurium* and *Francisella novicida*, chlorpromazine is strongly inhibitory to *F. novicida* growth (Dean & van Hoek, 2015a). Moreover, TCA amoxapine was demonstrated to resensitize methicillin-resistant *S. aureus* to oxacillin *in vitro* (Wilson et al., 2018). Also, TCAs drugs were reported to have anti-plasmid effects and to prevent the growth of intestinal pathogens such as *E. coli*, *Yersinia enterocolitica*, *Giardia lamblia*, *Plasmodium falciparum*, and *Leishmania* spp, reviewed in (Macedo et al., 2017a). Ketamine, a non-competitive NMDA (N-methyl-D-aspartate) was shown

effective against *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*, and *Candida albicans* (Evrensel & Ceylan, 2018).

The typical antipsychotics, namely Chlorpromazine, Thioridazine and trifluoperazine, have been shown to possess an *in vitro* antimicrobial activity against strains of *S. aureus*, *Shigella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus* and *E. coli* (Csiszar & Molnar, 1992; Hahn & Sohnle, 2014; Mazumder et al., 2001). Regarding the atypical antipsychotics, olanzapine was found to have a pronounced antibacterial activity toward *E. coli* at concentrations above 537 µg/mL (Morgan et al., 2014).

It is important to keep in mind that most of the previous cited studies used clinical isolates that do not necessarily represent the human gut microbiota or tested non- clinically relevant doses. This might bias the extrapolation of the gathered data to microbiome applications. To address this, a recent study conducted by our group investigated the antimicrobial impact of six different psychotropics: phenelzine, venlafaxine, desipramine, bupropion, aripiprazole and (s)-citalopram against 12 commensal bacterial strains representing the human gut microbiota. The data revealed an important antimicrobial activity (bacteriostatic or bactericidal) of different psychotropics against the tested strains, with desipramine (TCA) and aripiprazole (Atypical antipsychotics) being the most inhibitory (Ait Chait et al., 2020). However, phenelzine (MAOIs) and (S)-citalopram (SSRIs) were found to be more active against *Eubacterium rectale* and *Faecalibacterium prausnitzii*, both belonging to the Firmicutes phylum. Strains of the most dominant phyla of human microbiota such as *Akkermansia muciniphila*, *Bifidobacterium animalis* and *Bacteroides fragilis* were significantly altered, with minimum inhibitory concentrations (MICs) ranged from 75 to 800 µg/mL (Ait Chait et al., 2020).

4.2. Evidence from animal studies

Recent studies using animal models have provided considerable information into the potential antimicrobial of psychotropics on gut microbiota and their possible mechanisms of action (Table 1.1). The administration of the atypical antipsychotic, **olanzapine**, for 3 weeks induced a significant increase of *Firmicutes* levels and a decrease in *Bacteroidetes*, *Proteobacteria*, *Actinobacteria* in both male and female rats (Kieran J. Davey et al., 2012). Such alterations in microbiota were associated with metabolic changes in female rats including weight gain, increased

food and water intake, elevation of plasma free fatty acids, and the liver lipogenic enzyme, fatty acid synthase (Davey et al., 2012). However, when olanzapine is co-administered with an antibiotic cocktail (Neomycin, metronidazole and polymyxin B), all microbial as well as metabolic changes in female rats, were significantly reduced. For instance, no changes in the relative abundance of *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were noticed (Davey et al., 2013). Moreover, analysis of fecal microbiota of female C57BL/6J mice treated with olanzapine for 7 weeks revealed alterations in their microbiota characterized by an increase in the relative abundance of class *Erysipelotrichi* (phylum *Firmicutes*) and *Gammaproteobacteria* (phylum *Proteobacteria*), while relative abundances of members of class *Bacteroidia* (phylum *Bacteroidetes*) were decreased (Morgan et al., 2014). The same study indicated that olanzapine treatment induced a shift towards microbiota that promote weight gain in both mouse and human (Morgan et al., 2014). Bahr and coauthors have reported that **risperidone** (atypical antipsychotic) induced a significant weight gain in female mice, due to reduced energy expenditure, which correlated with an altered gut

Table 1.1. Summary of animal studies reporting the alterations of microbiome due to psychotropics administration.

Model	Subjects	Psychotropic compound	Class	Dose and duration	Gut microbiome changes (↑: Increase, ↓: Decrease)	References
Rats	Sprague–Dawley male and female	Olanzapine	Atypical antipsychotic	2 mg/kg/day or 4 mg/kg/day, 21 days	<i>Firmicutes</i> ↑, <i>Bacteroidetes</i> ↓, <i>Proteobacteria</i> ↓, <i>Actinobacteria</i> ↓ (more significant in female rats)	(Davey et al., 2012)
Mice	C57BL/6J female	Olanzapine	Atypical antipsychotic	50 mg/kg, 7 weeks	<i>Erysipelotrichi</i> ↑, <i>Gammaproteobacteria</i> ↑, <i>Bacteroidia</i> ↓	(Morgan et al., 2014)
Mice	C57BL/6J female	Risperidone	Atypical antipsychotic	80 µg/day, 2 months	<i>Erysipelotrichaceae</i> ↑ :(<i>Allobaculum</i> , <i>Turicibacter spp.</i>), <i>Mollicutes class</i> ↑: (<i>Aneroplasma spp</i>) <i>Bacteroides</i> ↑, <i>Alistipes spp.</i> ↓, <i>Akkermansia spp.</i> ↓ <i>Lactobacillus spp.</i> ↓,	(Sarah M. Bahr et al., 2015)
Rats	Sprague–Dawley male	Fluoxetine	SSRIs	10 mg/kg/day, 4 weeks	<i>Deferribacteres</i> ↓, <i>Prevotella 7</i> ↓, <i>Prevotella 9</i> ↓, <i>Succinivibrio</i> ↓	(Cussotto et al., 2019)
		Aripiprazole	Atypical antipsychotic	20 mg/kg/day, 4 weeks	Bacterial diversity ↑, <i>Firmicutes</i> ↑, <i>Peptostreptococcaceae</i> ↑, <i>Clostridiaceae</i> ↑, <i>Ruminococcaceae</i> ↑	
		Lithium	Mood stabilizer	150 mg/kg/day, 4 weeks	Bacterial diversity ↑, <i>Ruminococcaceae</i> ↑ <i>Bacteroides</i> ↓,	
		Valproate	Anticonvulsant	1.5 g/kg/day, 4 weeks	Bacterial diversity ↑, <i>S24-7 uncultbact</i> ↓, <i>Ruminococcaceae</i> ↓	

Rats	Wistar maternal vulnerability female	Fluoxetine	SSRIs	10 mg/kg/day, 21 days	<i>Prevotella</i> ↓, <i>Oscillospira</i> ↓, <i>Ruminococcus</i> ↓	(Ramsteijn et al., 2018)
Mice	CF-1 male	Fluoxetine	SSRIs	20 mg/kg/day, 29 days	<i>Lactobacillus (Lactobacillus johnsonii)</i> ↓, <i>Bacteroidales S24-7</i> ↓, <i>Alistipes</i> ↑, <i>Lachnospiraceae</i> ↑, <i>Lachnoclostridium</i> ↑, <i>Anaerotruncus</i> ↑	(Lyte et al., 2019)
Mice	Male BALB/c OlaHsd	Fluoxetine	SSRIs	10 mg/kg/day, 21 days	β -diversity richness ↓ <i>Ruminococcus</i> ↓, <i>Adlercreutzia</i> ↓	(Lukić et al., 2019)
		Escitalopram	SSRIs	10 mg/kg/day, 21 days		
		Venlafaxine	SNRIs	10 mg/kg/day, 21 days		
		Duloxetine	SNRIs	10 mg/kg/day, 21 days		
		Desipramine	TCA	20 mg/kg/day, 21 days		

↑: Increase, ↓: Decrease

microbiota (Sarah M. Bahr et al., 2015). Indeed, the authors reported an increase in the relative abundance of *Firmicutes* and a decrease in the abundance of *Bacteroidetes* following risperidone treatment. At genus level, an enrichment in *Allobaculum*, *Turicibacter* spp. (*Erysipelotrichaceae* family), *Aneroplasma* spp. (*Mollicutes* class) and *Bacteroidetes* spp. was detected, while species from the genera *Alistipes* and *Akkermansia* were depleted in risperidone treated animals compared to controls (Sarah M. Bahr et al., 2015).

More recently, the study of Cussotto and colleagues witnessed dysbiotic alterations in the gut microbiota composition of rats when treated with different psychotropics at different dosages for four weeks (Cussotto et al., 2019). The atypical antipsychotic, **Aripiprazole**, showed in turn strong effects on microbiota composition of rats following a 4-week treatment at 20 mg/kg/day. It decreased the relative abundance of several taxa namely *Clostridium*, *Ruminiclostridium*, *Intestinibacter* and *Eubacterium coprostanoligenes* (Cussotto et al., 2019). In addition, the mood stabilizer, **lithium**, and the anticonvulsant, **valproate**, increased the bacterial richness of rat microbiota compared to untreated animals (Cussotto et al., 2019). At the genus level, lithium increased the relative abundance of *Ruminococcaceae* and decreased *Bacteroides*, while valproate decreased the relative abundance of *S24-7 uncultbact* and increased *Ruminococcaceae* (Cussotto et al., 2019). Interestingly, the dysbiotic microbiota occurred following the psychotropics treatment in rat was associated with changes in Short-Chain Fatty Acids (SCFAs) levels. Thus, aripiprazole increased acetate and isovalerate concentration in the caecum while valproate decreased significantly the levels of propionate and butyrate while augmenting the levels of isovalerate. Those changes could be mapped to bacterial taxa known to be SCFA producers (e.g., *Bacteroidetes* or *Clostridium* spp.) (Cussotto et al., 2019). Among the tested antidepressants (fluoxetine, escitalopram and venlafaxine), only **fluoxetine** (SSRIs) induced small changes in the microbiota with a decrease of *Deferribacteres* and a complete inhibition of the growth of *Succinivibrio* and *Prevotella* caecal taxa (Cussotto et al., 2019). These affected genera are often associated with diverse physiological conditions. For instance, *Eubacterium coprostanoligenes* was found to have an hypocholesterolemic effect when orally administered to germ-free mice (Li et al., 1998). Also, *Clostridium sensu stricto* 1 was increasing in infants with high genetic risk of developing coeliac disease (Olivares et al., 2015).

An additional study conducted by Lyte and collaborators has also examined the effect of **fluoxetine** administration at 20 mg/kg/day for 29 days on the microbiota of healthy male mice (Lyte et al., 2019). The results revealed changes in body mass regulation associated with a shift of microbial communities towards dysbiosis, with lower relative abundance of *Lactobacillus johnsonii* and *Bacteroidales S24-7*. The authors suggest that depletion of certain microbial genera due to fluoxetine supplementation is partially responsible for some of the known side effects such as weight loss (Lyte et al., 2019). Another study reported the decrease in the abundance of genera *Prevotella*, *Oscillospira* and *Ruminococcus* in gut microbiota of female rats developing a depressive-like phenotype and treated with fluoxetine during pregnancy and lactation (Ramsteijn et al., 2018). A more recent study tested the effect of five different antidepressants (fluoxetine, escitalopram, venlafaxine, duloxetine and desipramine) on BALB/c mice gut microbiota. An increase of the microbial β -diversity was observed in all antidepressants-treated groups compared to controls, with a depletion of the genera of *Ruminococcus*, *Adlercreutzia* and an unclassified *Alphaproteobacteria*. It was also found that simultaneous supplementation of *Ruminococcus flavefaciens* attenuated the behavioural symptoms observed upon duloxetine administration, indicating an anti-antidepressant potential (Lukić et al., 2019).

4.3. Human observations

Research examining the relationship between psychotropics and human gut microbiota is still in its infancy. Few studies have linked the intake of psychotropic medications to human gut microbiota and little is known about how the psychotropics can impact the composition and the functionality of the microbiota (Table 1.2). The initial study in this area demonstrated, in five male psychiatrically ill children aged 9–13, that chronic treatment with **risperidone** was associated with a significantly lower ratio of Bacteroidetes/Firmicutes as compared with treatment-naïve psychiatric controls (Sarah M. Bahr et al., 2015). Additionally, *Clostridium*., *Lactobacillus*, *Ralstonia* and *Erysipelotrichaceae* family members were more abundant in participants treated with risperidone along with a rise in body mass index (BMI) (S M Bahr et al., 2015). Another study related the intake of **risperidone** to a significant microbiota dysbiosis in normal weight patients with first episode schizophrenia after 24 weeks of treatment (Yuan et al., 2018). Furthermore, these findings were supported by another cohort study (Flowers et al., 2017, 2019) that reported alterations in the gut microbiota composition with the administration of Atypical

Table 1.2. Summary of human studies reporting the alterations of microbiome due to psychotropics administration.

Cohort description	Mean age (years)	Psychotropic compound	Class	Gut microbiome changes	References
Five male psychiatrically ill children	11.7	Risperidone	Atypical antipsychotic	<i>Bacteroidetes: Firmicutes</i> ratio↓, <i>Clostridium sp.</i> ↑, <i>Lactobacillus sp.</i> ↑, <i>Ralstonia sp.</i> ↑, <i>Erysipelotrichaceae</i> family ↑	(S M Bahr et al., 2015)
Forty-one patients with a first episode of schizophrenia and normal body weight	23.1	Risperidone	Atypical antipsychotic	<i>Bifidobacterium spp.</i> ↑, <i>Escherichia coli</i> ↑, <i>Clostridium coccoides</i> ↓, <i>Lactobacillus spp.</i> ↓	(Yuan et al., 2018)
Forty-nine adults with bipolar disorder	46	Clozapine, olanzapine, risperidone, quetiapine, asenapine, ziprasidone, lurasidone, aripiprazole, paliperidone, iloperidone	Atypical antipsychotic	<i>Lachnospiraceae</i> ↑, <i>Akkermansia</i> ↓	(Flowers et al., 2017)
Seventeen patients with major depressive disorder	42.5	Escitalopram	SSRIs	Bacterial diversity ↑ (mainly α -diversity)	(Liśkiewicz et al., 2019)
313 IBS patients	45.4	SSRIs mainly Paroxetine	SSRIs	<i>Eubacterium ramulus</i> ↑	(Vich Vila et al., 2020)

↑: Increase, ↓: Decrease

Antipsychotic (AAP), notably a significant increase of *Lachnospiraceae* family abundance and a decrease of *Akkermansia* in AAP-treated females compared to untreated controls.

Few studies investigated the effect of antidepressants on gut microbiome structure in human subjects. In a large cohort study of 76 elderly participants on polypharmacy, antidepressants was strongly associated with the abundance of several taxa (Ticinesi et al., 2017). Recently, Liskiewicz and colleagues observed a significant increase in fecal microbiota biodiversity, mainly alpha diversity in hospitalized patients with depressive episodes, with no differences in taxa abundance after six weeks of hospitalization, and concomitant therapy using 5–20 mg of **escitalopram** (Liśkiewicz et al., 2019).

More recently, a Dutch cohort study of 313 patients with IBS who use SSRIs antidepressants, reported a significant increase in *Eubacterium ramulus* abundance. Interestingly, the increase of this taxa is mainly observed in **paroxetine** users (Vich Vila et al., 2020). In addition, the same authors confirmed the decrease of the pathway involved in peptidoglycan maturation in the multi-drug meta-analysis of SSRI antidepressant users compared to non-users (Vich Vila et al., 2020).

It is important to note that the direct antimicrobial effect of psychotropics might be part of their mechanism of action by mediating their effects through regulation of microbiota. This can be supported by the variability of psychotropics efficacy when considering microbiome variability between individuals. While lanicemine does not exhibit antidepressant effects in treatment-resistant depressed patients, ketamine shows rapid and sustained antidepressant effects, both being NMDAR antagonists (Qu et al., 2017a). Ketamine modulates the fecal microbiome in the susceptible mice after chronic social defeat stress, suggesting an antidepressant mechanism partly mediated by the modulation of gut microbiota (Qu et al., 2017a). More recently, the study conducted by Lukic and colleagues illuminated more this hypothesis, suggesting an additional mechanism to improve the therapeutic effect of psychotropics via modulation of microbiota (Lukić et al., 2019). These authors demonstrated that the introduction of a single *Ruminococcus* species (i.e., *R. flavefaciens*), is able to abolish antidepressive effects of an antidepressant. This confirms that changes in gut microbiota can be causally related to antidepressant properties of antidepressant drugs (Lukić et al., 2019).

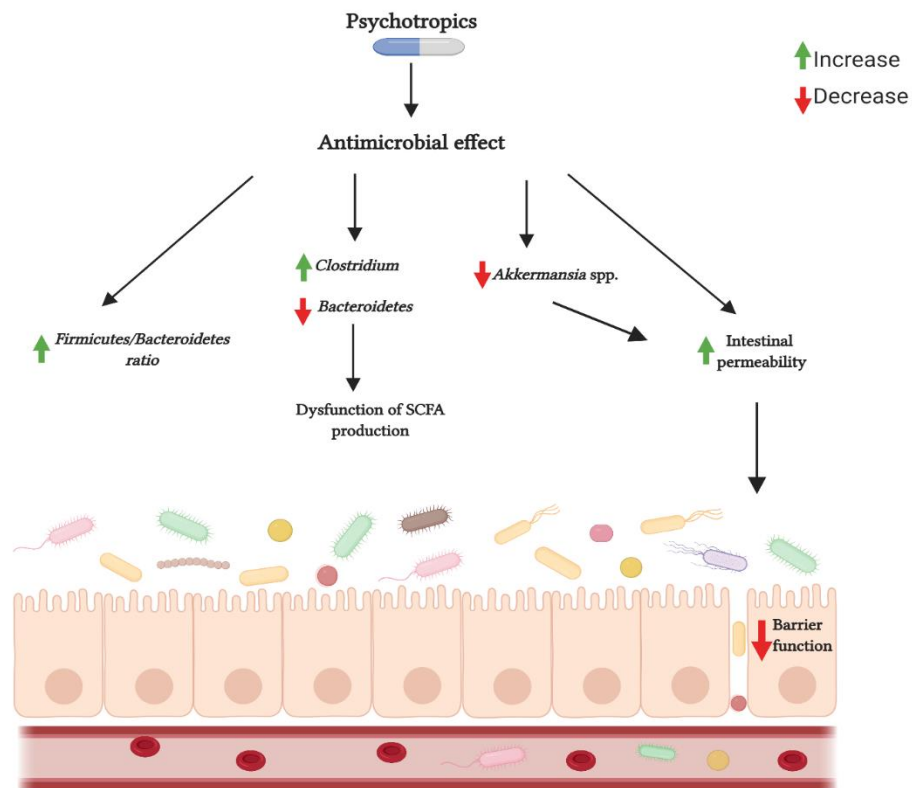


Figure 1.1. Psychotropics alter the gut microbiota to destabilize many metabolic pathways. They have a direct effect on the gut microbiota composition provoking dysbiosis and causing metabolic disturbances to the host.

5. Potential nutritional and therapeutic approaches associated with psychotropics’ dysbiosis

Taking the evidences mentioned before together, it is obvious that psychotropic medications are able to inhibit the growth of microbiota in the gut which can affect its balance and induce dysbiosis. Therefore, a considerable attention has been given to the following potential approaches of gut microbiota modulation to restore its balance.

5.1. Probiotics: lessons learnt from antibiotic-associated intestinal dysbiosis

Probiotics are defined as “live microorganisms that confer health benefits to the host when ingested in adequate amount” (C. Hill et al., 2014). Over the past decade, numerous studies have evaluated the health-promoting role and effectiveness of probiotics in improvement of intestinal health,

stimulation of the immune system, improvement of gut mucosal barrier integrity, inhibition of pathogenic bacterial growth, production of Short-Chain Fatty Acids (SCFAs) and prevention from various diseases (Cervantes-Barragan et al., 2017; Kim et al., 2018; Sakamoto et al., 2007). Probiotics are often proposed as a potential therapy to restore and improve dysbiotic microbiota related to different diseases (Blaabjerg et al., 2017; Sireswar et al., 2019). However, using probiotics for management and prevention of dysbiosis related to antipsychotic medications was not discussed before.

Because of the similarities between antipsychotics and antibiotics (chemical structure, mechanisms of action, etc.), we will focus in this section on how probiotics were successfully used to effectively treat antibiotics-induced microbiota dysbiosis. Indeed, antibiotic treatments has been shown to transiently or permanently alter the composition and the functionality of gut microbiome via depletion of one or several taxa (Becattini et al., 2016). Such alterations occur rapidly within days, leading to the development of different diseases such as inflammatory bowel disease (IBD), type 2 diabetes, obesity, asthma as well as chronic diarrhea (Becattini et al., 2016). The latter may arise from depletion of the normal gut bacterial residents giving opportunities for colonization by pathogens such as *Clostridium difficile* where antibiotic-associated diarrhea (AAD) being the most frequent one. Many previous studies have supported the role of probiotics, single or multi-strains to prevent and treat AAD and antibiotic-related dysbiosis by restoring the balance of intestinal microbiota (Barker et al., 2017; Grazul et al., 2016). Using animal model, the administration of *Lactobacilli* and *Bifidobacterium* strains in mice showed a reduction in the proportion of *Proteobacteria* and a better recovery of members of the phylum *Bacteroidetes* after antibiotics treatment (Grazul et al., 2016; Y. Shi et al., 2018; Ying Shi et al., 2017). Evidence of probiotic-mediated effect on gut microbiota were provided in a human trial whereby the administration of a four-strain capsule of *Lactobacillus* and *Bifidobacterium* together with antibiotics to patients treated for *C. difficile* was associated with significant reductions in the duration of related diarrhea compared with placebo (Barker et al., 2017). Another study reported a reduced level of *Verrucomicrobiaceae* in stools of subjects consuming the probiotic capsules compared to those given placebo (empty) capsules, knowing that this family was positively correlated with the susceptibility to *C. difficile* infection (De Wolfe et al., 2018). In another study, ingestion of a multi-strain mixture of *Bacillus subtilis* and *Enterococcus faecium* after an antibiotic treatment for *Helicobacter pylori* infection have reduced the antibiotic-induced changes to fecal bacterial and

fungal composition (Oh et al., 2016). Moreover, when antibiotics were administered to healthy volunteers, ingestion of *Saccharomyces boulardii* CNCM I-745 together with a seven-day regime of amoxicillin-clavulanate was associated with attenuation of microbiota shifts, including less *Escherichia coli* overgrowth (Kabbani et al., 2017). *Lactobacillus rhamnosus* (specially *L. rhamnosus* GG) and *Saccharomyces boulardii* were found to be the most appropriate probiotic for preventing and treating AAD (Goldenberg et al., 2015). Recently, our group found that strains of *Bifidobacterium* and *Lactobacillus* showed more resistance to tested psychotropics compared to other genera. For instance, probiotic *Lactobacillus rhamnosus* GG (LGG) showed high compatibility and survival rate with all tested psychotropics even with desipramine (TCA) being the most active one, highlighting its potential to prevent or treat dysbiosis related to psychotropics intake (Ait Chait et al., 2020).

Several mechanisms by which probiotics modulate the gut microbiota have been described and reviewed in (Fliss et al., 2011). Specific molecular mechanisms include inhibition of intestinal pathogens by the production of antibacterial compounds, competitive exclusion either by the consumption of limited nutrient resources or adherence to the epithelium, or stimulation of indigenous microbial activity (Mekonnen et al., 2020).

5.2. Prebiotics

According to the last International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus panel, a prebiotic is defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (Gibson et al., 2017). An ideal prebiotic should be 1) resistant to the gastric acidity and hydrolysing enzymes; 2) easily fermentable by gut bacteria; and 3) able to stimulate the viability and/or activity of beneficial microorganisms (Rastall & Gibson, 2006). Prebiotics are primarily dietary components of foods or are used as enrichment ingredients. Inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), human milk oligosaccharides (HMO) and lactulose are the most known and accepted prebiotics. Recently, another category of new prebiotics was included, such as resistant starch, xylo-oligosaccharides (XOS), sugar alcohols, lactosucrose, soya-oligosaccharides (SOS), isomalto-oligosaccharides (IMO), and pectic-oligosaccharides (POS) (Gibson et al., 2017). Beside their ability to induce the proliferation of intestinal beneficial bacteria, several studies demonstrated the essential role of prebiotics in conferring health benefits to human body including bone structure (Weaver et al.,

2011), improvement in lipid metabolism (Vulevic et al., 2013), anti-diabetic activity (Ebrahimzadeh Leylabadlo et al., 2020), and improve brain functions (Kao et al., 2016).

In addition, there is increasing evidence that prebiotics can be used to modulate the microbiota and alleviate gut dysbiosis. In this regard, (Johnson et al., 2015) reported that the combination of some fermentable fibers (pectin and inulin) with antibiotics (Gentamicin and ampicillin) attenuated the adverse effect of antibiotics on gut microbiota when tested in batch fermentation. Indeed, the fermentable fibers restored the growth of beneficial microbiota (*Bacteroides*, *Bifidobacterium*, *Feacalibacterium*, ...) and induced the production of SCFAs. Recently, (Kanwal et al., 2018) found that a polysaccharide isolated from the mushroom *Dictyophora indusiate* (used as a prebiotic), reversed the dysbiosis and increased beneficial microbiota, including *Lactobacillaceae* (lactic acid-producing bacteria), and *Ruminococaceae* (butyrate-producing bacteria) in male BALB/c mice after treatment with oral clindamycin and metronidazole. According to the same authors, this polysaccharide improved also the intestinal integrity by increasing the expression of tight-junction proteins and decreasing endotoxin/pro-inflammatory cytokine levels. (Kanwal et al., 2018). Moreover, the coadministration of the prebiotic B-GOS (bimuno galacto-oligosaccharide) to female Sprague-Dawley rats with olanzapine antipsychotics significantly attenuated olanzapine-induced weight gain. This coadministration also elevated the *Bifidobacteria* spp., and reduced species within the *Firmicutes* (*Coprococcus*, *Oscillibacter*, *C. coccoides*, *Roseburia intestinalis* cluster, *Clostridium* XVIII cluster) and *Proteobacteria* (*Escherichia/Shigella* spp.) phyla. These data suggest that B-GOS might be a good prebiotic to alleviate olanzapine induced weight gain which is correlated with psychotropic-associated gut microbiota dysbiosis (Kao et al., 2018).

5.3. Fecal microbiota transplantation (FMT)

During the last few years, fecal microbiota transplantation (FMT) has gained widespread attention as a therapeutic strategy to modulate the gut microbiota. It is known as the introduction of an intestinal microbiota obtained from the faces of a healthy donor into the patient's gastrointestinal tract (Borody et al., 2019). Usually, FMT is employed to treat gastrointestinal diseases caused by pathogenic or conditionally pathogenic microorganisms, mainly treatment of *Clostridium difficile* infection (CDI) (Borody et al., 2019). The risk of this latter is increased when intestinal microbiota flora is destroyed as a result of antibiotic use, and it does not respond to antimicrobial therapy (van

Nood et al., 2013). Many case reports, studies and meta-analyses have been confirmed the clinical efficiency (up to 90%) of FMT in the management of CDI. Fuentes and colleagues noted that FMT can immediately normalize the disturbed microbiota in patients suffering from CDI. Indeed, the analysis of their microbiota revealed an increase of the Bacteroidetes, *Clostridium* clusters IV and XIVa, *Fecalibacterium prausnitzii*, *Butyrivibrio crossotus*, *Enterococcus*, *Lactobacillus* and *Veillonella* abundance and a decrease in pathogenic bacteria, such as *C. difficile* and *Escherichia coli* in intestinal content (Fuentes et al., 2014).

Following the successful treatment of patients with severe CDI, attempts have been made to apply FMT in the treatment of various diseases associated with gut dysbiosis such as metabolic syndrome, diabetes (Kootte et al., 2017; Vrieze et al., 2012) and psychiatric disorders (Kang et al., 2018). As mentioned before in the manuscript, the gut microbiota can affect various psychiatric and neurodegenerative disorders, thus, FMT can be a promising therapeutic strategy for the clinical treatment of these disorders. In this regard, Kang and colleagues reported that treatment of children with ASD for 8 weeks with FMT improved their behavioral as well as their gastrointestinal symptoms by increasing the abundance of *Bifidobacterium*, *Prevotella* and *Desulfovibrio* overall bacterial diversity (Kang et al., 2018). Furthermore, (Cai et al., 2019) revealed that the transfer of 200 mL bacterial solution to the descending duodenum of an elderly patient was useful to cure her depression. The authors reported that she become euphoric, gain weight and have improved PHQ-9 score. Until now, evidence on the advantageous effects of FMT on human health is not enough and some studies showed its controversial results. More additional clinical trials are required to validate the efficiency of FMT in patient suffering from psychiatric disorders.

6. Conclusion and future directions

The current review highlighted the importance of considering the psychotropic medications when studying the dysbiosis of human gut microbiota in psychiatric disorders. Multiple studies have reported, albeit confusing, changes in microbiome abundance in mental illness patients. Therefore, the impact of psychotropic drugs should be included in the equation as confounding factors when investigating microbial biomarkers, knowing that patients with psychiatric disorders are usually put in long-term antidepressant medication. Of note, all the cited works in this literature have well-proven the strong antimicrobial potential of psychotropics. However, most of these studies have used clinical isolates that did not represent the human gut microbes or have used animal models

that differ from humans in terms of the structure of intestine and gut microbiota composition. Thus, the relevance of those findings derived from animals to humans remains to be determined. Even in the few human trials, considerable individual differences in microbiota, diet and other factors can compromise the conclusion. Therefore, the changes in gut microbiota reported in these studies cannot be simply interpreted as a cause of only psychotropics. It is worth noting that the ex vivo gastrointestinal models represent useful alternative tools to investigate the effect of psychotropics on gut microbiota and understand their interactions. Studies from antibiotic-induced dysbiosis have proven the efficiency of probiotics, prebiotics and the fecal microbiota transplantation to restore the balance in the gut microbiota. Similarities between antibiotics and psychotropics in terms of chemical structure and mechanisms of action suggest that these nutritional tools can be promising strategies to alleviate psychotropic-associated dysbiosis.

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Chapter 2

***In vivo* and *ex vivo* models for studying the gut microbiota**

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1. Introduction

The human gut hosts the most diverse microorganisms ecosystem found anywhere in the human body (Gill et al., 2006). Numerous studies have pointed to the importance of microbial community structure and its contribution to homeostatic processes, including digestion of nutrients, protection against invading pathogens, and immune enhancement (Tremaroli & Bäckhed, 2012). Conversely, disruptions in the gut microbiota composition, known as “dysbiosis”, have been linked to numerous disease states. Thus, several investigations are now elucidating the effect of therapeutic interventions (Vázquez-Baeza et al., 2018) and xenobiotic products (Maurice et al., 2013) on human health via alterations of the structure and function of the gut microbiota. However, studying the gut microbiota in humans and animals is challenging, as many limitations involving sampling the human gastrointestinal tract, ethical restrictions are met when conducting this kind of research (Williams et al., 2015). The development of *in vitro* and *ex-vivo* models could provide a unique opportunity to study the complex microbial interactions and perturbations *in vitro* in a controlled environment (Allen-Vercoe, 2013). In this chapter, we provide an overview of different gastrointestinal models that are currently available to study the human gut microbiome.

2. *In vivo* models

2.1. Human studies

The human gut microbiome studies can be conducted in a variety of individuals, including healthy human volunteers, hospital patients, or sudden death victims (McDonald, 2017). The randomized placebo-controlled, double-blinded human trials will always be the preferred ultimate research strategy for the most reliable investigation of human gastrointestinal microbial ecosystems (Williams et al., 2015). However, some important limitations can restrict such human trials. Firstly, human studies are limited by the inaccessibility of sampling along the intestinal tract and ethical issues in human subjects, limiting the experimental design (Macfarlane & Macfarlane, 2007). Human trials are also expensive, time-consuming, and high patient dropout rate (Macfarlane & Macfarlane, 2007). Moreover, there is a lack of complete host parameters control (e.g., diet, environment, age, and genetics), making a high degree of inter-individual variability, which results in difficulties in comparing the gut microbiota of control and treatment (McDonald, 2017).

2.2. Animal studies

Animals provide an alternative route to researchers to conduct more controlled experiments where they can manipulate the environmental and genetic factors influencing the gut microbiota. The most used animal models to study the gut microbiota include mice, rats, pigs, and zebrafish (Gérard et al., 2004; Rawls et al., 2004). Animals can be conventional (germ-free or colonized with their native gut microbiota) or gnotobiotic (inoculated with specific microbes or human feces) (Macfarlane & Macfarlane, 2007). There are several advantages of using animal models; for instance, there are (a) fewer ethical restrictions than human trials; (b) complete environmental control (diet, stress, etc.); (c) genetic control of subject population; and (d) accessibility of intestinal contents, tissues, and organs (Boureau, L. Hartmann, T. Karjalaine, 2000).

Gnotobiotic animals associated with human microbiota, also referred to as human microbiota-associated (HMA) animal models, have been used frequently to assess the effect of diet, toxic compounds, substrates, bacterial strains, or pathogens in gut microbiome research (Williams et al., 2015). They are also very often used to carry out immunological studies to study, for example, allergic phenomena and gastrointestinal infections caused by pathogens and test components capable of preventing and treating such diseases (Falk et al., 1998). These kinds of studies are difficult to conduct in humans for mainly ethical reasons. On the other hand, gnotobiotic animals also have some limitations. Even if the associated microbiota is of human origin, the intestinal physiology (e.g., pH, secretions, peristalsis) remains very different from that of humans. Also, the humanized mice tend to re-shift to mice microbiota overtime (Nguyen et al., 2015). In addition, studies carried out with gnotobiotic animals remain expensive because they require special equipment (e.g., Sterile isolator) and qualified personnel for animal husbandry (McDonald, 2017). It is also important to consider that 85% of bacterial genera present in the animal model gut microbiota, specifically murine model are not present in humans (McDonald, 2017). Overall, these observations show that clear differences can be observed at the level of specific genus/species abundances between the murine and human gut microbiota. The observed differences might be caused by intrinsic differences between these two mammalian systems and various confounding factors ranging from diet to exposure to pathogens (Nguyen et al., 2015). In addition, the lab rodents are known to consume their own feces - a practice called coprophagy. The ingested feces represent a self-re-inoculation that can confound gut microbiota (Bogatyrev et al., 2020).

3. *Ex-vivo* fermentation models

Because of the limitations mentioned above of *in vivo* studies, *ex-vivo* gut fermentation models can be used as an exciting tool permitting the study of complex microbial ecosystems to complement human and animal studies. Gut fermentation models are artificial systems that closely mimic some of the spatial, temporal, and environmental conditions within the human gut (Macfarlane & Macfarlane, 2007). The key objective of these models is to culture stable, reproducible, and complex microbial communities in a highly controlled environment (McDonald et al., 2013; Van den Abbeele et al., 2010). A variety of *ex-vivo* models were developed using single or multiple vessels inoculated with fresh human feces or defined microbial communities. The vessels are operated under controlled conditions of temperature, pH, growth medium, and transit time to mimic the microbial composition and activity in a specific region of the human gut (McDonald, 2017).

3.1. Batch Fermentation Models

Batch cultures represent the simplest forms of *ex-vivo* models used to study the human gut microbiota (Figure 2.1). These batches are usually performed in a closed system (sealed bottles or reactors) without adding or removing material following inoculation. The compound of interest (i.e., substrate or inhibitor) is added to the reactor and run typically for a short period of 24 - 48 h. This short incubation period is due to rapid progression to the stationary phase due to nutrient depletion and accumulation of inhibitory bacterial metabolites (Macfarlane et al., 1992). Batch cultures are inexpensive and facilitate the rapid testing of a wide variety of substrates in initial screening assays; however, they are limited to short-term studies as changes in substrate availability, pH, and redox potential can result in the selection of non-representative microbial populations over time (Macfarlane & Macfarlane, 2007).

3.2. Continuous fermentation models

Continuous fermentation systems or chemostat offer a relevant tool that can be used for long-term studies (from days to months) since there is a continuous replenishing of nutrients and removing of wastes (including toxic products) from the culture system (Payne et al., 2012). The continuous model ensures the control of environmental parameters, including pH, temperature, atmosphere, nutrient input, and waste removal, which allows the maintenance of stable conditions where microbial composition and activity reach a steady-state over time (Ziv et al., 2013). These systems

provide insight into compositional and metabolic changes in microbial communities over time in response to perturbations, including the introduction of pathogens (Crowther et al., 2014), probiotics, dietary substrates (Collins et al., 2018), or drugs (Newton et al., 2013).

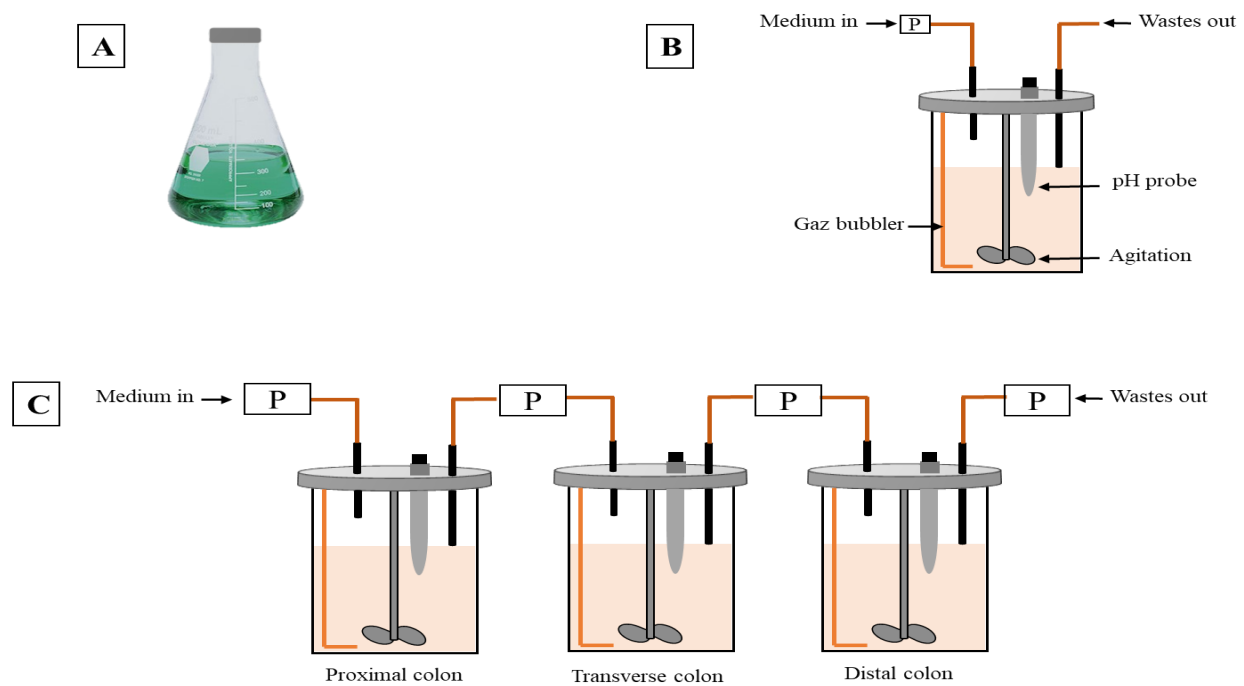


Figure 2.1: *In vitro* gut fermentation models used to culture human gut microbiota.

(A) Batch fermentation model. (B) Single-stage chemostat model simulating one segment of the gut. (C) Three-stage chemostat model simulating the ascending, transverse, and descending colon.

Continuous fermentation can be composed of one or multiple reactors that can be inoculated with the same fecal sample and run parallel to each other, allowing for comparison of one or more test reactors to a control reactor (Figure 2.1). Continuous single-stage chemostats are composed of one reactor and designed to simulate a specific segment of the intestinal tract (i.e., distal or proximal colon). Multistage chemostat systems are composed of more than one reactor simulating several intestinal tract segments and are often composed of three reactors set to mimic the ascending, transverse, and descending colon (three-stage chemostat) (Macfarlane et al., 1998; McDonald et al., 2013).

The chemostat bioreactors are usually seeded with a liquid fecal suspension; however, this kind of inoculum experience rapid washing of less competitive bacteria and are therefore limited in operational time to less than four weeks (Patrick De Boever, Roel Wouters, Va, 2001; Sghir et al., 1998). In addition, it is challenging to reproduce both the planktonic (acellular) and sessile (biofilm) state of bacterial populations in the colon (Macfarlane & Macfarlane, 2007). To solve these problems, a process of immobilization of the fecal microbiota has been developed, originally designed to mimic the infant gut (Flint et al., 2007). Here, fecal microbes were suspended in a porous polysaccharide matrix (gellan and xanthan gums) to form 1-2 mm diameter gel beads. These gel beads are then transferred to a first-stage inoculum reactor (PolyfermS model), usually used to inoculate a set of parallel second-stage reactors continuously. As the microbes grow in the gel beads, they form a high-cell density peripheral layer. These cells are continuously released from this layer to the growth medium. This model was adapted to achieve the goal of the current thesis.

Other artificial digestive systems have been developed to model additional gut environment features, such as the TIM-2 or SHIME models. In the TIM-2 model, there is the addition of functions that mimic peristaltic movements, water absorption, and metabolites from the colon (Minekus et al., 1999). The SHIME model consists of a series of five reactors that mimic the duodenum / jejunum, the ileum, and the three parts of the colon (Molly et al., 1993). Furthermore, the three-stage continuous model was improved to directly generate the anaerobic atmosphere from the microbiota metabolism, leading to different gas ratios of CO₂ and H₂ in each compartment (Feria-Gervasio et al., 2014).

3.3. Advantages, Challenges, and limitations of gut fermentation models

The *ex vivo* fermentation models represent an excellent system that can closely mimic microbial composition and activity in different human gut regions, offering unique advantages. Firstly, as continuous culture systems are standardized, they provide high reproducibility to assess the changes that occur due to exposure to perturbations or compounds (drugs, nutritional therapies, probiotics, prebiotics, antibiotics, and other medications) in the absence of confounding factors. This enables researchers to determine causality between the intervention and changes in community dynamics, which contrasts to most human studies, which rely on correlations between interventions and changes in the gut microbiota composition or functionality (Guzman-Rodriguez

et al., 2018). Importantly, there are no ethical constraints for using *ex vivo* models so that pathogens, toxic or radioactive compounds, can be used without ethical approval. Moreover, *ex vivo* models provide insights into different steps of the fermentation process by allowing a dynamic sampling over time in different consecutive regions of the human colon (Venema & van den Abbeele, 2013).

Despite all these mentioned benefits, the *ex vivo* models present some limitations. Such systems may be an oversimplification of the *in vivo* situation and do not always provide accurate models of what occurs *in vivo*, as they lack an epithelial mucosa, host immunological interactions, and neuroendocrine system functionality (Boureau, L. Hartmann, T. Karjalaine, 2000). It is also essential to properly validate *in vitro* models before they are used for gut microbiota studies. The three-stage chemostat system developed by Macfarlane et al. was validated using microbiological and chemical measurements taken from the intestinal contents from human sudden death victims (Macfarlane et al., 1998). However, other models have not been fully validated due to the ethical constraints involved in sampling different intestinal compartments.

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Chapter 3

Hypothesis, Objectives, and specific Aims

The impact of psychotropics on human gut microbiota remains under-explored. Here we postulate that psychotropics could induce gut microbiota breakdown through an antimicrobial mechanism and that probiotic strains could alleviate such associated dysbiosis. The purpose of the present study was to investigate:

- (i) the antimicrobial activity of some oral commonly prescribed psychotropics from different therapeutic classes on intestinal bacteria and probiotics *in vitro*;
- (ii) the impact of psychotropics on colonic microbiota diversity and metabolism *ex vivo*;
- (iii) growth and capacity of probiotics to alleviate antimicrobial effects of psychotropics on colonic microbiota in a simulated human colon.

Chapter 4

Unravelling the antimicrobial action of antidepressants on gut commensal microbes

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Abstract

Over the past decade, there has been increasing evidence highlighting the implication of the gut microbiota in a variety of brain disorders such as depression, anxiety, and schizophrenia. Studies have shown that depression affects the stability of gut microbiota, but the impact of antidepressant treatments on microbiota structure and metabolism remains underexplored. In this study, we investigated the in vitro antimicrobial activity of antidepressants from different therapeutic classes against representative strains of human gut microbiota. Six different antidepressants: phenelzine, venlafaxine, desipramine, bupropion, aripiprazole and (s)-citalopram have been tested for their antimicrobial activity against 12 commensal bacterial strains using agar well diffusion, microbroth dilution method, and colony counting. The data revealed an important antimicrobial activity (bacteriostatic or bactericidal) of different antidepressants against the tested strains, with desipramine and aripiprazole being the most inhibitory. Strains affiliating to most dominant phyla of human microbiota such as *Akkermansia muciniphila*, *Bifidobacterium animalis* and *Bacteroides fragilis* were significantly altered, with minimum inhibitory concentrations (MICs) ranged from 75 to 800 µg/mL. A significant reduction in bacterial viability was observed, reaching 5 logs cycle reductions with tested MICs ranged from 400 to 600 µg/mL. Our findings demonstrate that gut microbiota could be altered in response to antidepressant drugs.

Keywords: antidepressants, gut bacteria, antimicrobial activity, human microbiota, dysbiosis, depression.

1. Introduction

The gut microbiota represents a diverse community relatively stable during the adult age (Mehta et al., 2018) that plays a crucial role in host physiology, homeostasis, development, and metabolism (Bauer et al., 2016; Sampson & Mazmanian, 2015; Turnbaugh et al., 2009). Over the past decade, there has been increasing evidence highlighting the implication of the gut microbiota in a variety of brain disorders such as depression, anxiety, and schizophrenia (Codagnone et al., 2019; Kelly et al., 2016a). Studies have shown that depression affects the stability of gut microbiota, but the impact of antidepressant treatments on microbiota structure and metabolism remains underexplored (Jiang et al., 2015a). Indeed, gut microbiota could be altered during major depressive episodes (Jiang et al., 2015a) or in response to antidepressant treatments, which could be undervalued confounding factors (Cheung et al., 2019; Cusotto et al., 2019; Macedo et al., 2017b).

Antidepressant drugs have been increasingly shown to possess antimicrobial properties with possible implications in the microbiota-gut-brain axis. The anti-tuberculosis agent iproniazid was first to be used in the treatment of depression in the 1950s due to its euphoriant effects on tuberculosis patients, reviewed in (Macedo et al., 2017b). Since then, several classes of antidepressants, including monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), N-methyl-D-aspartate (NMDA) receptor antagonists, and tricyclic antidepressants (TCAs) have been assessed for their antimicrobial potency, with their mechanism of action being poorly investigated. For instance, SSRIs such as sertraline, fluoxetine and paroxetine are efflux inhibitors in bacteria cell walls and are effective on Gram-positive bacteria such as *Enterococcus* and *Staphylococcus* (Muhammad Ayaz, Subhan, Ahmed, Khan, Ullah, Ullah, et al., 2015b). In addition, several studies highlighted the antifungal potential of SSRIs fluoxetine, sertraline, and paroxetine against *Aspergillus* spp., *Candida parapsilosis*, and *Candida albicans* (Muhammad Ayaz, Subhan, Ahmed, Khan, Ullah, Ullah, et al., 2015b; Costa Silva et al., 2017b; Gu et al., 2016b). In addition, several SSRIs have been reported to have antimicrobial properties at high concentrations while having antimicrobial enhancer properties at lower concentrations. This synergistic effect is confirmed by decreases in the minimum inhibitory concentrations of antibiotics when combined with antidepressants (L. Li et al., 2017b). Likewise, Ketamine, an NMDA antagonist, was shown effective against *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*, and

Candida albicans (Evrensel & Ceylan, 2018). Another class of antidepressant drugs, the TCAs, was reported to have anti-plasmid effects and to prevent the growth of intestinal pathogens such as *E. coli*, *Yersinia enterocolitica*, *Giardia lamblia*, *Plasmodium falciparum*, and *Leishmania* spp, reviewed in (Macedo et al., 2017b).

Besides, other evidence gathered from animal studies suggested that the antidepressants modulate the composition of the intestinal microbiota (Cusotto et al., 2019; K J Davey et al., 2013; Lukić et al., 2019; Lyte et al., 2019). Administration of TCA desipramine causes important side effects and results in a higher incidence of infections generating gingivitis and dysbiosis of oral microbiota (Gimenez-Bastida et al., 2018). A prior study revealed that ketamine also modulates the fecal microbiome in the susceptible mice after chronic social defeat stress, suggesting an antidepressant mechanism partly mediated by the modulation of gut microbiota (Qu et al., 2017b). However, all the existing previous studies were carried out in animal models or using isolated strains (references or clinical isolates) that do not necessarily represent the human gut microbiota to enhance the efficacy of existing chemotherapeutic agents such as antibiotics. Limited studies have investigated the effect of antidepressant medications on the growth of commensal microbial residents of the human gut microbiota. For instance, of 1000 non-antibiotics drugs, oral antipsychotics were able to reduce the *in vitro* growth of gut bacterial strains (Maier et al., 2018). Likewise, Cusotto et al. (Cusotto et al., 2019) reported the *in vitro* sensitivity of two commensal bacteria, notably *Escherichia coli* APC105 and *Lactobacillus rhamnosus* 6118 toward two SSRIs, fluoxetine and escitalopram.

The chronic use of antidepressant drugs presenting antimicrobial effects may be related to the development of adaptive alterations in gut microbiota, with potentially deleterious effects (Macedo et al., 2017b). The purpose of the present study was to investigate the antimicrobial effect of some oral commonly prescribed antidepressants from different therapeutic classes against commensal bacteria representative of the predominant phyla found in the human gut microbiota.

2. Material and methods

2.1. Antidepressants

The six (6) antidepressants tested in this study are listed in Table 4.1. The choice of these drugs was based on their common prescription, mode of action, and therapeutic class. Venlafaxine

hydrochloride, bupropion hydrochloride, aripiprazole and (S)-citalopram oxalate were purchased from TCI America (Portland, USA) while phenelzine sulphate salt and desipramine hydrochloride were from Sigma Aldrich (St. Louis, MO, USA). The stock solutions were prepared following the manufacturer's recommendations (please refer to Table S4.2, Appendices) to achieve a concentration of 10 mg/mL, filter sterilized and then stored at -20°C until use. Working solutions of 1.8 mg/mL and 1.2 mg/mL were prepared from the stock solutions in the respective growth media before each experiment.

2.2. Media, bacterial strains and culture conditions

De Man, Rogosa and Sharpe (MRS), Brain Heart Infusion (BHI) and Fastidious Anaerobe Broth (FAB) media were obtained from Criterion (Santa Maria, CA, USA). Mucin from porcine stomach and yeast extract were purchased from Sigma (St. Louis, MO, USA). Twelve (12) commensal bacterial strains were purchased from the American Type Culture Collection (Table S4.1, Appendices). The intestinal strains were selected in a way to represent the main abundant phyla in the human gut, notably Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Verrucomicrobia. All strains were cultured in their recommended media (Table S4.1, Appendices). Frozen stocks of strains were maintained at -80°C until use. Bacteria strains were grown in their appropriate media a least three times at 37°C before each experiment to obtain a robustly and uniformly growing culture. The enzyme Oxyrase for broth purchased from Sigma Aldrich (St. Louis, MO, USA) was added to culture media at 1% to promote the growth of the anaerobic strains.

2.3. Determination of the antibacterial activity

Agar well diffusion method

The antimicrobial activity of antidepressants was determined visually using the agar well diffusion assay, as previously described (Hammami et al., 2009). Briefly, appropriate media containing 7.5 g agar/L was cooled to 45°C, seeded with an overnight culture of each intestinal strain at 1% (v/v) and poured into a sterile Petri dish (25 mL). After solidification, 7 mm diameter wells were made using the wide end of a sterile glass pipette and filled with 80 µL of ½ dilution series of antidepressant solutions starting from 10 mg/mL to 0.625 mg/mL. The plates were kept at 4°C for 2 h and then incubated for 24 h at 37°C. The diameter of the inhibition zone around the well was measured.

Table 4.1. List of antidepressants tested in this study.

Drug	Class	Mode of action
Phenelzine	Monoamine-oxidase inhibitor (MAOIs)	Inhibition of the breakdown of neurotransmitters (norepinephrine, serotonin, dopamine) by blocking the monoamine oxidase enzyme.
Venlafaxine	Serotonin-norepinephrine reuptake inhibitors (SNRI)	↑ synaptic levels of 5-HT and NE by blocking the reuptake of the neurotransmitters into the presynaptic neuron
(S)-Citalopram	Serotonin-specific reuptake inhibitors (SSRI)	↑ synaptic levels of 5-HT by blocking the reuptake of the neurotransmitter into the presynaptic neuron
Desipramine	Tricyclic antidepressants (TCA)	Inhibition of noradrenaline and serotonin reuptake by neurons
Bupropion	Atypical antidepressants	It has predominantly antagonist activity on postsynaptic D2 (Dopamine) receptors and partial agonist activity on presynaptic D2 receptors.
Aripiprazole	Atypical antidepressants	It has predominantly antagonist activity on postsynaptic D2 (Dopamine) receptors and partial agonist activity on presynaptic D2 receptors.

Upward arrow signifies increase, 5-HT serotonin, D: dopamine.

Broth microdilution method and determination of Minimum Inhibitory Concentration

The antibacterial effect of the antidepressants was performed using the broth microdilution method as described in the approved CLSI standard reference method for antimicrobial susceptibility testing by broth diffusion for aerobic (CLSI, 2018b) and anaerobic bacteria (CLSI, 2018a). Briefly, a 96-well microplate (Randor, PA, USA) was filled by distributing 100 µL of appropriate media (corresponding to each strain). Medium alone and medium with each strain inoculum were used as a negative and positive control on the same microplate. Then, 100 µL of each working antidepressant solution was added to each well (C1 to C12) and 2-fold serially diluted to reach final concentrations ranged from 800 to 150 µg/mL. Wells thus containing 100 µL of media were inoculated with 100 µL overnight culture of the intestinal strains diluted to a concentration of 10^5 - 10^6 CFU/mL. Plates were incubated anaerobically at 37°C for 24 h. Reading of each well was performed with measuring the optical density (OD) at 650 nm in a microplate reader (Tecan Spark, Austria) (Hammami et al., 2009). The MIC of each antidepressant was determined as the lowest concentration that inhibits the growth of the target strain. Also, samples incubated for 16 h were centrifuged to remove the drug-containing media and resuspended in the appropriate drug-free media. The viable bacterial strains were then determined by standard plate counting method on the appropriate media solidified with agar 1.2% and expressed as colony-forming units (CFU) per ml.

2.4. Statistical analysis

Statistical analysis was performed using GraphPad Prism v8.3. Data were expressed as the mean \pm standard deviation (SD) of triplicate experiments. One-way analysis of variance (ANOVA) followed with Tukey's multiple comparison was applied to determine the statistically significant difference ($P < 0.05$) among experimental variables.

3. Results

3.1. Antibacterial activity on solid media

The antibacterial activity of different doses (0.625-10 mg/mL) of the tested antidepressants was first assessed by the well diffusion method. As shown in Figure 4.1, these drugs display a dose- and drug-dependent antibacterial effect. Desipramine and aripiprazole showed the most inhibitory effect against all the tested strains with respective diameter of inhibition zone ranging from 13 to

35 mm and 15 to 31 mm (Table S4.3, Appendices). At lesser extent, phenelzine and (s)-citalopram showed moderate antibacterial activity against some intestinal strains with diameter inhibition zone going from 9 to 19 mm (Table S4.3, Appendices). Besides, minimal inhibition zone (around 9 mm) was observed in some strains with bupropion; however, no zone was detected with venlafaxine (data not shown). *Akkermansia muciniphila* and *Clostridium leptum* were the most sensitive strains to tested antidepressants (Figure 4.1), while *Lactobacillus rhamnosus* being the most resistant (Table S4.3, Appendices).

3.2. Growth kinetics and determination of minimum inhibitory concentrations (MICs)

The antimicrobial activity of tested antidepressants against 12 commensal intestinal strains was quantified using the micro-broth dilution method. Figures 4.2; 4.3 and 4.4 illustrate the growth kinetics of some commensal gut bacteria. In the presence of increasing concentrations of antidepressants, the growth curves were dose-dependent, with strains being totally or partially inhibited. Desipramine was very active against most of the tested intestinal strains (10/12) with MIC values varying from 75 to 800 µg/mL (Table 4.2). For instance, 75 µg/mL of desipramine was enough to inhibit the growth of *A. muciniphila* partially, while higher concentrations (>150 µg/mL) inhibited its growth completely. *Faecalibacterium prausnitzii* and *Eubacterium rectale* were the least susceptible to desipramine (MIC >800 µg/mL). The tested strains were also sensitive to aripiprazole at MIC values ranging from 200 to 800 µg/mL. Beside *A. muciniphila* which was highly sensitive to aripiprazole (MIC = 200 µg/mL), other bacteria including, *Lactobacillus casei*, *Enterococcus faecium*, *Bacteroides fragilis*, and *C. leptum* were all inhibited at a dose of 300 µg/mL (Table 4.2). Comparatively, phenelzine exhibited a higher inhibitory effect against *E. rectale* and *F. prausnitzii* with respective MIC values of 300 and 400 µg/mL, while (s)-citalopram inhibited *E. rectale* at MIC value of 300 µg/mL. At the highest tested concentration (800 µg/mL), venlafaxine and bupropion presented MICs >800 µg/mL or no inhibitory activity against the tested strains.

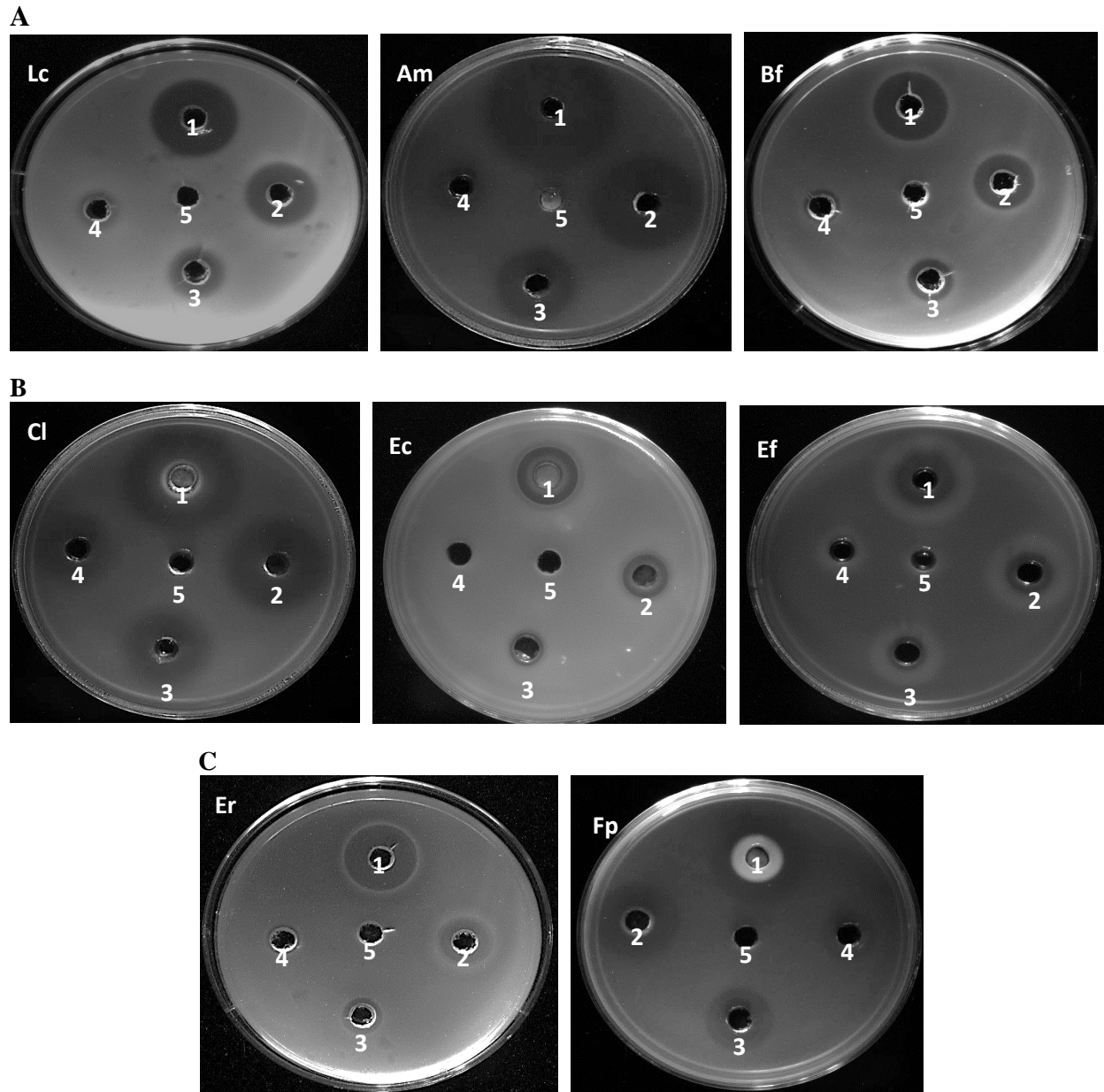


Figure 4.1. Inhibition zone of desipramine (A), aripiprazole (B) and phenelzine (C) against some intestinal bacteria strains.

Lc: *L. casei*, Am: *A. muciniphila*, Bf: *B. fragilis*, Cl: *C. leptum*, Ec: *E. coli*, Ef: *E. faecium*, Er: *E. rectale*, Fp: *F. prausnitzii*. (1: 10 mg/mL; 2: 5 mg/mL; 3: 2.5 mg/mL; 4: 1.25 mg/mL; 5: 0.625 mg/mL).

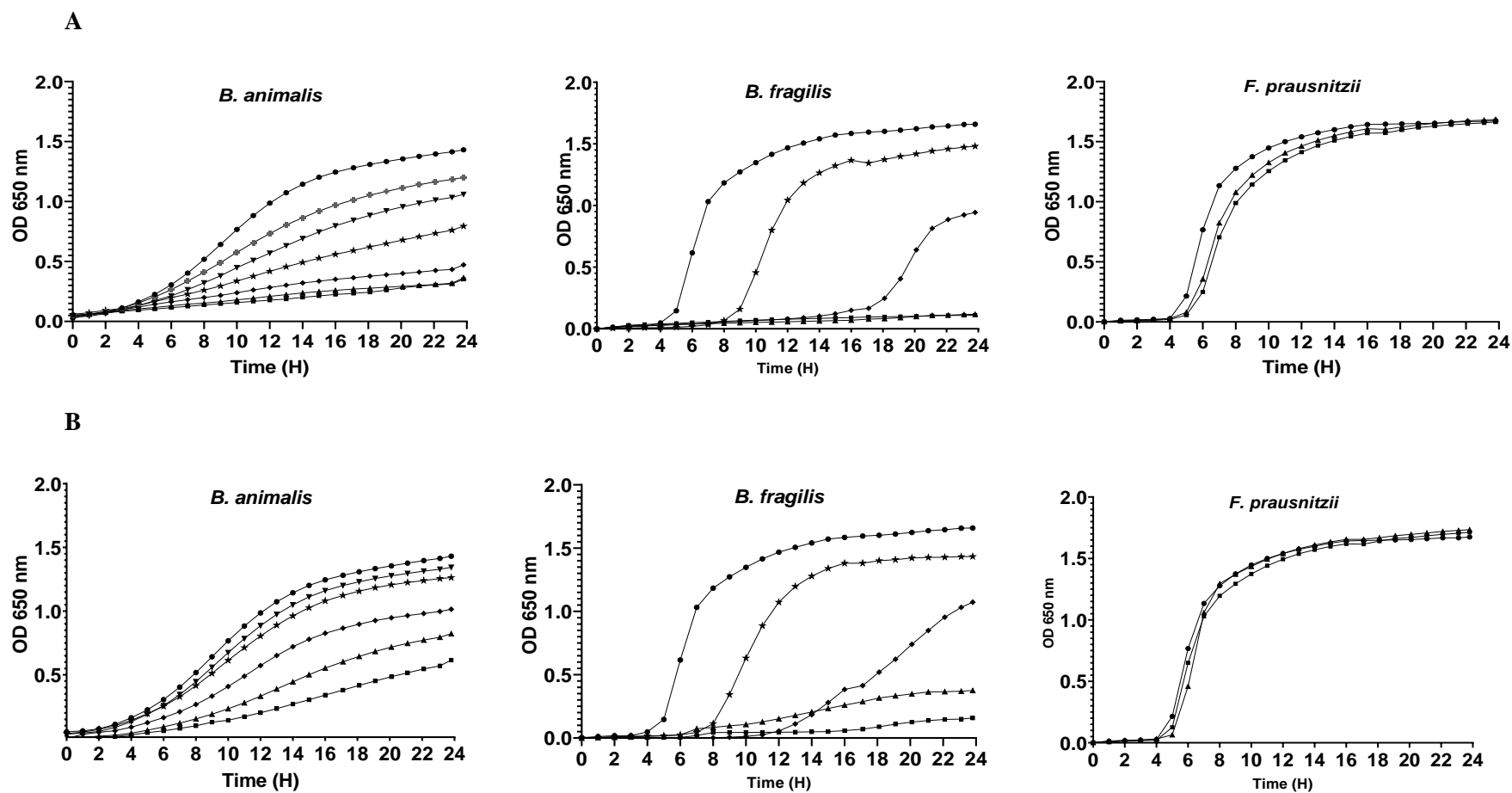


Figure 4.2. Growth of some intestinal strains in the presence of (A) Desipramine and (B) Aripiprazole.

Concentrations ($\mu\text{g/mL}$) of antidepressants were 0 (circle), 800 (square), 600 (triangle), 400 (diamond), 300 (star), 200 (inverted triangle) and 150 (cross).

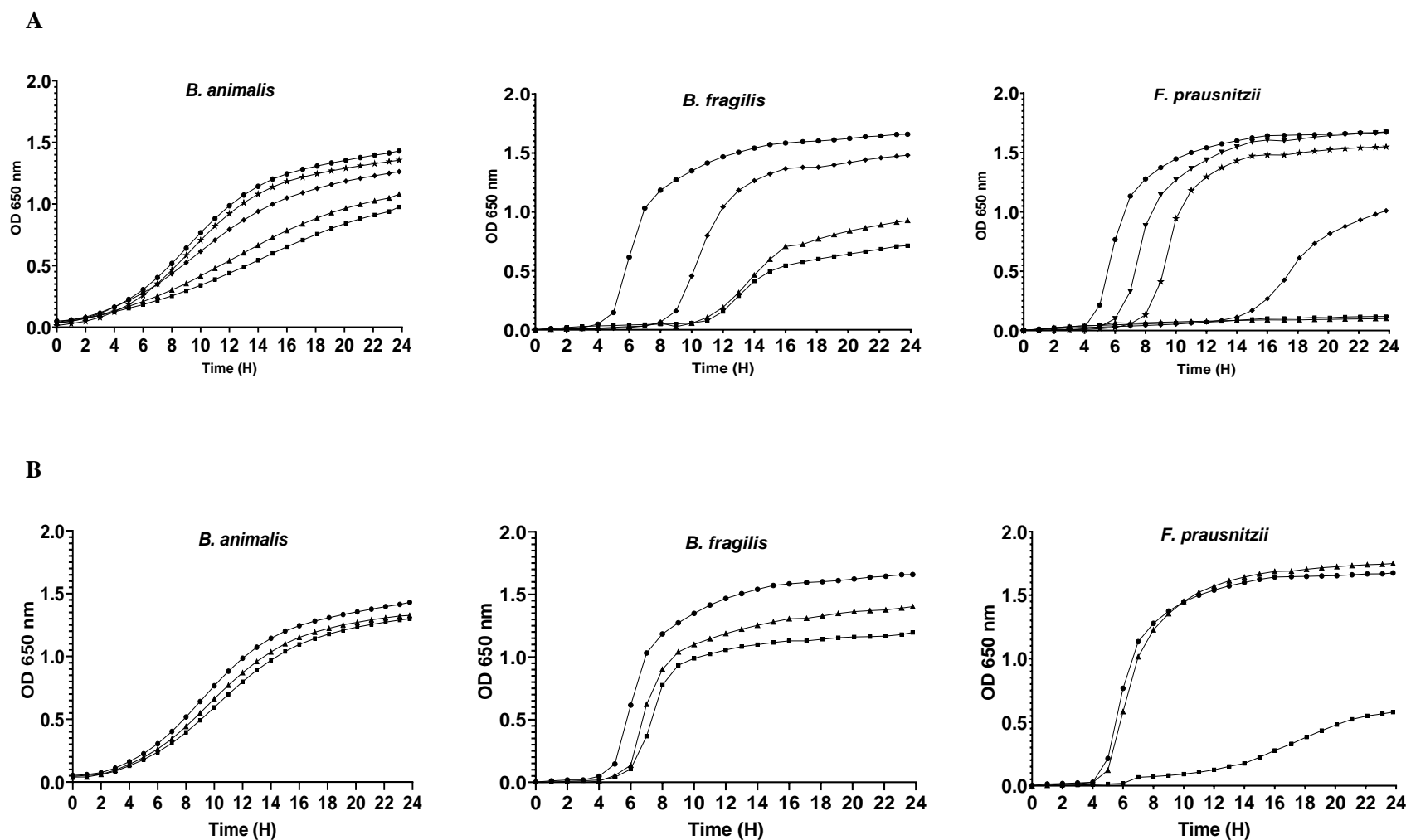
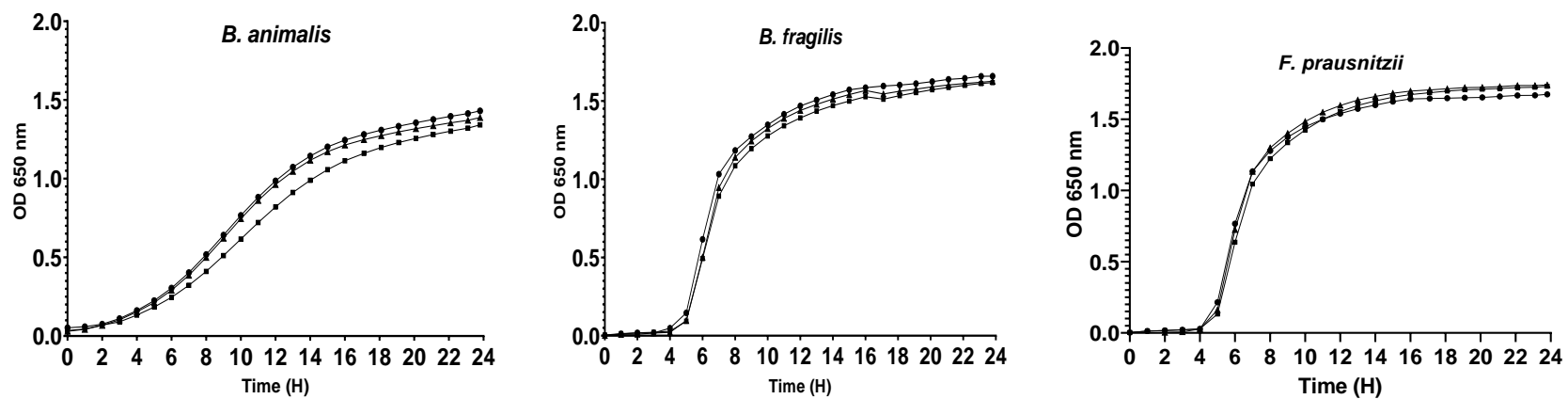


Figure 4.3. Growth of some intestinal strains in the presence of (A) Phelzine and (B) (s)-Citalopram.

Concentrations ($\mu\text{g/mL}$) of antidepressants were 0 (circle), 800 (square), 600 (triangle), 400 (diamond), 300 (star), 200 (inverted triangle) and 150 (cross).

A



B

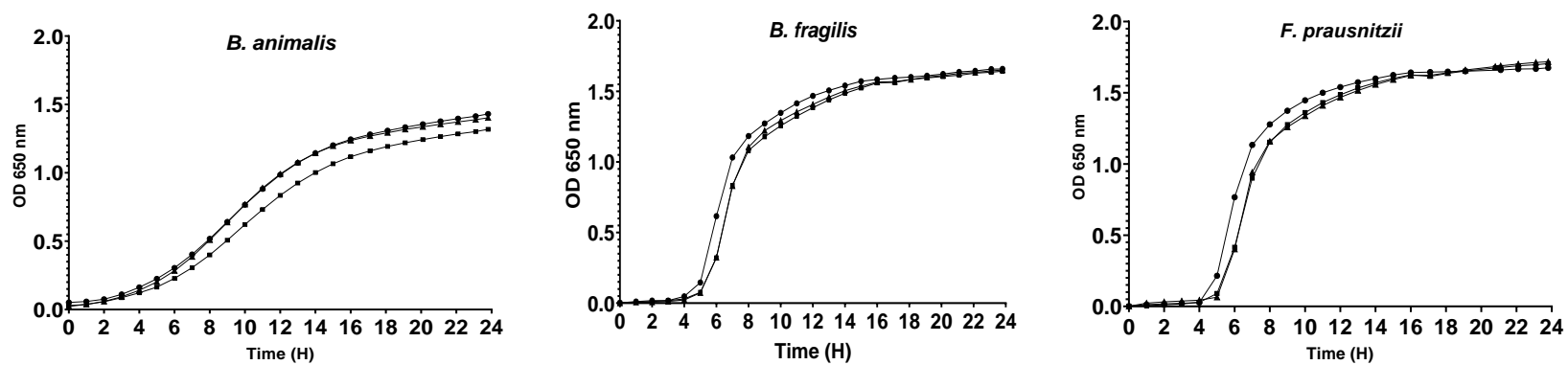


Figure 4.4. Growth of some intestinal strains in the presence of (A) Venlafaxine and (B) Bupropion.

Concentrations ($\mu\text{g/mL}$) of antidepressants were 0 (circle), 800 (square), 600 (triangle), 400 (diamond), 300 (star), 200 (inverted triangle) and 150 (cross).

Table 4.2. Minimal inhibitory concentration (MICs) of antidepressants against commensal gut bacteria.

Intestinal strains	Minimal inhibitory concentration (µg/mL)					
	Phenelzine	Venlafaxine	Desipramine	Bupropion	Aripiprazole	Citalopram
<i>L. reuteri</i> ATCC 23272	> 800	NI	600	NI	800	> 800
<i>L. rhamnosus</i> ATCC 53103	> 800	NI	800	NI	600	> 800
<i>L. casei</i> ATCC 393	> 800	NI	300	> 800	300	800
<i>B. animalis</i> ATCC 25527	800	> 800	200	> 800	400	> 800
<i>E. faecium</i> ATCC 35667	> 800	800	600	> 800	300	> 800
<i>E. rectale</i> ATCC 33656	300	NI	> 800	> 800	> 800	300
<i>F. prausnitzii</i> ATCC 27768	400	NI	> 800	> 800	> 800	800
<i>B. fragilis</i> ATCC 25285	600	> 800	400	> 800	300	800
<i>P. aeruginosa</i> ATCC 27853	600	800	300	800	600	800
<i>E. coli</i> ATCC 25922	800	NI	150	> 800	600	800
<i>C. leptum</i> ATCC 29065	800	> 800	300	> 800	300	800
<i>A. muciniphila</i> ATCC BAA-835	> 800	> 800	75	> 800	200	> 800

NI: No Inhibition at the maximal tested concentration (800 µg/mL).

3.3. Bacteria viability in the presence of different antidepressant concentrations

The logarithmic reductions of the viable bacterial cells in presence of antidepressants after 16 h incubation is presented in Figure 4.5. Increased doses of antidepressants significantly reduced bacterial counts ($P < 0.05$). For instance, desipramine strongly and significantly ($P < 0.05$) affected the cell viability of tested intestinal strains, being strain-dependant. Indeed, cells of *A. muciniphila* and *E. coli* was completely inhibited at respective concentrations of ≥ 300 and $200 \mu\text{g/mL}$ of this drug (Figure 4.5). No viable *A. muciniphila* cells were detected in samples treated with either desipramine or aripiprazole at 600 or $800 \mu\text{g/mL}$ compared to initial inoculum counts ($5.58 \pm 0.19 \log_{10} \text{cfu/mL}$), suggesting a bactericidal effect of these drugs (Figure S4.2C, Appendices). At concentrations up to $300 \mu\text{g/mL}$ of desipramine, the viable cell counts decreased significantly ($P < 0.05$) by more than 5 log cycles (i.e. *L. reuteri* and *B. fragilis* at $800 \mu\text{g/mL}$), 4 log cycles (i.e. *C. leptum* at $800 \mu\text{g/mL}$ and *L. reuteri* at $600 \mu\text{g/mL}$), 3 log cycles (i.e. *B. animalis* at $600 \mu\text{g/mL}$), 2 log cycles (i.e. *P. aeruginosa* at $600 \mu\text{g/mL}$), 1 log cycle (i.e. *L. casei* at $400 \mu\text{g/mL}$) or less than 1 log (i.e. *B. animalis* at $300 \mu\text{g/mL}$). No reduction was detected against the two strains *E. rectale* and *F. prausnitzii*. Likewise, aripiprazole completely inhibited the cells of *E. coli* at $800 \mu\text{g/mL}$ and *A. muciniphila* at 800 and $600 \mu\text{g/mL}$ (Figure S4.2C, Appendices). Reductions of 5 log cycles were obtained for some of the other intestinal strains. The number of *E. rectale* and *F. prausnitzii* was not affected by the different concentrations of aripiprazole. Comparatively, phenelzine and (s)-citalopram (Figure 4.5) were more active towards *E. rectale* and *F. prausnitzii*, with reductions varying from 2 to 5 logs when tested at $400 \mu\text{g/mL}$ or up. No significant reductions were obtained in the presence of different concentrations of venlafaxine and bupropion (Figure S4.1, Appendices).

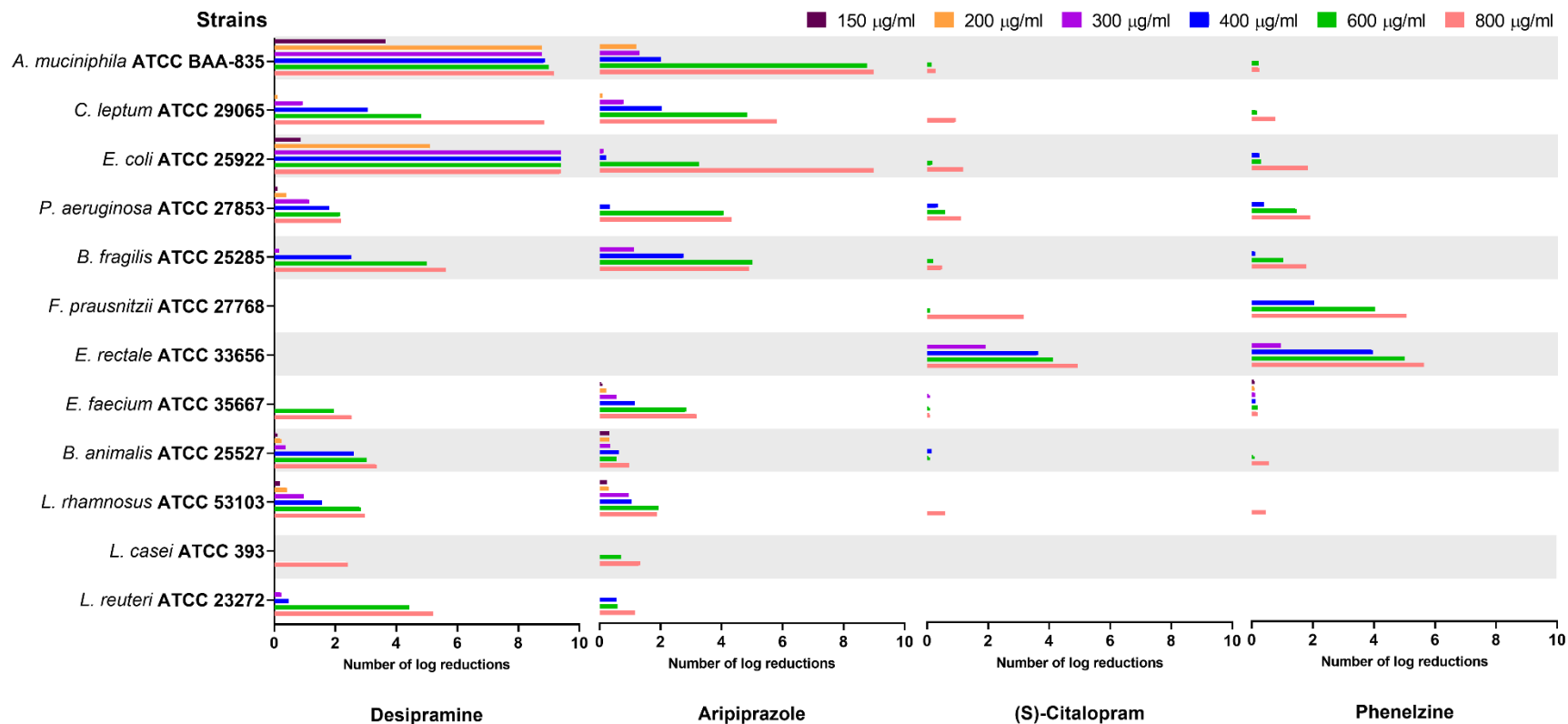


Figure 4.5. Logarithmic reductions of the growth of the reference strains in the presence of the antidepressants.

4. Discussions

There is an increasing interest on how therapeutic drugs could affect and alter the human gut microbiota composition and function (Maier et al., 2018). While some knowledge is accumulating on the antimicrobial impact of some antidepressants on isolated strains or the gut microbiota of animal models, information about other classes of antidepressants and representative species from the human gut is poorly investigated. There is an urgent need to clarify the real contribution of the antimicrobial role of antidepressants and the subsequent consequences to gut microbiota structure and metabolism. In this study, we investigated the *in vitro* effect of commonly prescribed antidepressants from different classes on commensal bacterial strains members of the human gut microbiota. The results clearly demonstrated that most of the tested antidepressants exerted an important dose-dependent inhibitory effect (bactericidal in some cases) on the growth of the tested bacterial strains.

Desipramine, belonging to tricyclic antidepressants class, showed the most potent antibacterial activity and significant ($P < 0.05$) growth reduction, with *A. muciniphila* (Verrucomicrobia family) and *E. coli* (Proteobacteria group) being the most sensitive microorganisms at MIC values of 75 and 150 $\mu\text{g/mL}$, respectively. Information about the antimicrobial activity of desipramine, in particular toward human gut strains, is missing from the literature. In an *in vivo* study, (Lukić et al., 2019) reported that desipramine was able to reduce the richness and increase beta diversity of Male BALB/c mice gut microbiota. Also, the same authors found a reduction in abundance at the genus level of *Ruminococcus*, *Adlercreutzia*, and an unclassified Alphaproteobacteria in mice treated with desipramine. Likewise, administration of desipramine was also shown to cause important side effects and results in a higher incidence of infections generating gingivitis and dysbiosis of oral microbiota (Gimenez-Bastida et al., 2018). Other representatives of the tricyclic antidepressants group were previously shown to possess an *in vitro* antimicrobial effect toward human pathogenic species, such as amitriptyline against *Staphylococcus* spp., *Bacillus* spp., and *Vibrio cholerae* (Mandal et al., 2010); and imipramine, which inhibited the growth of *E. coli* and *Yersinia enterocolitica* (Csiszar & Molnar, 1992). Another FDA-approved TCA drug, maprotiline, has shown the potential to reduce the severity of *Francisella* infection by decreasing virulence without being bactericidal (Dean & van Hoek, 2015b). Maprotiline and chlorpromazine have strong antibiofilm activity against *Francisella* (Dean & van Hoek, 2015b). Besides its antibiofilm

inhibitory activity in *Salmonella* Typhimurium and *Francisella novicida*, chlorpromazine is strongly inhibitory to *F. novicida* growth (Dean & van Hoek, 2015b). Moreover, TCA amoxapine was demonstrated to resensitize methicillin-resistant *S. aureus* to oxacillin *in vitro* (Wilson et al., 2018). In addition, members of TCA drugs were reported to possess anti-plasmid effects and to inhibit intestinal pathogens such as *E. coli*, *Yersinia enterocolitica*, *Giardia lamblia*, *Plasmodium falciparum*, and *Leishmania* spp, reviewed in (Macedo et al., 2017b).

Interestingly, **aripiprazole** and **bupropion**, belonging both to the atypical group of antidepressants, displayed different effects, with aripiprazole having a pronounced antibacterial activity and bupropion exhibiting no significant growth inhibition. Some previous studies have shown that aripiprazole exerts an inhibitory effect on gut microbiota (Cussotto et al., 2019; Maier et al., 2018). For instance, Maier et al. (2018) demonstrated, in an *in vitro* large-scale study, that several non-antibiotics drugs inhibit the growth of human gut bacteria, with *Akkermansia* levels being reduced in the presence of atypical antipsychotics (including aripiprazole). Our study revealed a high antibacterial sensitivity of *Akkermansia* to aripiprazole and desipramine, with bactericidal effects at tested concentration range. In rats, the administration of aripiprazole for 4 weeks was associated with modulation of the relative abundance of firmicutes genera, including *Clostridium*, *Ruminiclostridium*, *Intestinibacter* and *Eubacterium coprostanoligenes* (Cussotto et al., 2019).

Being the most commonly prescribed class of antidepressants to treat the major depressive disorder, the selective serotonin reuptake inhibitors (SSRIs) tested in this study, **(S)-citalopram** was found to be more active against *E. rectale* and *F. prausnitzii*, both belonging to the Firmicutes phylum. Many isolated *in vitro* studies conducted with SSRI drugs, using reference strains or clinical isolates (not necessarily representing the gut microbes), showed an antimicrobial effect (Macedo et al., 2017b). According to (Cussotto et al., 2019), Escitalopram, an enantiomer of (s)-citalopram, and fluoxetine were able to completely inhibit the growth of *E. coli* APC105 and *L. rhamnosus* 6118, both resident of the human gut, at a concentration of 600 µg/mL. In addition, citalopram was reported to exert an inhibitory effect against some pathogenic strains of *E. coli* with MIC value over than 800 µM (Mohammad Ayaz et al., 2015; Bohnert et al., 2011), and against *P. aeruginosa* strains with MIC ranged 4,000-6,000 mg/mL (Mohammad Ayaz et al., 2015). Other studies were demonstrated the antimicrobial effect of more SSRIs drugs such as

sertraline, fluoxetine, citalopram and paroxetine on *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Bacillus* and *Clostridium* strains (Muhammad Ayaz, Subhan, Ahmed, Khan, Ullah, Ullah, et al., 2015b; Kalayci et al., 2015; Munoz-Bellido et al., 2000). In addition, several studies highlighted the antifungal potential of SSRIs fluoxetine, sertraline, and paroxetine against *Aspergillus* spp., *Candida parapsilosis*, and *Candida albicans* (Muhammad Ayaz et al., 2015; Costa Silva et al., 2017b; Gu et al., 2016b). Several SSRIs have been reported to have antimicrobial properties in high concentrations while having antimicrobial enhancer properties in lower concentrations. This synergistic effect is confirmed by decreases in the minimum inhibitory concentrations of antibiotics when combined with antidepressants. For example, sertraline has been shown to affect bacterial transcription and increase the susceptibility of resistant *Escherichia coli* APEC_O2 to tetracycline *in vitro* (L. Li et al., 2017b). Nevertheless, high-dose treatments with sertraline as an adjuvant for the treatment of antibiotic-resistant *E. coli* infections were reported to exacerbate the pathological outcome of infection in chickens (Kromann et al., 2017b). Other studies using animal models provided *in vivo* evidence for the antimicrobial activity of SSRIs (Cussotto et al., 2019; Lukić et al., 2019; Ramsteijn et al., 2020).

Venlafaxine from the therapeutic class of serotonin-norepinephrine reuptake inhibitors (SNRIs) did not show any antibacterial effect on the growth of tested bacterial strains. This finding is in agreement with (Cussotto et al., 2019) who tested *in vitro* against *L. rhamnosus* and *E. coli* at maximal concentrations of 600 µg/mL. Moreover, venlafaxine was found to be inactive when tested against *E. coli* and *P. aeruginosa*; however, this drug augmented the antibacterial effects of antibiotics towards resistant strains (Muhammad Ayaz et al., 2015).

The monoamine-oxidase inhibitor (MAOIs), **Phenelzine**, showed a remarkable antibacterial effect on some strain's representative of the firmicutes phylum (*E. rectale* and *F. prausnitzii*). Little is known about the antimicrobial activity of phenelzine. This may be explained by the more consideration directed to other classes of antidepressants.

Variation in the antibacterial activity of antidepressants between the different therapeutic classes was observed in this study, suggesting potential differences in their mechanisms of inhibitory action. Even these latter are not fully understood, the one proposed mechanism for the action of SSRIs is inhibition of efflux pumps (Munoz-Bellido et al., 2000) and decrease of the activity of DNA gyrase for TCAs antidepressants (Macedo et al., 2017b). The mechanisms underlining the

drug-induced alterations in gut microbiota are only partly known. Indeed, the findings from this work highlight the variability in MIC values of antidepressants towards the strains of different species where some antidepressants found to exhibit a bacteriostatic or bactericidal effect. These differences in microbe's inhibition may facilitate the intestinal abundance changes by selecting some bacteria and promote the overgrowth of others, causing a shift of microbial communities towards dysbiosis or eubiosis.

The antimicrobial activity of antidepressant against gut microbiota could be considered as a side effect, but also possibly as mechanism of antidepressant action in the gut. Indeed, while lanicemine does not exhibit antidepressant effects in treatment-resistant depressed patients, ketamine shows rapid and sustained antidepressant effects, both being NMDAR antagonists (Qu et al., 2017b). Ketamine modulates the fecal microbiome in the susceptible mice after chronic social defeat stress, suggesting an antidepressant mechanism partly mediated by the modulation of gut microbiota (Qu et al., 2017b). Therefore, the antimicrobial effect of antidepressants could be also an important mechanism for alleviating intestinal dysbiosis observed in patients with MDD (Macedo et al., 2017b).

Importantly, we should also take into consideration that the concentrations below the MICs or the sub-inhibitory MICs, even they did not inhibit the growth of the intestinal strains, they delayed their growth in the first hour of incubation triggering the reduction of the growth rate. It was proved in case of antibiotics that the continuous growth in the presence of sub-inhibitory concentrations could select resistant bacteria and promote the evolution of resistance development (Andersson & Hughes, 2014). Moreover, the antidepressants impaired differentially specific microbiota genera that are commonly correlated with human health and dysbiosis. For example, *B. fragilis* member of Bacteroidetes family has been shown to have beneficial roles such as stimulating immune development (Mazmanian & Kasper, 2006). Bifidobacteria are known for their ability to protect the gut, boost the immune system, and control inflammatory responses (O'Callaghan & van Sinderen, 2016).

An important aspect of the current study is to extrapolate the *in vitro* findings to the human gut level in a way to understand the link between antipsychotic-induced microbiota dysbiosis and metabolic dysfunction. In fact, very few observational studies in humans have examined the behaviour of the gut microbiome following antidepressant treatment. The chronic use of the

atypical antipsychotic, risperidone, in children, gradually decreased the Bacteroidetes: Firmicutes ratio, which is associated with a mass body gain (S M Bahr et al., 2015). Additionally, Flowers et al. (Flowers et al., 2017) demonstrated that, in adult subjects with bipolar disorder, the atypical antipsychotic (AAP) class increased significantly *Lachnospiraceae* family abundance and decreased *Akkermansia* genus. This latter species is known to have beneficial anti-inflammatory properties and can protect against gut barrier dysfunction and fat mass development (Plovier et al., 2017). More recently, an increase in fecal microbiota biodiversity, mainly alpha diversity was shown in human patients after six weeks of concomitant therapy using 5–20 mg of escitalopram (Liśkiewicz et al., 2019).

To convert the *in vitro* observations to *in vivo* human gut level, we need to understand whether the antipsychotics medications reach the gastrointestinal tract (GIT) at sufficient concentrations to exert an antimicrobial effect. It is difficult to estimate the real concentrations of orally administered psychotropics in the human GIT since these drugs are affected by many factors like dose, solubility, distribution of fluids volume, transit time and uptake and metabolization by human cells and by bacteria. In this work, we focused on the effective concentrations in the colon (specifically ascendant colon), a part of the GIT that grows the most abundant microbial populations and essential site of fermentation (Donaldson et al., 2016), and the colon concentrations were estimated based on fecal excretion data gathered from previously published works and DrugBank (Wishart et al., 2018), and the maximal daily doses for each antidepressant (please refer to Table S4.4, Appendices). To calculate the approximative colonic concentrations, we assumed the volume of the colon from two different studies. Schiller et al.(2005) reported the mean fluid colon volume after a meal being 18 mL, while Pritchard et al.(2014) reported a volume of 480 mL. As we can observe from Table S4.4 and according to (Schiller et al., 2005), the estimated concentrations are higher than the tested concentrations in this study. For example, if we assumed the minimum daily concentration for desipramine as 25 µg/mL, and the remaining amount in feces as 30%, the approximative concentration in the colon will be 1389 µg/mL, 16-fold higher than the highest concentration tested for desipramine in this work. Despite the difference in colonic volume reported in reference studies (Pritchard et al., 2014; Schiller et al., 2005), the real drug concentration depends on how much of free-water available to dissolve the chemicals and should be somewhere in between, thus requiring more research. Another point to take into consideration is that the proportions excreted in feces given for the different antidepressants do not reflect the

real remaining amount of these drugs, because of the gradual solubilization through the passage from the different sections of the colon. This means that higher exposure will be in the ascendant colon, which represent the microbial-enriched regions (Donaldson et al., 2016). Also, the exposure time and the cumulative effect of drugs in the colon may be a determinant factor to increase the risk of antimicrobial activity, since the antidepressants are taken daily and for an extended period. Indeed, according to (Pratt et al., 2017), 25 % of individuals in the USA have used antidepressants for more than 10 years between 2011 and 2014. In addition, considering the clinical context, where polypharmacy and comorbidities play an important role, combination of several drugs even at low concentration could influence the gut microbiota structure and function (Vich Vila et al., 2020). Beside antibiotics, non-antibiotic drugs can also contribute to antimicrobial resistance (Maier et al., 2018; Vich Vila et al., 2020). This impact on resistome profile seems likely considering the long-term use of antidepressants.

Little is known about the microbial drug metabolising enzymes in the GIT that could influence both compound structure and microbiome profile. Gut microbiota in general can metabolize xenobiotics either directly, mainly through reduction or hydrolysis, or indirectly through affecting host drug metabolism (Spanogiannopoulos et al., 2016). Some of the bacterial strains employed in our study are known to metabolize other drugs. For example, *Lactobacillus*, *Bacteroides*, and *Enterococcus* spp. were capable of metabolizing sulfasalazine via reduction (Peppercorn & Goldman, 1972). However, no evidence is available regarding their effect on the tested antidepressants degradation, but we can assume that the bacterial-xenobiotic crosstalk is bidirectional and can apply to our case of psychotropics.

Finally, multiple studies have reported albeit confusing changes in microbiome abundance in depression. For instance, Mason et al. (Mason et al., 2020) have recently reported a depletion of *C. leptum* and *Bacteroides* in depression and anxiety, but no information is reported about medication. Of note, *A. muciniphila*, *C. leptum*, and *B. fragilis*, important anti-inflammatory microbial groups, were found in our study very affected by tested antidepressants. Therefore, the impact of antidepressant drugs should be included in the equation as confounding factors when investigating microbial biomarkers, knowing that patients with MDD are usually put in long-term antidepressant medication.

5. Conclusion

Our findings indicate clearly the strong antimicrobial effect of antidepressants from different chemical classes against gut commensal bacteria representative of the predominant phyla found in the human gut microbiota. The chronic use of antidepressant drugs presenting antimicrobial effects may be related to the development of adaptive alterations in gut microbiota, with potential deleterious effects. The antimicrobial activity of antidepressant against gut microbiota could be considered as a side effect, but also possibly as mechanism of antidepressant action in the gut. There is an urgent need to clarify the impact and mechanisms of the antimicrobial activity of antidepressants and the subsequent consequences to gut microbiota structure and metabolism. The present study provides new insights into the existing interplay between psychotropic chemicals and microbiota while further investigations are still needed.

Author contributions statement

Y.A., T.A.T. and R.H. conceived and designed the study. Y.A. acquired the data. Y.A. and W.M. analyzed and interpreted the data. Y.A., W.M., T.A.T., and R.H. drafted or revised the article. All authors discussed the results and commented on the manuscript.

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Declaration of competing interest

The authors declare that there is no conflict of interest.

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Chapter 5

Aripiprazole affects gut microbiota while probiotics are able to alleviate related dysbiosis in a simulated human colon

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The results provided in this chapter are in preparation for publication.

Abstract

Growing evidence highlight that therapeutic non-antibiotic compounds strongly affect human health by modulating gut microbiota composition and metabolism. Here, we studied the impact of aripiprazole, an atypical antipsychotic, on the composition of gut microbiota and its metabolic activity, and the role of probiotics to attenuate related dysbiosis using an *ex-vivo* model of the human colon. Following 48h fermentation, aripiprazole altered the diversity of gut microbiota. At the phylum level, aripiprazole distinctly lowered the relative abundances of Firmicutes and Actinobacteria but increased Proteobacteria proportion. *Lachnospiraceae*, *Lactobacillaceae*, and *Erysipelotrichaceae* families were also declined by aripiprazole treatment compared to the control group. In addition, aripiprazole reduced the level of butyrate as measured by GC. A probiotic combination (*Lactobacillus rhamnosus* and *Bifidobacterium longum*) alleviated gut microbiota alteration and increased the butyrate production to a similar level as the control. These findings provide evidence that aripiprazole alters the gut microbiota composition and function, while the probiotic can mitigate related dysbiosis.

Keywords: gut microbiota, aripiprazole, dysbiosis, probiotics, microbiome diversity.

1. Introduction

Considerable evidence suggests that gut microbiota interacts with the brain and plays a key role in the pathogenesis of mental illnesses (Cryan & Dinan, 2012; Szyszkowicz et al., 2017). Multiple studies investigating microbiome biomarkers have reported, albeit confusing, changes in microbiome abundance related to mental diseases. A growing body of research has recently demonstrated that several pharmaceutical non-antibiotic compounds, including psychotropics, drugs used to treat mental illnesses, influence the human gut microbiota and/or microbial isolated strains. Indeed, psychotropics were reported to exhibit a high potential antimicrobial effect, similar to antibiotics, through which they can alter the microbiota composition (Ait Chait et al., 2020; Cussotto et al., 2019; Flowers et al., 2017). For instance, Mason et al. (2020) have recently reported depletion of *C. leptum* and *Bacteroides* in depression and anxiety, but no information was reported about medication. Alterations of gut microbiota in mental disorders may be either directly related to the disease pathogenesis but also to the chronic use of psychotropic medications, which could be undervalued confounding factors (Ait Chait et al., 2020). For example, *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Clostridium leptum*, and *Bacteroidetes fragilis*, important anti-inflammatory microbial groups linked to disease status, were found significantly affected by several classes of psychotropics (Ait Chait et al., 2020). Therefore, the antimicrobial effect of psychotropic drugs should be considered of utmost importance factor, knowing that patients with mental disorders are generally put in long-term medication. While some knowledge accumulates on the antimicrobial impact of some psychotropics on isolated strains or the gut microbiota of animal models, information about other classes of psychotropics and representative species from the human gut is under-investigated.

Ex vivo fermentation models are powerful approaches to investigate the impact of psychotropic drugs on human gut microbiota functionality without host effects in a highly controlled environment (Payne et al., 2012). These models allow the strict control of physiologic parameters, such as retention time, pH, temperature and anaerobiosis, and medium composition used to mimic the diet. Colonic models from simple short-term batch fermentation to multistage long-term continuous flow models were developed (Tanner et al., 2014). Continuous models further control the medium flow rate for culturing microbiota in steady-state conditions, allowing a fiber to develop its full effect along the entire trophic chain, thereby increasing the experiment's physiological relevance. In particular, continuous fermentation systems with immobilized gut

microbiota were shown to simulate the high-cell density, biodiversity, and long-term stability of the intestinal microbiota (Zihler Berner et al., 2013). This prevents washout of less competitive bacteria and ensures the repeated exposure of a single microbiota to different compounds (Payne et al., 2012; Tanner et al., 2014).

Numerous strategies were proposed to restore the balance of a dysbiotic microbiota due to psychotropic medication, reviewed in (Chapter 1; Ait Chait et al., 2020). Of particular interest, many previous studies have supported the role of probiotics (single or multi-strain) to prevent and treat antibiotic-associated diarrhea and antibiotic-related dysbiosis by restoring the balance of intestinal microbiota (Barker et al., 2017; Grazul et al., 2016). Therefore, interventions with probiotics could improve microbiota composition and reduce the adverse effects of psychotropics. This study aimed to investigate the impact of aripiprazole, an atypical antipsychotic drug, on human colonic microbiota diversity and metabolism using *ex vivo* in a simulated human colon. In addition, the growth and capacity of a probiotic mixture to alleviate aripiprazole-related dysbiosis was also investigated.

2. Materials and methods

2.1. Psychotropics and probiotics

One psychotropic was investigated in this study, namely aripiprazole (atypical antipsychotics), purchased from TCI America (Portland, USA). This drug was selected following demonstration of potentially antimicrobial effect within intestinal bacterial pure cultures from our previous work (Ait Chait et al., 2020). Stock solutions of aripiprazole at a concentration of 20 mg/mL were prepared according to the manufacturer's recommendations, filter-sterilized (0.22 µm), and then stored at -20°C until use.

Lactocaseibacillus rhamnosus HA-114 and *Bifidobacterium longum* R0175 were kindly supplied by Lallemand Health Solutions (Montreal, Canada) and used hereafter as a probiotic mixture. The strains were cultured from the lyophilized powder in MRS broth supplemented with 0.1% HCL-cysteine and maintained at -20°C until use. Before each experiment, the bacteria were sub-cultured three times at 37°C to obtain a robustly and uniformly growing culture.

2.2. Feces collection and immobilization

Fecal samples were collected fresh from two healthy adult donors (D1: female; age 36 and D2: male; age 39). The volunteers were not suffering from any known colonic conditions, were not taking pre- or probiotic supplements, and had not received any psychotropics (no psychiatric illness) or antibiotics treatment for at least three months prior to sample collection. The two donors provided their written, informed consent. Sampling of fecal material for inoculation of colonic fermentation has been approved by The University of Ottawa Research Ethics Board (certificate H-02-18-347; 29/07/2019). The feces were processed to slurries by dilution in reduced peptone water (20%, w/v), homogenized, and further immobilized in 1–2 mm gel beads consisting of gellan gum (2.5%, w/v), xanthan (0.25%, w/v), and sodium citrate (0.2%, w/v) under anaerobic conditions as described previously in details (Le Blay et al., 2012). The immobilized microbial community from each donor was used to run one independent fermentation experiment.

2.3. Experimental set-up and fermentation procedure

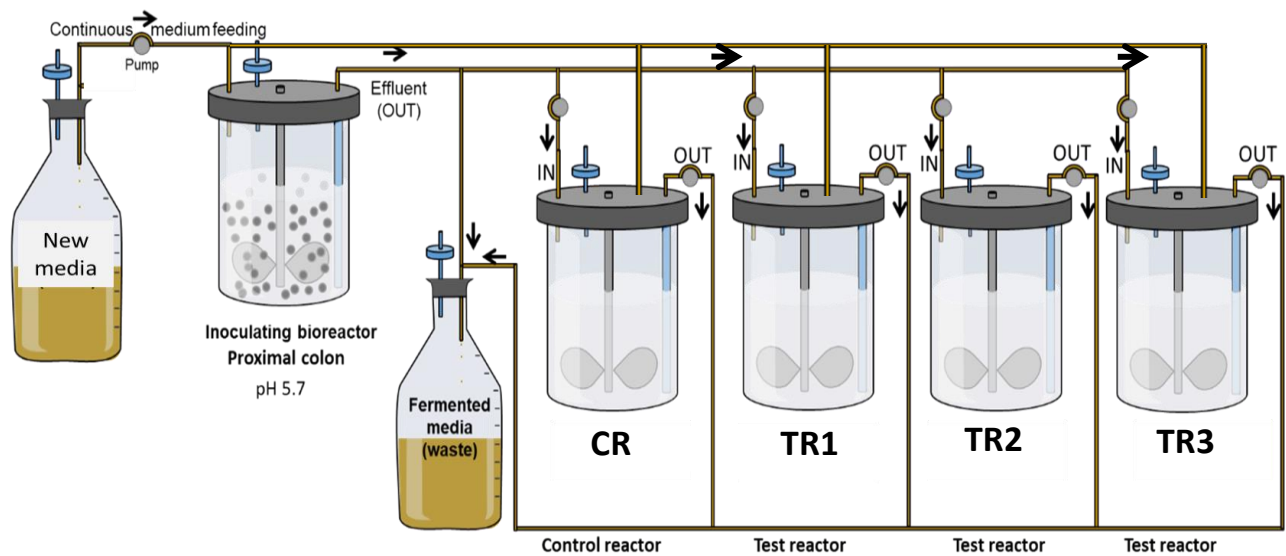
The continuous fermentation was carried out for 42 days using an *ex-vivo* model simulating the large intestine (NuGUT Research Platform, University of Ottawa) as previously described (Le Blay et al., 2012; Le Lay et al., 2015). This model consists of a two-stage design comprising an Inoculation Reactor (IR) with immobilized fecal microbiota used to continuously inoculate four second-stage reactors operated in parallel (Figure 5.1). Each reactor was set up to reproduce the physiological and microbiological conditions of the adult proximal colon (pH 5.7, stirring at 120 rpm, 37°C, and mean retention time of 8 h). Anaerobiosis was ensured through continuous headspace flushing of N₂ and CO₂ at a 0.9:0.1 ratio, and a constant pH of 5.7 was maintained by the addition of 2.5 M NaOH.

The fermentation procedure was initiated by transferring 60 mL of immobilized gel beads into the IR (1L BioFlo® 120 vessel; Eppendorf, Mississauga, ON, Canada) containing 140 mL fresh sterile Macfarlane culture medium prepared as previously described (G.T. Macfarlane et al., 1998). During the first 48 h, the colonic model was run as a batch culture to allow beads colonization, and the nutritive medium was replaced by a fresh medium every 12 h. Following the 48 h, the medium flow was switched to continuous mode for the rest of the experiment. After a stabilization period of 15 days, the microbial community in IR was used to inoculate four-second stage

DASGIP® bioreactors (Eppendorf, Mississauga, ON) : one CR (Control Reactor: non-treatment control) and three Treatment Reactor TR1-3 that were challenged with different parallel treatment periods (Figure 4.1). The working volume of 100 mL was maintained in each sub-bioreactor by inoculation of 5% (v/v) (0.62 mL/h) IR effluent and addition of 95% (v/v) (11.88 mL/h) fresh fermentation medium. Then, the entire second stage bioreactors were run for another 2 days to reach the stability of the microbial community. Once the stabilization is reached in all reactors, the bioreactors were subjected to treatment every 24 h as follow:

- CR bioreactor: served as non-treatment control
- TR1 bioreactor: challenged with aripiprazole at a final concentration of 400 µg/mL, simulating an estimated single daily dose of psychotropics.
- TR2 bioreactor: challenged with the probiotic mixture (*L. rhamnosus* and *B. longum*) added at a final concentration of 10⁹ CFU/mL each.
- TR3 bioreactor: challenged with aripiprazole 400 µg/mL and the probiotic mixture.

Effluent samples (2 mL) were taken from bioreactors at 0, 2, 4, 6, 8, 12, 24, and 48 h of treatment. The collected samples were separated (centrifugation at 14 000 g, 5 min, 4 °C) to pellet used for metagenomic DNA extraction and supernatant used for Short Chain Fatty Acids (SCFA) analysis. Each fermentation experiment was conducted in duplicate for each fecal sample donor.



IR+CR

Constant conditions (Human proximal colon)

TR 1-3

Stab 1 > T1 > W > Stab 2 > T2 > W > Stab 3 > T3 > W > Stab 4 > T4 > W

Figure 5.1. Experimental reactor set-up of the *ex vivo* PolyFermS fermentation model. IR: inoculum reactor, containing immobilized donor feces (30% v/v); CR: control reactor; TR1-TR3: test reactors 1-4; Stab: stabilization period; T: treatment period; W: wash period.

2.4. Microbial community analysis

DNA extraction

Genomic DNA was extracted from the pellet of fecal slurry and fermentation samples using a Fast DNA Spin Kit (MP Biomedicals; Solon, OH, USA) following the manufacturer's instructions. The mechanical lysis was performed in 2 cycles of 40 s each at a speed of 6.0 m/s in a Bead Mill-24 Homogenizer (Fisher Scientific; Ottawa, ON, Canada) with 5 min cooling on ice between the two cycles (Mottawea et al., 2016). The amount of extracted DNA was quantified using the Qubit fluorometer (Invitrogen; Carlsbad, CA, USA) and stored at -20 °C until used for further analysis.

16S rRNA gene sequencing

The microbial profile of fecal slurry and fermentation samples was assessed using tag-encoded 16S rRNA gene Miseq-based (Illumina, CA, USA) high throughput sequencing. The V3-V4 regions of the 16S rRNA gene were amplified using dual-barcoded primers, and the amplicon

library for sequencing was constructed using Illumina standard protocol. The amplicon libraries were pooled in equimolar amounts and paired end sequenced with Illumina MiSeq platform (NuGUT Research Platform, University of Ottawa) using 600 bp MiSeq Reagent Kit v3 (Illumina; San Diego, CA, USA) as per standard protocol.

Sequences were quality filtered, deblur denoised, and clustered into observed features based on 97%-similarity using the Greengenes database (v13.8) via QIIME 2.2020.8 software (Caporaso et al., 2010). Observed features that occurred in less than 10% of the samples were removed. The remaining observed features were rarefied into an equal number of 4,000 reads per sample using QIIME. Alpha diversity was estimated with observed features, Shannon entropy, Pielou_Evenness, and Faith_pd. Beta diversity among samples was calculated using Bray-Curtis distance and visualized using Principal Coordinate Analysis (PCoA). The contribution of different treatments to the diversity of gut microbiota community was assessed from the Bray-Curtis distance matrix using the permutational multivariate analysis of variance (PERMANOVA)-pairwise and 999 permutations (Bolyen et al., 2019). To identify differential taxa among different treatments, linear discriminant effect size analysis was conducted on the relative abundance of different taxa levels (Segata et al., 2011). Samples were labeled with the treatment type as the sample class and the time points as the subclass. Taxa with \log_{10} LDA score ≥ 2 and $p < 0.05$ were considered significant. When required Kruskal-Wallis test was applied for statistical analysis, and P-values were corrected using the two-stage Benjamini, Krieger, and Yekutieli false discovery rate (FDR) procedure.

2.5. Determination of SCFA content

The production of Short-chain fatty acids (SCFA; butyrate, acetate, and propionate) in fermentation samples from all sub-reactors was determined using Gas Chromatography coupled to Flame Ionization Detector (GC-FID) (Shimadzu GC-2030) as previously described (Monk et al., 2015). In brief, supernatants collected from fermentation samples were centrifuged (14000 g, 30 min, 4°C) and filter sterilized (0.22 μm). A mixture of formic acid and 2-ethyl butyric acid was used as an internal standard and added to each sample at a concentration of 0.5 mM. Around 100 μL of each sample was injected in a capillary column Stabilwax-DA (60 m \times 0.25 μm ; Restek) with a run time of 20 min. The peaks were identified and quantified with standards from

MilliporeSigma (Oakville, ON, Canada). Results were expressed as the concentration of SCFAs in mM. All samples were analyzed in duplicates (two technical measures).

2.6. Statistical Analysis

Data from Gas Chromatography analyses were analyzed using GraphPad Prism v8.3. (GraphPad Software, USA) in order to assess the significance of results among treatments at the same time and among different time points within each treatment. Data are expressed as means \pm Standard deviations (SD), and an ANOVA test with Bonferroni as a post hoc test for multiple comparisons was used (P-values < 0.05).

3. Results

3.1. Diversity of the gut microbiota

The observed features and alpha diversity (Shannon entropy, Faith-pd, and Peilou -Evenness) were evaluated for the microbiota from the four bioreactors between 0 and 48 h (Figure 5.2). The diversity of the total bacterial community decreased significantly during the 48 h of treatment with aripiprazole as indicated by observed features and Shannon entropy indices (Figure 5.2-A, B). Aripiprazole treatment significantly reduced bacteria species evenness in all statistical comparisons, indicated by decreases in Peilou-Evenness indices of alpha diversity (Fig. 5.2-D). However, bacterial species richness, indicated by Faith-pd indices, was not affected by aripiprazole administration (Figure 5.2-C). The probiotic addition also increased the diversity of gut microbiota compared to the group treated with aripiprazole alone or in combination with probiotics.

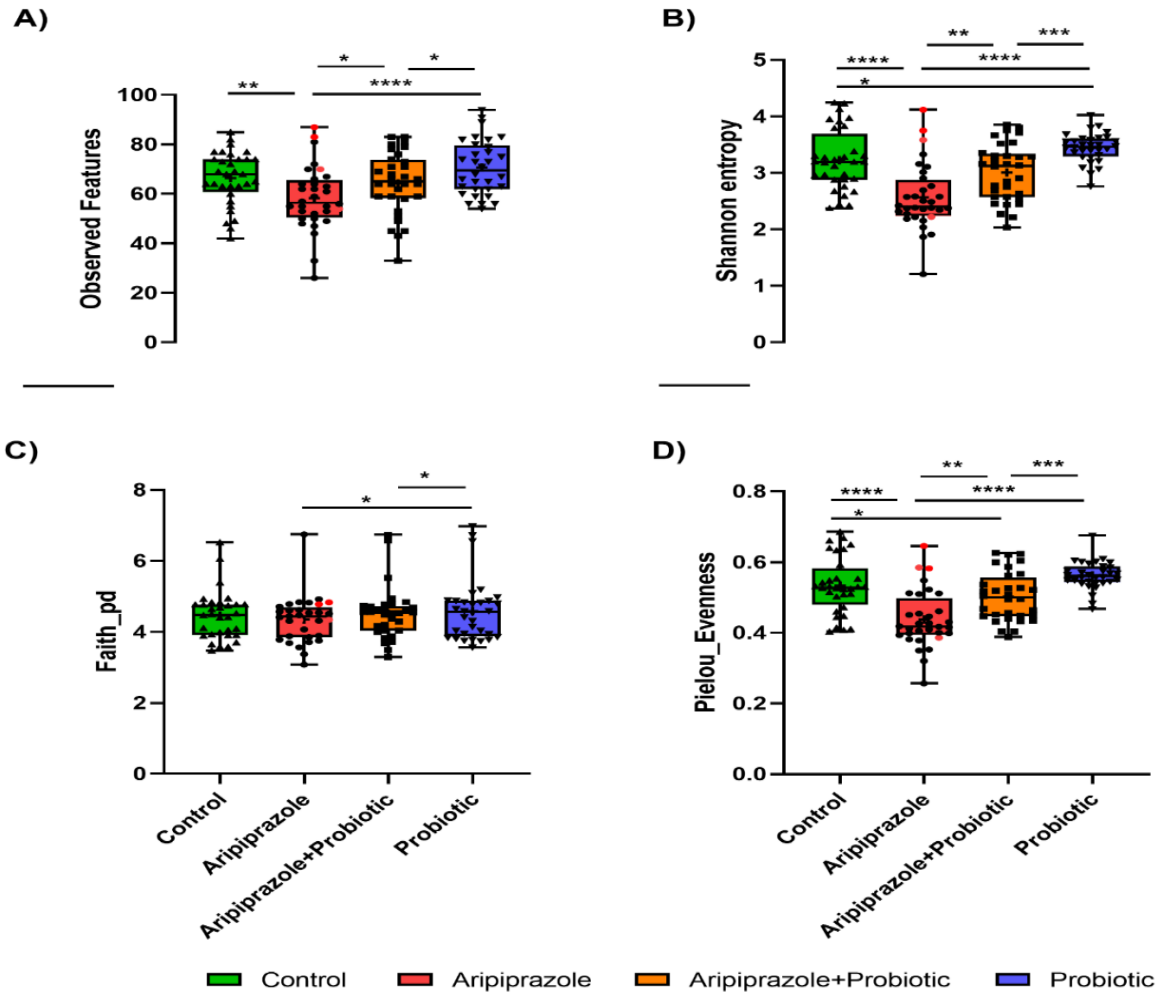


Figure 5.2. Modulation of microbiota composition following treatment with aripiprazole and probiotics. (A) observed futures, (B) Shannon index, (C) phylogenetic measures and (D) evenness of all treated samples (n = four biological replicates each). Results were calculated from rarefied 4000 reads per sample using QIIME2. 2020.6 version. Middle lines represent mean. Data were analyzed using the Kruskal- Wallis test and Two-stage Benjamini, Krieger, and Yekutieli FDR procedure; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. red dots represent the zero time points.

Beta diversity was calculated through principal coordinates analysis (PCoA) based on Bray-Curtis distances (Figure 5.3). Results revealed that the microbiota community from the two donors were highly distinct, forming two separate clusters ($p = 0.001$; Figure 5.3B). In addition, (PCoA) revealed a clear separation between experimental replicates within each donor ($p = 0.001$; Figure 5.3C) and distinct clustering of the aripiprazole and other treatment groups ($p = 0.001$; Figure 5.3A).

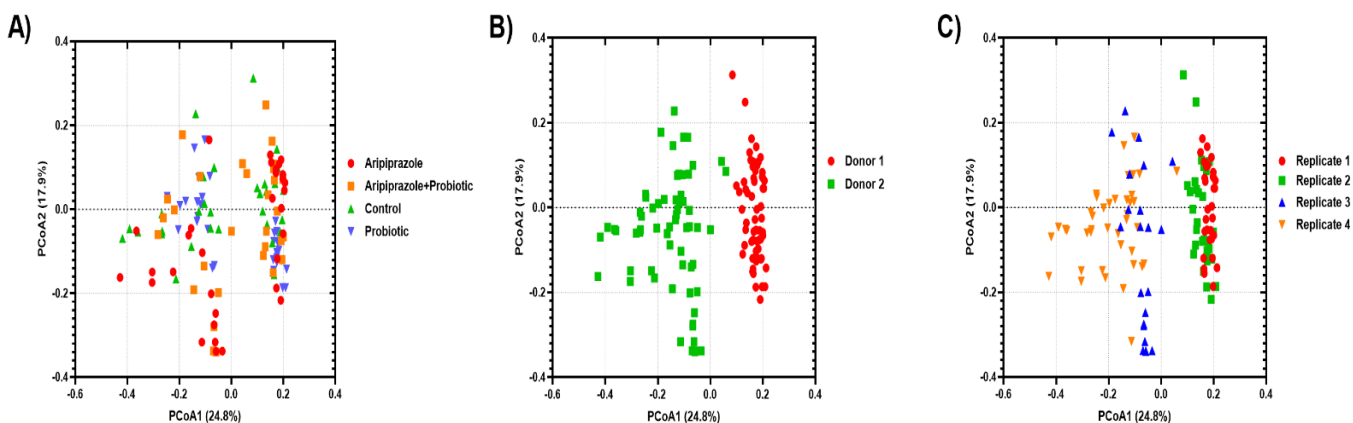


Figure 5.3. Plots of Principal Coordinate Analysis (PCoA) based on Bray-Curtis distances among the identified microbiota in different samples, showing clustering based on the type of treatment, donor, and replicate. The samples were colored, as indicated in legends. PCoA1 and PCoA2 represent the top two coordinates that captured the highest microbial variability among samples, and the percentage shown indicates the fraction of variation represented by each coordinate.

3.2. Effect of aripiprazole alone or in combination with probiotics on gut microbiota composition

The composition of gut microbiota was evaluated according to treatment. The relative abundance kinetics of the intestinal microbiota in the different treatment groups are presented in Figure 5.4. The most identified phyla among the different treatment groups comprised Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria, with Firmicutes and Actinobacteria the dominant phyla at different time points. The aripiprazole treatment significantly decreased the relative abundance of Firmicutes and Actinobacteria ($P < 0.05$), while significantly increased Proteobacteria abundance following 24 h of treatment, compared to control. The simultaneous addition of both probiotic mixture and aripiprazole to bioreactors significantly attenuated the

drug's inhibitory effect by increasing the level of Firmicutes and Actinobacteria while decreasing the abundance of Proteobacteria phylum, compared to Aripiprazole treatment alone.

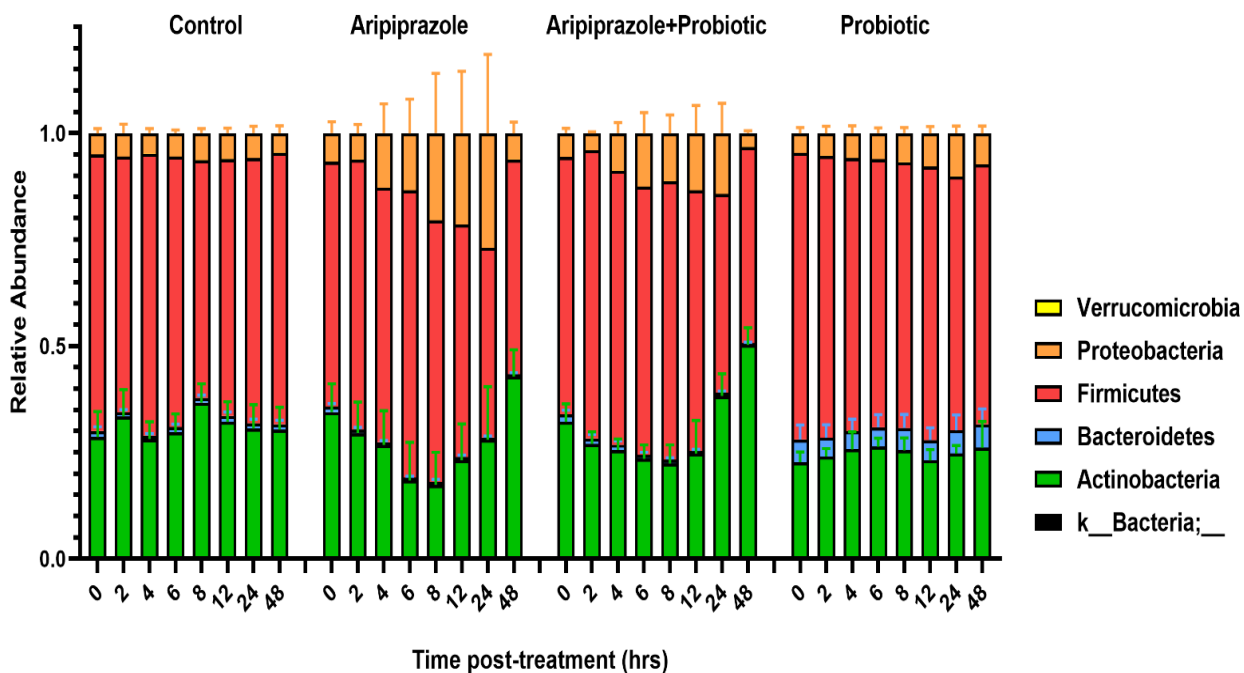


Figure 5.4. Microbial community structure. Bar plot of prevalence at the phylum level.

In keeping with the observations mentioned above, the calculation of the Linear discriminate analysis (LDA) effect size showed that the aripiprazole treatment led to a major shift in microbiota composition, as illustrated in Figure 5.5. At the genus level, *Roseburia* genera was significantly depleted after the administration of aripiprazole compared to control. At the family level, *Lachnospiraceae*, *Lactobacillaceae*, *Erysipelotrichaceae*, and an unclassified *Lactobacillaceae* were four families that showed a decreasing trend following aripiprazole treatment (figure 5.5-A). The microbiota supplemented with the probiotic mixture showed enrichment in *Lactobacillaceae*, *Erysipelotrichaceae* family, and Firmicutes phylum compared to baseline control and aripiprazole treatments (figure 5.5-C, D).

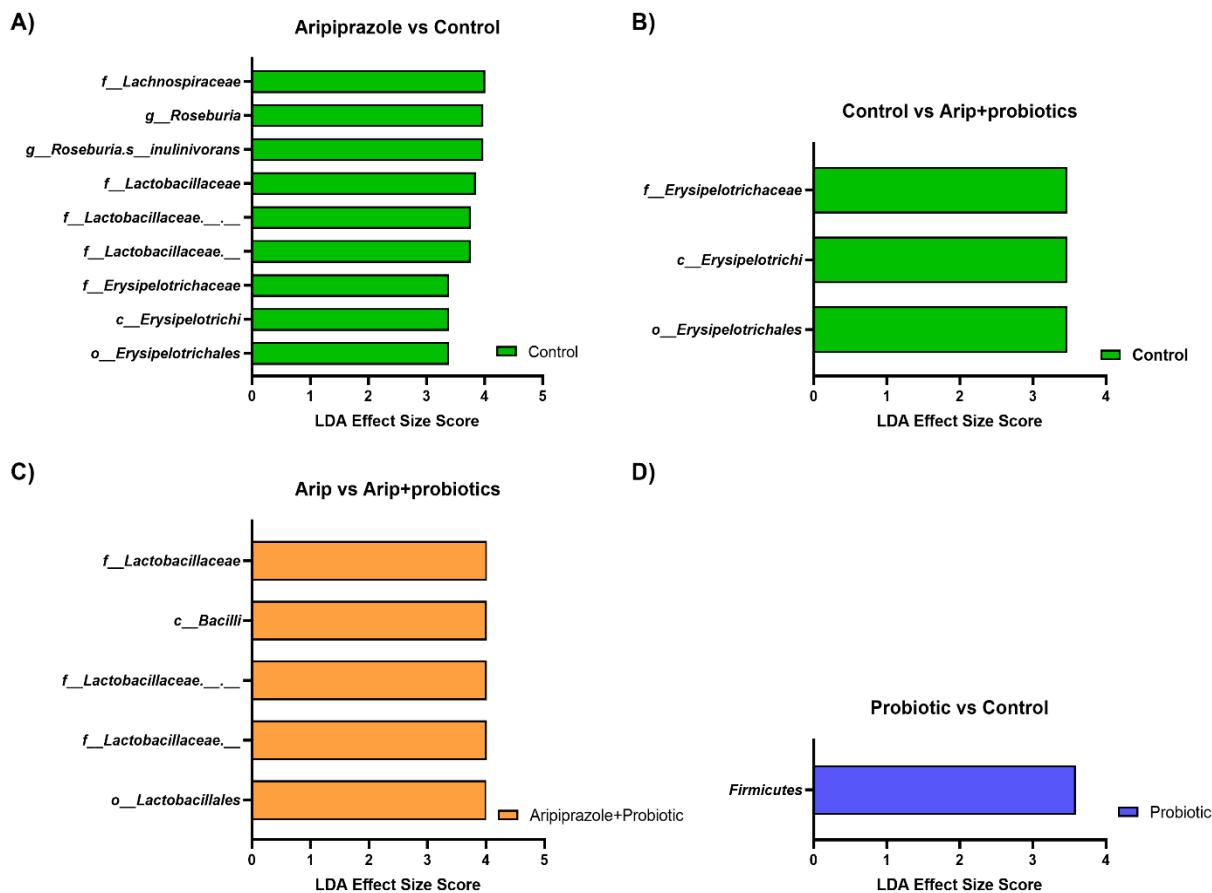


Figure 5.5. Histograms of the linear discriminant analysis (LDA) scores showing microbial taxa that vary significantly in abundance between different treatments.

3.3. Effect of aripiprazole alone or in combination with probiotics on microbiota metabolism

To further understand the effect of aripiprazole, we used the GC method to determine microbiota SCFA changes. The three major SCFA generated by gut microbiota, namely butyrate, acetate, and propionate, were identified and quantified in all treatment groups (please refer to appendices S5.1-4). As shown in figure 5.6, all identified SCFA concentrations were significantly decreased ($p < 0.05$) during exposure to aripiprazole, compared to the control group. When the probiotic mixture was added to microbiota, the concentrations of SCFA were higher than the aripiprazole group and similar to the control group (Figure 5.6).

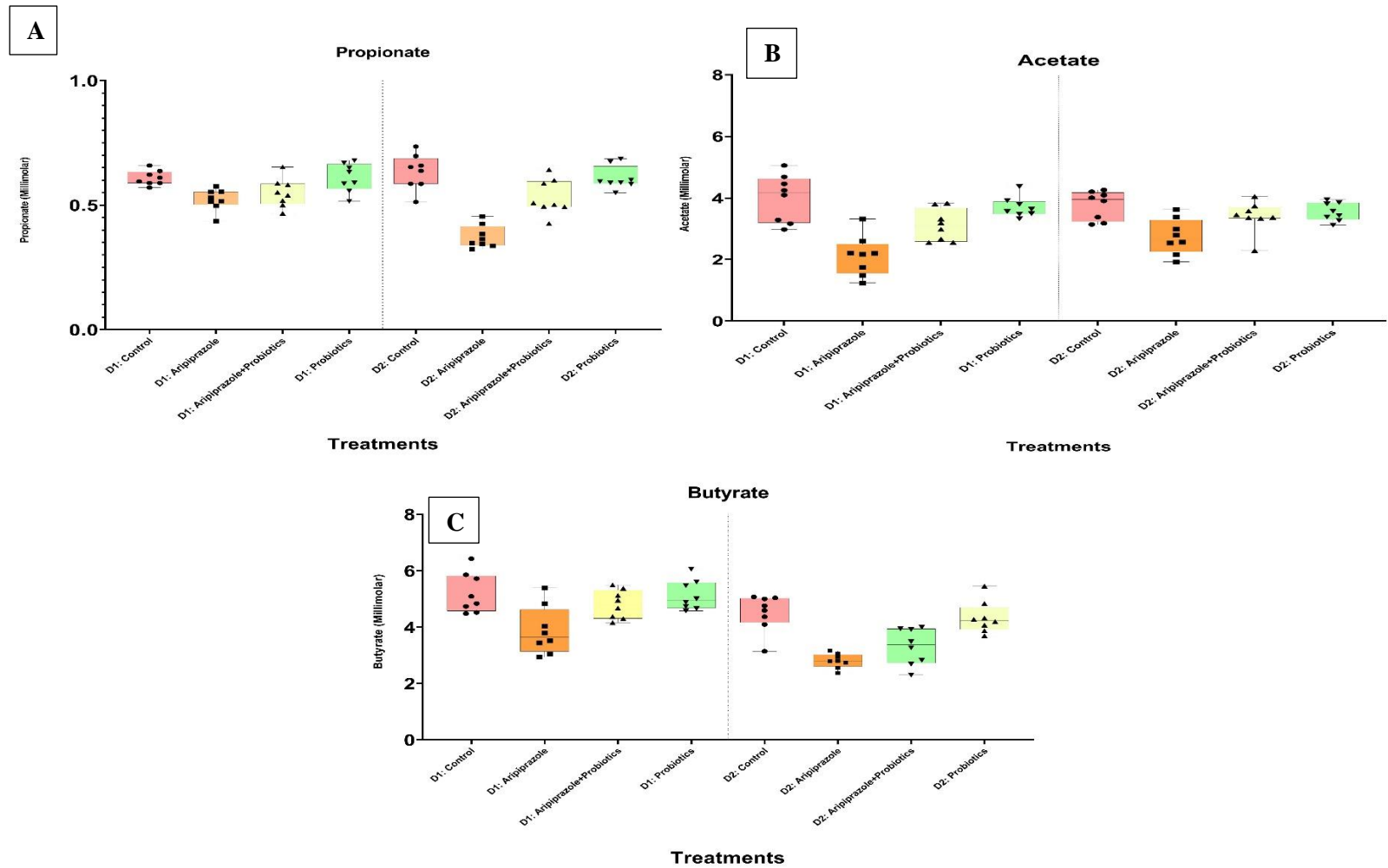


Figure 5.6. Short-chain fatty acids (SCFAs) concentration measured by GC over 48 h with all treatment groups. (A) propionate, (B) acetate, and (C) butyrate. Each time point is represented with 4 biological replicates \times 2 technical measures.

4. Discussion

The present study aimed to investigate the impact of an atypical antipsychotic drug, namely aripiprazole, on human gut microbiota, including its ability to alter the colon microbiota composition and metabolism. An ex-vivo continuous fermentation system, mimicking adult proximal colon conditions, was used to achieve this aim.

Aripiprazole is a quinolinone derivative mostly prescribed for schizophrenia treatment, treatment, and prevention of mania's recurrence and control of agitation and disturbed behavior in schizophrenia. Additionally, in the USA, it has been licensed by the food and drug administration (FDA) in 2002 for the treatment of irritability associated with autistic spectrum disorder (ASD) (Deb et al., 2014).

The findings of the current study confirmed the expectations of our first investigation (Ait Chait et al., 2020) that psychotropic medications can induce major alterations in human gut microbiota leading to dysbiosis due to their highly antimicrobial potential. Significant differences were found among each treatment group in the microbial structure by 16S rDNA sequencing. The analysis revealed that aripiprazole shifted the microbiota composition and decreased its diversity. This is in line with previous *in vivo* and *in vitro* studies that demonstrated the growth inhibition effect of the gut microbiota and the modulation of its structure by aripiprazole and other psychotropics (Cussotto et al., 2019; Flowers et al., 2017; Maier et al., 2018).

At the phylum level, the aripiprazole induced an increase in the abundance of Proteobacteria and a decrease in the prevalence of Firmicutes and Actinobacteria. Members of the Proteobacteria phylum have a low abundance in healthy humans, for which the load is suggested to be a potential diagnostic criterion for dysbiosis and disease (Shin et al., 2015). The increase in these bacteria's abundance causes the gut microbiota to enter a pathogenic state, which is extremely easily infected and destroyed by exogenous pathogenic microbes (Garrett et al., 2010).

The phylum Firmicutes, including *Lachnospiraceae*, *Lactobacillaceae*, *Erysipelotrichaceae* families, and the genera *Roseburia*, were most suppressed by aripiprazole treatment. Previous studies reported that *Lachnospiraceae*, especially *Roseburia* species often associated with a healthy state, are some of the main SCFA producers (La Rosa et al., 2019). In addition, *Roseburia* and *Blautia* species represent the genera most involved in controlling gut inflammatory processes,

atherosclerosis, and maturation of the immune system, demonstrating that the end products of bacterial metabolism (butyrate) mediate these effects (Kasahara et al., 2018). Other data pointed to an inverse correlation (Spearman's rank correlation analysis; $p < 0.05$) between different taxa of *Lachnospiraceae* and major depressive disorder (MDD) (Zheng et al., 2016). Similarly, other studies investigating altered microbiota composition in patients with MDD have reported that the relative proportion of *Erysipelotrichaceae* was significantly lower in the MDD than in the healthy control group (Jiang et al., 2015b). Some studies have reported an association between enrichment of *Erysipelotrichaceae* and disease phenotypes such as Parkinson's disease, obesity, and inflammation (Lai et al., 2018).

The addition of the probiotic mixture composed of *L. rhamnosus* HA-114 and *B. longum* R0175 simultaneously with aripiprazole had a protective effect on gut microbiota and tended to protect diversity level close to the control group. Previous studies reported that probiotics could alleviate gut microbiota impairment due to antibiotic treatments and help the host develop homeostasis after perturbation (Ducatelle et al., 2015). Other studies have reported the promising positive effects of probiotics on gut microbiota (Berni Canani et al., 2016). The similarities between several psychotropics and antibiotics (chemical structure, mechanisms of action, etc.) suggest a positive role for probiotics to treat drug-induced microbiota dysbiosis. This study provided the first evidence on the potential of probiotics to alleviate psychotropic-related microbiota dysbiosis.

Microbial SCFAs have been shown to contribute significantly to host health within the gut and in the periphery (Morrison & Preston, 2016). Here, we found a decrease in the concentrations of the three identified SCFAs, namely butyrate, acetate, and propionate, following the aripiprazole treatment, probably due to the depletion of SCFA-producing bacteria. However, probiotic mixture supplementation markedly improved the level of total SCFAs. Butyrate represents the main energy source for colonocytes, where it induces numerous metabolic and immunological functions (Smith et al., 2013). The depletion of major butyrate producers, including *Lactobacillus*, is a common characteristic of IBD microbiota (Gevers et al., 2014). Schulthess et al. have recently shown that preconditioning macrophages with butyrate induced their antimicrobial effects, restricting intestinal bacterial growth and increasing resistance to entero-pathogens (Schulthess et al., 2019). In addition, the microbiota of young adults with depression were depleted in major butyrate producers and pathways of SCFA generation (Liu et al., 2020).

The antimicrobial activity of psychotropics against gut microbiota could be considered a side effect and possibly a mechanism of antidepressant action in the gut. Indeed, while lanicemine does not exhibit antidepressant effects in treatment-resistant depressed patients, ketamine shows rapid and sustained antidepressant effects, both being NMDAR antagonists (Qu et al., 2017b). Ketamine modulates the fecal microbiome in the susceptible mice after chronic social defeat stress, suggesting an antidepressant mechanism partly mediated by the modulation of gut microbiota (Qu et al., 2017b). Therefore, the antimicrobial effect of psychotropic drugs could also be an important mechanism for alleviating intestinal dysbiosis observed in patients with MDD (Macedo et al., 2017b). However, results from this study demonstrated potential dysbiosis inducing properties for psychotropic drugs with a considerable deleterious shift in microbiome and metabolism. The chronic use of psychotropic drugs presenting antimicrobial effects may be related to the development of adaptive alterations in gut microbiota, with potentially deleterious effects.

Moreover, aripiprazole impaired differentially specific microbiota genera that are commonly correlated with human health and dysbiosis. The impact of psychotropic drugs should be put in the equation as confounding factors when investigating microbiome biomarkers in mental diseases, knowing that patients are usually put in long-term antidepressant medication. There is an urgent need to clarify the impact and mechanisms of the antimicrobial activity of psychotropics and the subsequent consequences to gut microbiota structure and metabolism.

5. Conclusion

In summary, aripiprazole induced significant alterations in human gut microbiota composition and metabolic profile, eventually leading to gut dysbiosis. Many of the observed compositional and metabolic changes for aripiprazole were in accordance with previous *in vitro* and *in vivo* data on the inhibitory effect of psychotropics, thus confirming the suitability of the *ex vivo* simulated human colon for the study of their impact on gut microbiota structure and function. The probiotic mixture, namely *L. rhamnosus* HA-114 and *B. longum* R0175, had a protective effect and can alleviate gut microbiota disturbances induced by aripiprazole by mitigating gut dysbiosis, which suggests that probiotics are conducive to maintaining gut homeostasis. These findings provide fresh insight into the adverse effects of psychotropic medications and the potential of probiotics in modulating related gut dysbiosis.

Acknowledgments

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Chapter 6

Discussion and conclusion

This research project investigated the impact of the most commonly used psychotropic drugs on human gut microbiota using *in vitro* and *ex vivo* methods. Additionally, the potential of commercial probiotics to attenuate the psychotropic-related microbiota dysbiosis was studied. Several studies on mental illnesses patients have recently demonstrated the bidirectional interactions between the gut and the brain in three major systems, neuroimmune, neuroendocrine, and sensory neural pathways. These studies also revealed significant changes in the relative abundance of the most dominant phyla of microbiota, leading to a dysbiotic state. However, such studies investigating microbiome biomarkers usually do not take into consideration the psychotropic medications as a potential confounding factor due to their potential antimicrobial effect.

The chapter 3 described our first step to determine the *in vitro* antimicrobial effect of commonly used psychotropics on gut microbiota. Limited are the studies describing the effect of psychotropics on gut microbiota. Indeed, the first investigation in this field used some psychotropics such as chlorpromazine as an alternative to antibiotics to fight the resistant bacteria. Most of the tested bacteria were pathogenic strains such as *Staphylococcus*, *Salmonella*, etc. More recent studies used a murine model to explore the impact of psychotropics on the gut microbiota. However, the use of isolated pathogenic bacteria or murine microbiota does not represent the human microbiota profile and its function. This study's novelty consists of testing the antimicrobial activity of several psychotropics on intestinal bacterial strains representing the most common phyla residing in the human gut. Here, we found that psychotropics showed a highly antimicrobial potential against the tested bacteria. Each psychotropic drug showed a specific antimicrobial activity profile toward the intestinal bacteria, being bactericidal in some cases. These results are important as they inform us how the antimicrobial effect of psychotropics can lead to depletion of some phyla and the overgrowth of others, shifting the microbiota from homeostasis to a dysbiotic state. In addition, we also tested probiotic bacteria to evaluate their survival under exposure to psychotropics. Results revealed an interesting resistance of probiotics to different tested

concentrations. This first step guided us in selecting psychotropics and probiotics in the second part of this work.

The goal of chapter 4 was to study the effect of psychotropics on gut microbiota using an *ex vivo* continuous fermentation model mimicking the adult proximal colon. The chosen psychotropic was aripiprazole for its significant antimicrobial effect against the intestinal strains, whereas *L. rhamnosus* and *B. Longum* were selected as probiotics and tested in combination for their compatibility with aripiprazole. We succeeded in developing two distinct gut microbiota communities using two healthy donors' feces immobilized in small beads. To our knowledge, this the first study using the *ex vivo* simulated colon to investigate the impact of psychotropics on human gut microbiota. The results revealed important microbiota composition changes following the treatment with 400 µg/mL aripiprazole, resulting in a shift from a healthy status dominated mainly by Firmicutes and Actinobacteria to a pathogenic profile dominated by Proteobacteria. In terms of metabolism, the concentration of the short-chain fatty acids was drastically reduced. However, we demonstrated that the probiotics' addition exerted a protective effect and attenuated the dysbiosis triggered by aripiprazole. Therefore, probiotics represent a potent nutritional strategy that could prevent microbiota dysbiosis and altered metabolism when administered concomitantly with psychotropics. The chronic use of antidepressant drugs presenting antimicrobial effects may be related to the development of adaptive alterations in gut microbiota, with potentially deleterious effects. The antimicrobial activity exerted by psychotropics in our study on microbiome suggests that gut dysbiosis observed in patients with mental illnesses could be a side effect to the medication, but also possibly as a mechanism of antidepressant action in the gut. The present study provides new insights into the existing interplay between psychotropic chemicals and microbiota, while further investigations are still needed.

Although we should be careful about generalizing our findings to the overall interplay of psychobiotics with human gut microbiota, this study illustrates the inhibitory effects of such drugs on human gut microbiota leading to a shift in microbiota composition and a dysfunctional metabolism. Additionally, our findings do offer proof of principle that probiotic supplementation potentially represents an effective strategy for tackling the side effects induced by psychotropic medication. Future comprehensive metabolomic and longer-term studies may give more in-depth insights into the bidirectional role of psychotropics on the gut microbiota structure and

metabolism. However, our results already show that short-term administration of psychotropics affects gut microbiota, while probiotics hold the potential to alleviate related dysbiosis.

For future works, the next direction to take in this area of research would be to:

- Investigate the dosage of psychotropics in the colon.
- Study the interplay dynamics between psychotropics and gut microbiota (i.e., metabolization of psychotropics).
- Study the combined effects of multiple psychotropics on gut microbiota for a long time, thus reflecting more real-life situations.
- Study the effectiveness of probiotics on patients with mental illnesses
- Combine the results of this study with clinical trials with a large cohort of patients with sufficient individual and regional variability to elucidate the effects of psychotropics on microbiota structure and the health implications of their interactions.

Appendices

Appendices for Chapter 4:

Table S4.1. Selected intestinal bacterial strains for the investigation of antibacterial activity.

Bacterial intestinal strains	Culture medium	Culture conditions
<i>Lactobacillus reuteri</i> ATCC 23272	de Man Rogosa et Sharp (MRS)	Aerobic, 37°C
<i>Lactobacillus casei</i> ATCC 393	MRS	Aerobic, 37°C
<i>Lactobacillus rhamnosus</i> ATCC 53103	MRS	Aerobic, 37°C
<i>Bifidobacterium animalis subsp. animalis</i> ATCC 25527	MRS	Anaerobic, 37°C
<i>Enterococcus faecium</i> ATCC 35667	MRS	Aerobic, 37°C
<i>Pseudomonas aeruginosa</i> ATCC 27853	Brain Heart Infusion (BHI)	Aerobic, 37°C
<i>Escherichia coli</i> ATCC 25922	BHI	Aerobic 37°C
<i>Akkermansia muciniphila</i> ATCC BAA-835	BHI + 0.5% mucin	Anaerobic, 37°C
<i>Eubacterium rectale</i> ATCC 33656	Fastidious Anaerobe Broth (FAB) + 0.5% yeast extract	Anaerobic, 37°C
<i>Faecalibacterium prausnitzii</i> ATCC 27768	FAB + 0.5% yeast extract	Anaerobic, 37°C
<i>Bacteroides fragilis</i> ATCC 25285	FAB + 0.5% yeast extract	Anaerobic, 37°C
<i>Clostridium leptum</i> ATCC 29065	FAB + 0.5% yeast extract	Anaerobic, 37°C

Table S4.2. Solvents used for psychotropics' stock solutions

Psychotropics	Solvent
Phenelzine	Water
Venlafaxine	Dimethylsulfoxide
(S)-Citalopram	Methanol
Desipramine	Water
Bupropion	Methanol
Aripiprazole	Acetic acid

Table S4.3. Inhibition zones diameters (mm) of some psychotropics against bacterial strains

Psychotropic Strain	Desipramine			Aripiprazole			Phenelzine			(S)-Citalopram			
	Concentration (mg/ml)	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5
<i>L. rhamnosus</i> ATCC 53103		13 ± 0.22	10 ± 0.12	NZ	24 ± 0.10	9 ± 0.11	NZ	13 ± 0.12	NZ	NZ	9 ± 0.10	NZ	NZ
<i>B. animalis</i> ATCC 25527		25 ± 0.12	18 ± 0.20	NZ	19 ± 0.11	9 ± 0.11	NZ	12 ± 0.22	NZ	NZ	NZ	NZ	NZ
<i>E. faecium</i> ATCC 35667		14 ± 0.21	11 ± 0.21	9 ± 0.10	17 ± 0.12	12 ± 0.21	9 ± 0.11	22 ± 0.21	17 ± 0.12	9 ± 0.14	14 ± 0.11	10 ± 0.12	NZ
<i>E. rectale</i> ATCC 33656		21 ± 0.13	16 ± 0.11	10 ± 0.12	18 ± 0.12	13 ± 0.11	11 ± 0.21	12 ± 0.11	14 ± 0.10	9 ± 0.12	11 ± 0.12	NZ	NZ
<i>F. prausnitzii</i> ATCC 27768		22 ± 0.21	18 ± 0.11	15 ± 0.21	21 ± 0.10	17 ± 0.22	14 ± 0.11	20 ± 0.14	16 ± 0.11	13 ± 0.11	13 ± 0.11	9 ± 0.11	NZ
<i>B. fragilis</i> ATCC 25285		20 ± 0.22	17 ± 0.20	14 ± 0.11	19 ± 0.11	15 ± 0.12	12 ± 0.12	13 ± 0.21	9 ± 0.10	NZ	12 ± 0.21	9 ± 0.13	NZ
<i>E. coli</i> ATCC 25922		21 ± 0.12	18 ± 0.12	16 ± 0.12	15 ± 0.11	12 ± 0.12	9 ± 0.11	19 ± 0.20	14 ± 0.22	10 ± 0.22	16 ± 0.12	11 ± 0.21	9 ± 0.10
<i>C. leptum</i> ATCC 29065		35 ± 0.10	29 ± 0.11	22 ± 0.21	31 ± 0.12	22 ± 0.22	19 ± 0.21	17 ± 0.11	14 ± 0.21	9 ± 0.23	20 ± 0.11	15 ± 0.22	11 ± 0.11
<i>A. muciniphila</i> ATCC BAA-835		32 ± 0.20	27 ± 0.12	20 ± 0.23	30 ± 0.13	22 ± 0.21	18 ± 0.22	21 ± 0.12	13 ± 0.12	10 ± 0.11	18 ± 0.13	14 ± 0.14	9 ± 0.13

NZ: no zone detected

Table S4.4. Approximate concentrations of antidepressants reaching the human colon.

Drugs	Preclinical /clinical (mg / day) (min – max)	% reaching the colon ¹	Concentrations reaching the colon (mg/ml)		Tested concentrations (µg/ml)
			Colon volume 1* (min-max)	Colon volume 2** (min – max)	
Phenelzine	45 – 75	21	727 - 1212	20-33	75-800
Venlafaxine	75 – 225	13	750 - 2250	20-61	75-800
(S)- Citalopram	20 – 60	10	154 - 462	16-188	75-800
Desipramine	25 – 300	30	577 - 6923	21-94	75-800
Bupropion	100 - 450	10	769 - 3462	11-34	75-800
Aripiprazole	10 – 30	55	423 - 1269	4-13	75-800

¹source: <https://www.drugbank.ca>

*: Colon fluid volume = 18 mL, according to (Schiller et al., 2005)

** : Colon volume = 480 mL, according to (Pritchard et al., 2014)

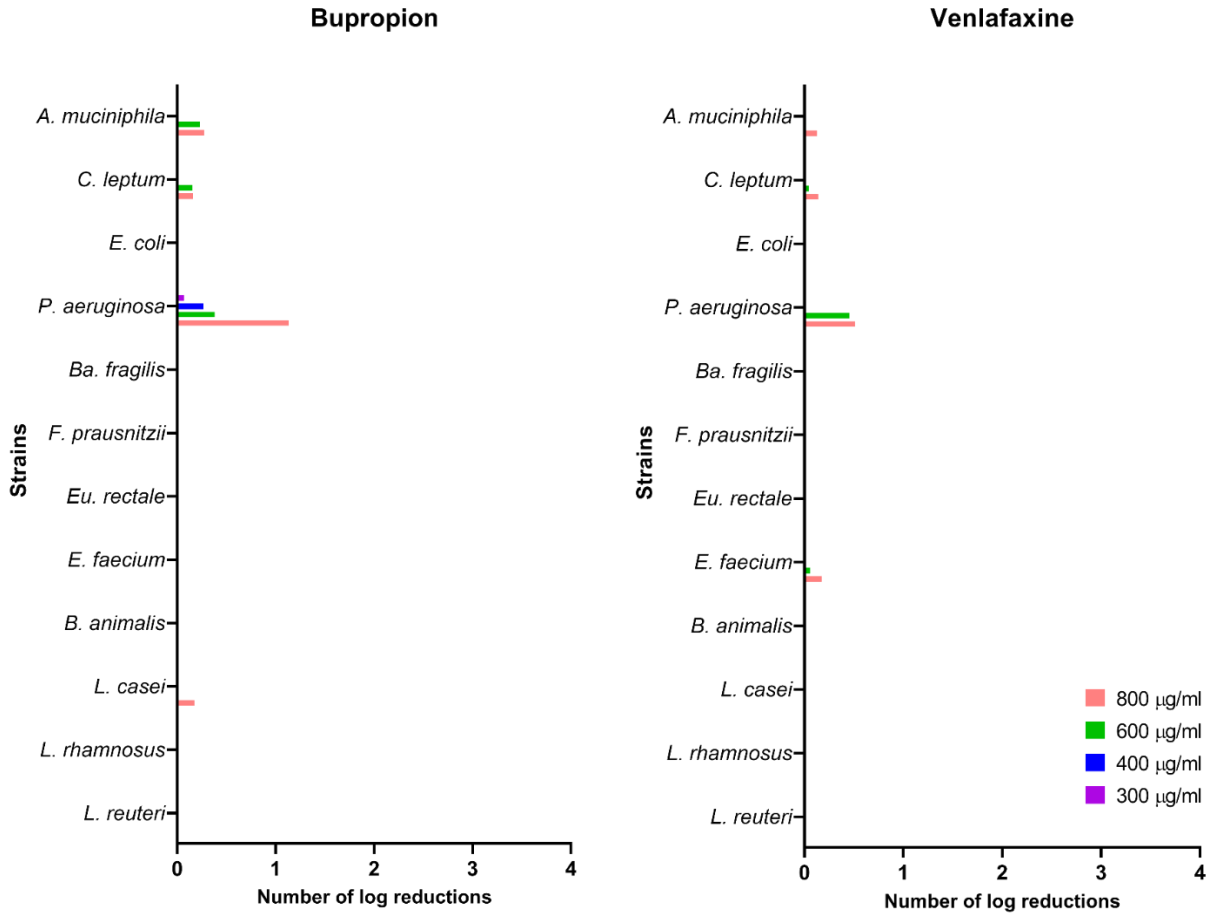


Figure S4.1. Logarithmic reductions of the growth of the commensal strains in the presence of bupropion and venlafaxine.

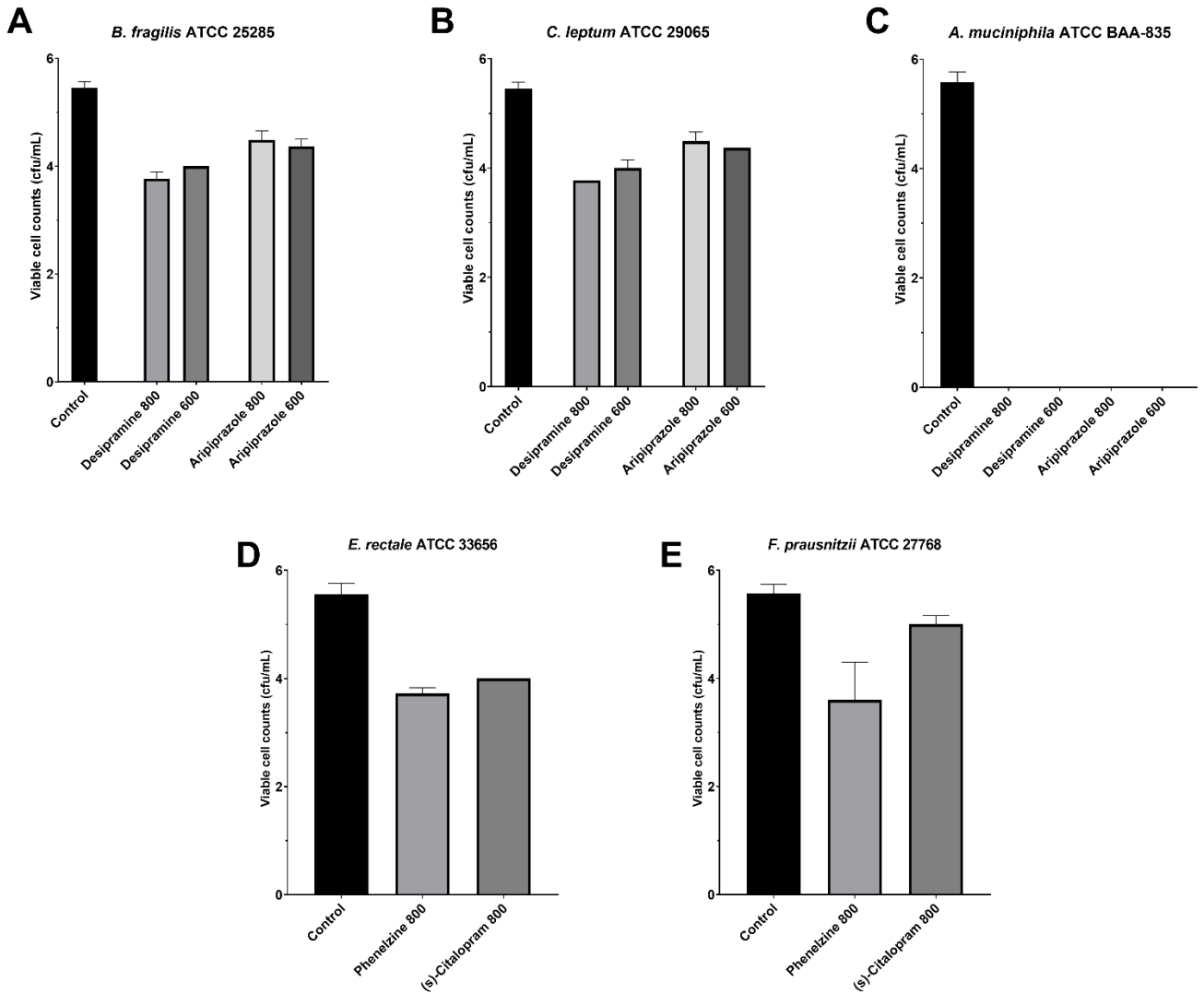


Figure S4.2. Bactericidal effect of desipramine, aripiprazole, phenelzine, and (s)-citalopram incubated for 16h at 600 and 800 $\mu\text{g}/\text{mL}$ against tested bacterial strains. All treatments were statistically significant compared to control, which corresponds to initial inoculum cell counts (CFU/mL).

Appendices for Chapter 5:

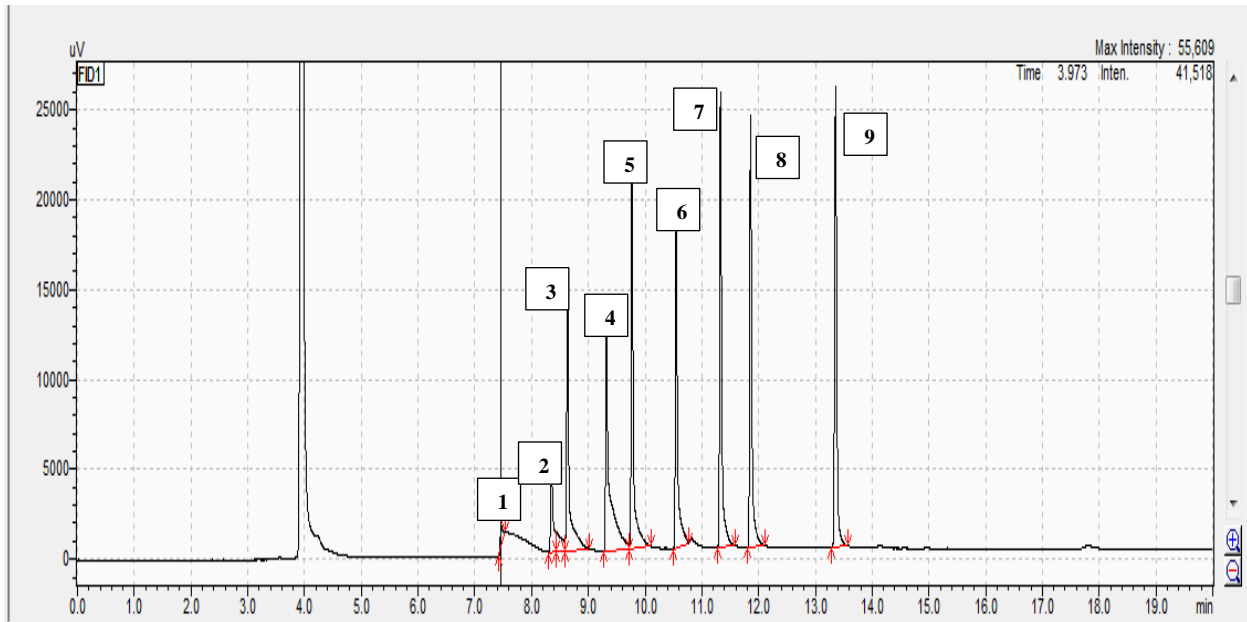


Figure S5.1: Gas Chromatography chromatograms of standards samples. 1: acetic acid, 2: propionic acid, 3: isobutyric acid, 4: butyric acid, 5: isovaleric acid, 6: valeric acid, 7: isocaproic acid, 8: hexanoic acid, 9: n-heptanoic acid.

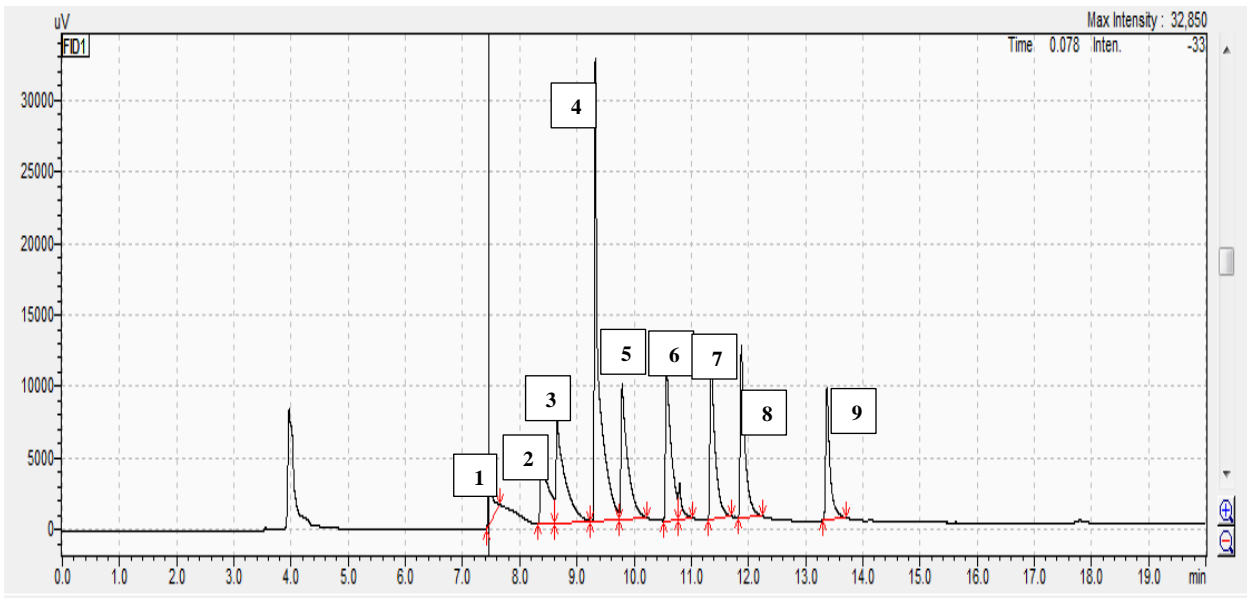


Figure S5.2: Gas Chromatography chromatograms of bioreactor control sample at time 0 before any treatment with aripiprazole or probiotics. 1: acetic acid, 2: propionic acid, 3: isobutyric acid, 4: butyric acid, 5: isovaleric acid, 6: valeric acid, 7: isocaproic acid, 8: hexanoic acid, 9: n-heptanoic acid.

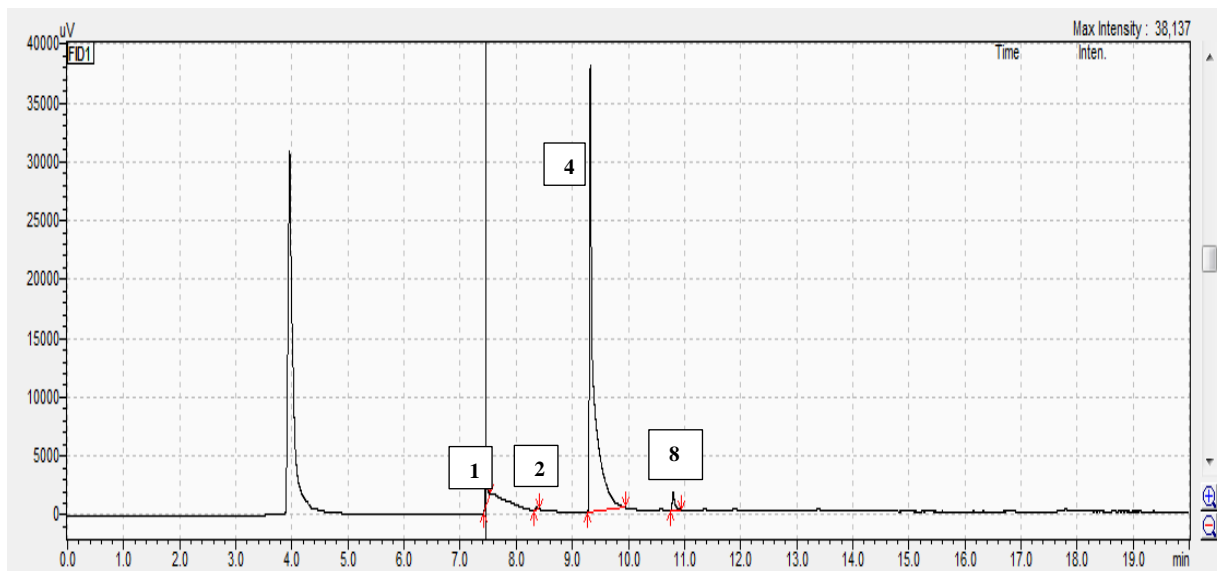


Figure S5.3: Gas Chromatography chromatograms of bioreactor sample treated with aripiprazole at time 6.1: acetic acid, 2: propionic acid, 4: butyric acid, 8: hexanoic acid.

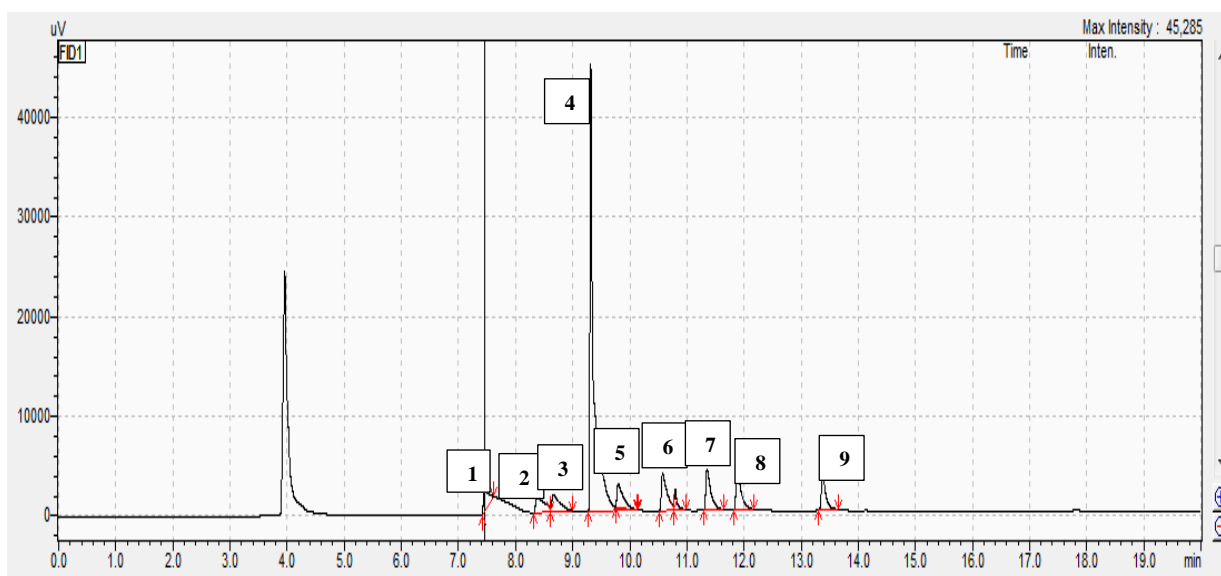


Figure S5.4: Gas Chromatography chromatograms of bioreactor sample treated with both aripiprazole and probiotics at time 6.1: acetic acid, 2: propionic acid, 3: isobutyric acid, 4: butyric acid, 5: isovaleric acid, 6: valeric acid, 7: isocaproic acid, 8: hexanoic acid, 9: n-heptanoic acid.

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