**Lactobacillus plantarum** PS128 ameliorates 2,5-Dimethoxy-4-iodoamphetamine-induced tic-like behaviors via its influences on the microbiota–gut-brain-axis

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Abstract

We previously reported a novel psychobiotic strain of *Lactobacillus plantarum* PS128 (PS128) which could ameliorate anxiety-like & depression-like behaviors and modulate cerebral dopamine (DA) and serotonin (5-HT) in mice. Here, we examine the possibility of using PS128 administration to improve tic-like behaviors by using a 5-HT2A and 5-HT2C receptor agonist 2,5-Dimethoxy-4-iodoamphetamine (DOI). PS128 was orally administered to male Wistar rat for 2 weeks before two daily DOI injections. We recorded the behaviors immediately after the second DOI injection and compared the results with control and haloperidol treatment groups. PS128 significantly reduced tic-like behaviors and pre-pulse inhibition deficit in a threshold-dose of 10⁹ CFU per day. Brain tissue analysis showed that DOI induced abnormal DA efflux in the striatum and prefrontal cortex, while PS128 ingestion improved DA metabolism and increased norepinephrine (NE) levels in these two regions. In addition, PS128 ingestion increased DA transporter and β-arrestin expressions and decreased DOI-induced phosphorylation of DA and cAMP regulated phosphoprotein of molecular weight 32 kDa (DARPP-32) at Thr34 and extracellular regulated protein kinases (ERK). PS128 ingestion also modulated peripheral 5-HT levels and shaped the cecal microbiota composition, which helps to alleviate DOI-induced dysbiosis. These results suggested that PS128 ameliorated DOI-induced tic-like hyper-active behaviors via stabilizing cerebral dopaminergic pathways through its modulation of host’s microbiota–gut–brain axis. Thus, we believe there are potentials for utilizing psychobiotics to improve syndromes caused by DA dysregulation in DA-related neurological disorders and movement disorders such as Tourette syndrome.

1. Introduction

Over the past decade, a multitude of studies had revealed that gut microbiota communicates with the central nervous system (CNS) through neural, immune, and endocrine pathways, and theses bidirectional communication pathways constitute the microbiota–gut–brain–axis (MGBA) which is vital for both physical and mental health (Collins et al., 2012; Cryan and Dinan, 2012; van de Wouw et al., 2017).
Probiotics, live microorganisms that confer health benefits on the host, have wide application in fermented food products and health supplements. Numerous studies have exploited the beneficial effects of probiotics which include improvements in gastrointestinal (GI) tract disorders (McKernan et al., 2010; Liu et al., 2011), metabolic disorders (Huang et al., 2013), defeation impediments (Riezzo et al., 2018), and modulating immune systems (Mei et al., 2013; Liu et al., 2014). Probiotics could also benefit the homeostasis of MGBA of the host, which could improve mental stabilities (Kim et al., 2018). For example, *Bifidobacterium infantis* 35624 had been shown to modulate cerebral monoamines metabolism, alter peripheral interleukin-6 (IL-6) levels, and alleviate depression-like behaviors in the maternal separation (MS) model of rat (Desbonnet et al., 2010). A recent study showed that both live and heat-killed *Lactobacillus paracasei* PS23 could reverse corticosterone-induced monoamines and neurotropic proteins reduction in hippocampus, and alleviate anxiety- and depression-like behaviors in mice (Wei et al., 2019). Furthermore, *Lactobacillus rhamnosus* GG had been shown to reduce obsessive-compulsive disorder (OCD)-like behavior in a 5-HT1A/1B Receptor agonist RU-24969 treated mice model and the effects were comparable to selective serotonin reuptake inhibitor (SSRI) fluoxetine (Kantak et al., 2014). In view of their effects on modulating behaviors, neurotransmitters and neurotropic factors, these probiotics strains are now named “psychobiotics” (Diman et al., 2013). Many believed that psychobiotics have great potential to improve psychiatric symptoms in patients with mental disorders (Zhou and Foster, 2015; Foster et al., 2016; Bermudez-Humaran et al., 2019).

We previously reported that chronic administration of *Lactobacillus plantarum* PS128 (PS128), a psychobiotic isolated from Fu-tsai (Chao et al., 2009; Liu et al., 2015), could ameliorate anxiety-like behaviors in naive mice and germ-free mice (Liu et al., 2016a, 2016c). In addition, in an early-life stress mice model oral administration of PS128 could improve depression-like behaviors, modulate dopamine (DA) and serotonin (5-HT) levels in prefrontal cortex (PFC) and striatum, and change serum levels of corticosterone and IL-6 (Liu et al., 2016c). Furthermore, in a recent clinical study on autism spectrum disorder (ASD) patients aged 7–15 in Taiwan, PS128 significantly ameliorated anxiety, hyperactivity/impulsivity and opposition/defiance behaviors and improved the total score of the Swanson, Nolan, and Pelham-IV-Taiwan version (SNAP-IV) psychometric scale in younger population (aged 7–12) compared with the placebo group (Liu et al., 2019). In another study of triathlon athletes, PS128 supplementation alleviated intensive exercise-induced oxidative stress and inflammatory state. Moreover, PS128 supplementation substantially increased the plasma branched-chain amino acids levels and improved exercise performance of these athletes (Huang et al., 2019). These mice and human studies of PS128 reveal not only its novel effects on physical and mental health but also its great potential for improving neurological disorders involving DA and 5-HT neurotransmissions. Hence, it warrants the further exploration of the potential of PS128 administration on relieving neurological disorders associated with the imbalance of dopaminergic or serotonergic neurotransmission such as Tourette syndrome (TS), attention deficit hyperactivity disorder (ADHD), obsessive compulsive disorders (OCD) and schizophrenia. Therefore, here we used a 5-HT7A and 5-HT2C receptor agonist 2,5-Dimethoxy-4-iodoamphetamine (DOI)-induction model to study the potential of PS128 on alleviating tic-like behaviors and the underlying DA and 5-HT changes.

Many studies had utilized pharmacological manipulations in animal models to study the relationship between various neuromodulators and neurological disorders. The 5-HT receptor (5-HTRs) agonists had been used in preclinical studies as in vivo models for TS (Wright et al., 1991; Handley and Dursun, 1992; Vickers et al., 2001; McCann and Isoda, 2013; Roessner et al., 2013), OCD (Flahiser-Grinberg et al., 2008; Kantak et al., 2014; Alonso et al., 2015) and schizophrenia (Aghajanian and Marek, 2000; Weiss and Feldon, 2001; Valsamis and Schmid, 2011; Mouri et al., 2013) studies. Some authors suggested that the 5-HT-induced behaviors in animal model of TS could highlight the complex relationship between different neuromodulator systems (Handley and Dursun, 1992; Bronfeld et al., 2013). In this regard, DOI had been used to generate sensorimotor gating deficit (Sipes and Geyer, 1994; Kehne et al., 1996), stereotype behaviors, perseverative behaviors, and regional contraction or reflex of muscles in rodents (Fone et al., 1991; Wright et al., 1991; Dursun and Handley, 1996; Vickers et al., 2001). Furthermore, some animal studies had demonstrated that DOI application could increase dopaminergic neuron activity and DA release in the ventral tegmental area (VTA) and medial prefrontal cortex (mPFC) (Iyer and Bradberry, 1996; Bortolozzi et al., 2005; Pehek and Hernan, 2015). On the other hand, DOI might inhibit striatal DA release (Ng et al., 1999) just like other 5-HT2C agonists (Alex et al., 2005; Burke et al., 2014). From our previous mice studies we had demonstrated that PS128 administrate could influence brain monoamines mainly in the PFC and striatum (Liu et al., 2016a, 2016c), therefore the DOI induction model seemed to be an ideal model to study the possible effects of PS128 on irregular neurotransmissions, especially on mesocortical and nigrostriatal DA pathways.

In the current study we focused on assessing whether PS128 could ameliorate the DOI-induced tic-like behaviors in rat and further examined the possible mechanisms underlying the effects of PS128 by analyzing the changes in central monoamine system and peripheral 5-HT concentration. In addition, we further analyzing the gut microbiota in each experimental group to investigate the possible effects of PS128 on the gut-brain axis.

2. Material and methods

2.1. Experimental animals and housing

Male Wistar rats (6 weeks old, weight 220–330 g) were purchased from BioLASCO Taiwan Co., Ltd (Taipei, Taiwan). Rats were quarantined after transportation and then housed in the specific pathogen-free room at the Laboratory Animal Center of National Yang-Ming University. The room was kept at a constant temperature (22 ± 1 °C) and humidity (55–65%) with a 12 h light/dark cycle and the water and chow were provided *ad libitum* (Lab Diet Autoclavable Rodent Diet 5010, PMI Nutrition International, MO, USA). All animal studies were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee, National Yang-Ming University (IACUC No. 10404080).

2.2. Preparation of *L. plantarum* PS128

PS128 was inoculated in Man Rogosa Sharpe (MRS) broth (Difco Corp., MD, USA), cultured at 37°C for 18 h, and then harvested by centrifugation at 6000 × g for 10 min. The pellet was re-suspended in MRS broth supplemented with 12.5% glycerol to a final concentration of 10^10 colony-forming units per milliliter (CFU/mL). The re-suspended solution was then aliquoted in freezertubes and stored at −80°C. Before the oral administration, aliquot of PS128 were thawed in 37°C water bath for 1 h and then centrifuged at 6000 × g for 10 min. The supernatant was removed and the bacterial pellet was re-suspended in saline.

2.3. Experimental procedures and tic-like behavior counting

All rats were randomly assigned into different experimental groups and acclimatized for 1 week before the experiment procedure started. The PS128 group received daily oral gavage of PS128 of intended CFU for 15 continuous days while control group received saline (1 mL per day) and positive control group received haloperidol (1 mg/kg in 1 mL saline per day; Sigma–Aldrich, MO, USA) during the same period. For tic-like behaviors induction, rats were given intravenous (i.v.) injection of DOI (200 μg/kg; Sigma–Aldrich, MO, USA) as a primary treatment on
day 14 and intraperitoneal (i.p.) injection of DOI (1 mg/kg in phosphate-buffered saline, PBS) as the secondary treatment on day 15 (Fig. 1A). The control group received PBS 100 μL (i.v.) on day 14 and on day 15 each. After the i.p. injection, rats were individually transferred to another clear cage for video recording immediately. After video recording, all rats were anesthetized by i.p. injection of pentobarbital sodium (50 mg/kg) and placed in a clean cage until rats were unconscious and unresponsive. Then the blood samples were collected by cardiac puncture and brain, intestinal tissues, and cecal content samples were also collected for further analysis.

From the 35-min recording period we counted the numbers of back muscle contraction (BMC), wet dog shake (WDS) and stereotyped behaviors. The BMC were counted only if a clear-cut powerful contraction sweeping from the back of the neck to the tail was present (Fone et al., 1991; Cheer et al., 1999). The WDS were counted when a paroxysmal shudder of the head, neck and trunk were present (Bedard and Pycock, 1977; Fone et al., 1991; Handley and Dursun, 1992; Cheer et al., 1999). Perseverative head or body grooming or self-gnawing were counted as...
stereotyped behaviors. During the primary setup stage of DOI induction model, the counters were trained for explicitly identify the BMC, WDS, and stereotyped behaviors. As for the formal experimental stage, the recording video file names were converted into random number-code and were analyzed by at least two different counters.

2.4. Startle response recording for prepulse inhibition (PPI) evaluation

We used the Med Associates Startle Reflex System (SOF-825 Startle Reflex, Med Associates, Virginia, USA) to evaluate the PPI. All experiment configuration and system setting were based on the manufacturer’s guidelines and related references (Jaworski et al., 2005; Valsamis and Schmid, 2011). We give the rats three training sessions before the oral administration of PS128, saline, or haloperidol. Rat was fixed in an acrylic animal holder on a calibrated platform in a sound-proof chamber with 68 dB ambient background noise. After 5 min acclimatization, rat was given 32 startle-elicitng sound bursts (120 dB, 40 ms). In 24 of the 32 startle stimuli we gave a prepulse stimulus 100 ms before the startle stimulus. The prepulse stimulus was a 20 ms pure tone of 75, 80 or 85 dB; there were 8 trials for each intensity. The remaining 8 trials were startle stimulus alone trials. The inter-trial interval was set at 1 min. Then on day 15, 5 min after the i.p. injection of PBS or DOI we give the rats three more startle sessions which was the same as the training session. The startle responses were recorded and calculated by the Startle Reflex Software. The PPI was evaluated by calculating the difference between the startle response without the prepulse stimulation (startle alone, S1) and the startle response with prepulse stimulation (prepulse and startle, S2), the difference then used to calculate the percentage change over S1. PPI (%) = [(S1 - S2) / S1] × 100.

2.5. Quantification of monoamines and their metabolites by high-performance liquid chromatography – electrochemical detection (HPLC-ECD)

2.5.1. Instrumentation and conditions

The HPLC-ECD system consisted of a S1130 HPLC pump system (Sykam, Eresing, German), an on-line S5300 sample injector (Sykam, Eresing, German), a DECADE II SDC electrochemical detector (Antec, Zoeterwoude, Netherlands), and a reversed-phase column (Kinetex C18, 2.6 μm, 100 × 2.1 mm I.D.; Phenomenex, CA, USA). The potential for oxidation of monoamines and their metabolites by high-performance liquid chromatography (HPLC)-electrochemical detection (ECD) was measured against an Ag/AgCl reference electrode at room temperature (25 °C). For the quantification of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindolacetic acid (5-HIAA), and 5-hydroxytryptamine or serotonin (5-HT), the mobile phase was pumped at a constant flow rate of 0.2 mL/min. The mobile phase contained 0.1 M NaH2PO4, 2 mM KCl, 0.74 mM 1-octanesulfonic acid (sodium salt), 0.03 mM ethylenediaminetetraacetic acid (EDTA), and 8% methanol and was adjusted to pH 3.74 with H3PO4 (Cheng et al., 2016). The pump was set at +700 mV with respect to an Ag/AgCl reference electrode at room temperature (25 °C). For the quantification of norepinephrine (NE) and 3-Methoxy-4-hydroxyphenylglycol (MHPG), the mobile phase was pumped at a constant flow rate of 0.1 mL/min and contained 0.1 M NaH2PO4, 2 mM KCl, 1.8 mM 1-octanesulfonic acid, 0.1 mM EDTA, and 10% methanol and was adjusted to pH 3.00 with H3PO4 (Bidel et al., 2016).

2.5.2. Sample preparation and data analysis

Rats were anesthetized by pentobarbital sodium injections (50 mg/kg, i.p.). The striatum and prefrontal cortex (PFC) were separated from the brain. The ileum and colon segments were pulverized and collected (approximately 0.1 g, wet weight). All rat tissue samples were lysed by sonication in the perchloric acid (PCA) buffer (0.1% perchloric acid, 0.1 mM EDTA, and 0.1 mM Na2S2O5) then centrifuged at 12,000 × g for 10 min and the supernatant were collected. The serum samples were mixed with PCA buffer containing 10% trichloroacetic acid (TCA) of the same volume. Before samples were injected into the HPLC-ECD system for analysis, all samples were first filtered by 4 mm syringe filter fitted with 0.22 μm polyvinylidene difluoride (PVDF) membrane (Millipex-GV, Millipore, USA). Diluted filtrates (20 μL) were injected into the chromatographic system. The concentrations of monoamines and their metabolites in the samples were interpolated using standards (Sigma–Aldrich, MO, USA) ranging from 1 to 100 ng/mL with DataApex Clarity chromatography software (version 5.0.3.180, DataApex Ltd., Prague, Czech).

2.6. Western blot analysis

The striatum and PFC of rats were lysed by sonication in RIPA lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 0.5% Sodium deoxycholate, 1% NP-40, 0.1% sodium dodecyl sulfate, SDS; pH = 7.5) containing protease inhibitor (Roche complete™, Mini, EDTA-free Protease Inhibitor Cocktail, Merck, Darmstadt, German) and phosphatase inhibitor (Halt™ Phosphatase Inhibitor Cocktail, Thermo Fisher, MA, USA). Tissue lysate was placed on ice and homogenized by vortex in a 5-min interval for 30 min, then the lysates were centrifuged at 12,000 × g for 10 min. Protein concentration in supernatant were determined by the Bradford Assay (Bio-Rad, CA, USA) using bovine serum album as standards (Sigma–Aldrich, MO, USA) ranging from 2 to 10 μg/mL. Protein sample was loaded onto a 12% SDS-PAGE gel for electrophoresis then the separated proteins were transferred onto the Roche PVC membrane (Merck, Darmstadt, German) and incubated with blocking buffer (5% skim milk in tris-buffered saline containing 0.1% Tween-20, TBST) for 1 h at room temperature. Subsequently, the membranes were probed with the primary antibodies overnight at 4 °C. The primary antibodies used were anti-DAT (1:500), anti-D2R (1:1000), anti-pDARPP32-34 (1:500), anti-pDARPP32-75 (1:500), anti-pERK (1:1000), anti-DARPP32 (1:1000) (Santa Cruz Biotechnology, CA, USA), anti-ERK (1:1000), and anti-p-β-arrestin (1:1000) (Cell Signaling Technology, MA, USA). After washing twice with TBST, membrane was incubated with secondary antibody conjugated with horseradish peroxidase in blocking buffer for 1 h at room temperature. Then the membrane was washed twice in TBST and the signal from the antibody-protein complex was obtained by enhanced chemiluminescence (ECL) of Millipore Immobilon™ Western Chemiluminescent HRP Substrate (Millipore Corporation, MA, USA). Images were analyzed using Luminescent Image Analyzer (LAS-4000, FUJIFILM Holdings Corporation, Tokyo, Japan) and the NIH ImageJ software (https://imagej.nih.gov/ij/).

2.7. Analysis of cecal microbiome by 16S rRNA pyrosequencing

2.7.1. DNA extraction from cecal samples of rats

Bacterial DNA was extracted and purified by previously published methods with slight modifications (Nakayama et al., 2015; Li et al., 2017). Briefly, 100 mg of cecal sample was washed three times by PBS and re-suspended in extraction buffer (100 mM Tris–HCl, 40 mM EDTA, 1% SDS; pH 9.0) with 0.3 g of glass beads (0.1 mm in diameter) and 500 μL of buffer-saturated phenol. The mixture was vigorously shaken for 30 s in the FastPrep FP120 homogenizer (Q-Biogene, CA, USA) at a speed setting of 5.0 m/s. The cecal sample was then centrifuged at 12,000 × g for 5 min. After centrifugation, 400 μL of the supernatant was added into an equal amount of phenol-chloroform-isooamyl alcohol (25:24:1, v/v). The mixture was shaken again in the FastPrep FP120 at a speed of 4.0 m/s for 45 s then centrifuged again at 12,000 × g for 5 min. The DNA was precipitated with 3 M sodium acetate (pH 5.4) and isopropanol. The DNA was then air-dried and dissolved in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). The concentration of DNA was adjusted to 10 ng/μL and the samples were stored at −20 °C.

2.7.2. 454 pyrosequencing of 16S rRNA genes and QIIME pipeline sequences analysis

The V1-V2 region of bacterial 16S rRNA gene was amplified by the
first PCR reaction with a bacterial universal primer set (27F: 5′-AGATTTGATCMTGGCTCAG-3′; 338R: 5′-TCTTGCTCCTCCGTTAGG AGT-3′). The reaction mixture (25 μL) contained DNA (10 ng), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl₂, 200 μM of each deoxynucleoside triphosphate (dNTP), 5 pmol of each primer, and 0.625 U Ex Taq HS (Takara Bio, Shiga, Japan). The first PCR condition was as follow: 98°C for 2.5 min; 15 cycles of 98°C for 15 s, 50°C for 30 s, and 72°C for 20 s; and finally 72°C for 5 min. The DNA amplicons from the first PCR reaction were barcoded by second PCR reaction with bar-coding primer set of 27Fmod with different 10-bp barcode sequence tags (Nakayama, 2010) and 338R in the reaction mixture. The reaction mixture (50 μL) contained DNA (10–100 ng), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μM of each dNTP, 10 pmol of each primer, and 1.25 U Ex Taq HS. The PCR condition was set as follow: 98°C for 2.5 min; 20 cycles at 98°C for 15 s, 54°C for 30 s, and 72°C for 20 s; and finally 72°C for 5 min. The final PCR products were purified by QIAquick PCR Purification Kit (Qiagen, CA, USA) according to the manufacturer’s protocol. The DNA amplicons were sequenced on the genome sequencer (GS) FLX 454 pyrosequencer (Roche, Basel, Switzerland) at the Veteran Yang-Ming (VYM) Genome Research Center of National Yang-Ming University.

The 16S rRNA gene sequences were processed by using the Quantitative Insights Into Microbial Ecology (QIIME v1.9.0) pipeline to classify microbial constituents and compare memberships between samples (Caporaso et al., 2010). The following quality check parameters were used: minimum quality score of 27, no primer mismatch, read length of 300 to 400 bp, maximum 3 ambiguous bases, and 6 bases in homopolymer runs. A total of 193,711 sequences were used to construct operational taxonomic units (OTUs) consisting of sequences with 97% sequence identity. Chimeric sequences were removed by ChimeraSlayer (Haas et al., 2011) and OTUs comprising fewer than three reads were excluded from further analyses (2854 OTUs). As a result, 1391 OTUs with quality-filtered sequences were obtained. Taxonomy classification was assigned according to the Greenegnes reference sequence database (gg_13_5) (DeSantis et al., 2006) with a confidence threshold value of 80% with the uclust consensus taxonomy assigner (Edgar, 2010). The bacterial composition of each sample was determined for each taxonomic level by applying the summarize_taxa_through_plots.py function of QIIME to the OTU table with the assigned taxonomy dataset.

2.7.3. Principal component analysis

The bacterial family diversity of each sample was analyzed based on relative abundance of each family by principal component analysis (PCA). The R package (ggbiplot) was used to generate PCA plots by using the first two principal components according to group.

2.7.4. Alpha- and beta-diversities of bacterial communities

Alpha diversity (as measured by the number of species observed, the phylogenetic diversity whole-tree, and Shannon indices) and Beta diversity were estimated with QIIME. The average values of the number of observed OTUs, the phylogenetic diversity whole tree, and the Shannon indices over 10 iterations were calculated for each rarefied OTU composition by using 1000 reads per sample. Beta diversity analysis was performed using phylogenetic distance matrices simulated by UniFrac analysis (Lopez Pujalte and Knight, 2005). To visualize bacterial community populations between groups, a PCoA plot was generated by using the make_2d_plots script bundled with QIIME. The QIIME script compare_categories using an Analysis of similarity (ANOSIM) (Clarke, 1993) test was used to consider whether there were significant differences in sample groupings using distance matrices.

2.8. Statistical analysis

All data were expressed as mean ± SEM. All statistical analyses were performed using Prism (version 7, Prism, CA, USA) or R software. Differences between experimental groups were analyzed by one-way analysis of variance (ANOVA) with Tukey’s post hoc test, the Kruskal–Wallis Rank Sum Test (Alpha diversity), or the Analysis of similarity (ANOSIM) test (Beta diversity); p < 0.05 was considered statistically significant.

3. Results

3.1. L. plantarum PS128 ameliorates tic-like behaviors and rescues the PPI deficits in the DOI-induced rat model of TS

We utilized the DOI-induced tic-like behaviors model by injecting DOI (1 mg/kg, i.p.) to rat (Bedard and Pycock, 1977; Fone et al., 1991; Wright et al., 1991; Cheer et al., 1999; Tizabi et al., 2001). We observed remarkable BMC responses 5 min after injection. Intermittent WDS and a few stereotype behaviors like grooming were also noted during the 60 min observation period (Fig. S1A & B). With the intention to achieve stable behavioral induction, we administrated daily DOI (1 mg/kg, i.p.) injection for four days and recorded the tic-like behaviors every day immediately after each injection. We noticed that both BMC and WDS occurrence were significantly enhanced in the 2nd day compared with the 1st day while the tic-like behavior counts showed no significant differences among the 2nd, 3rd and 4th day, indicating that two daily DOI injections could successfully induce stable tic-like behaviors (Fig. S1C & D). Following this 2-day continuous injection protocol, we also tested the combinations of different injection methods, i.e. i.v.+i.v., i.v.+i.p., i.p.+i.p. We found that the i.v.+i.p. group showed more significant tic-like behavioral expressions than other groups (Fig S1E). Therefore, we employed the two-time DOI-induction method (1st-day i.v. 200 μg/kg + 2nd-day i.p. 1 mg/kg) and recorded the tic-like behaviors or startle responses immediately after the 2nd-day injection to evaluate the effects of PS128 administration (Fig. 1A). Haloperidol, a DA antagonist commonly used in tics treatment, was administrated in another group of rat by oral gavage (1 mg/kg, daily) as the positive control. In our preliminary experiments we already demonstrated that the haloperidol treatment could mitigate the tic-like behaviors and PPI deficit (Fig. S1F & G, Fig. 1E), which is consistent with previous studies (Handley and Dursun, 1992; Gaynor and Handley, 2001).

The two-time administration of DOI successfully induced prominent BMC (F(4, 44) = 42.5, p < 0.001, Fig. 1B) and WDS (F(4, 45) = 6.63, p < 0.05, Fig. 1C). We quantified the total tic-like behaviors as the sum of BMC, WDS, and the stereotype behavior counts (Fig. 1D). DOI induction also reduced the percentage of PPI (F(4, 32) = 13.3, p < 0.001, Fig. 1E). Oral gavage of PS128 (10¹⁰ CFU, daily) alleviated the DOI-induced BMC (F(4, 44) = 42.5, p < 0.01), total tic-like behaviors (F(4, 31) = 22.4, p < 0.01), and the reduction on percentage of PPI (F(4, 32) = 13.3, p < 0.05) but not on WDS (Fig. 1B-E). Oral gavage of PS128 of same dosage to normal rats did not produce any abnormal behaviors compared with the saline control group (Fig. 1B-E). We further evaluated the effective dosage by daily oral gavage of low (10⁸ CFU), medium (10⁹ CFU), and high (10⁶ CFU) doses of PS128 and found that both medium and high dose groups showed improvement on total tic-like behaviors (F(15, 63) = 13.2, p < 0.01, p < 0.01, respectively) and PPI deficits (F(5, 31) = 7.98, p < 0.05, p < 0.05, respectively) whereas the low dose group did not show any obvious effect on DOI-induced behaviors (Fig. 1F-G).

3.2. L. plantarum PS128 modulates the metabolism of monoamines in the striatum and prefrontal cortex

To investigate the effects of PS128 on the amimergic neurotransmitters in brains of normal and DOI-treated rats, we used HPLC-ECD to measure levels of monoamines and their metabolites in the striatum and prefrontal cortex (PFC). The results showed that PS128 ingestion alone could increase NE (F(4, 45) = 6.51, p < 0.001) and caused a lower NE turnover rate (F(4, 44) = 3.57, p < 0.05) in the
Reduced the striatal DA level ($F(4, 44) = 11.3, p < 0.001$) and turnover rates of DA ($F(4, 45)= 12.1, p < 0.01$) compared with the Saline group (Table 1). PS128 ingestion also increased the striatal NE levels ($F(4, 45)= 6.51, p < 0.05$) and decreased the turnover rates of DA ($F(4, 45) = 12.1, p < 0.01$) compared with the Saline + DOI group (Table 1). There was no significance difference), HVA ($F(4, 44) = 4.92, p < 0.05$), and NE levels ($F(4, 45)= 4.51, p < 0.01$) of DOI-treated rats, whereas haloperidol increased the striatal DOPAC (approaching statistical significance), HVA ($F(4, 45) = 4.92, p < 0.05$) and DA turnover ratio ($F(4, 45) = 14.4, p < 0.05$) compared with the Saline + DOI group (Table 1). In the PFC, PS128 ingestion alone slightly increased DA and NE and decreased NE turnover ratio ($F(4, 45) = 9.72, p < 0.05$) compared with the Saline group (Table 1). DOI induction significantly increased DA ($F(4, 43) = 7.39, p < 0.01$) and NE levels ($F(4, 45) = 13.7, p < 0.01$) and decreased the turnover rates of DA ($F(4, 45) = 12.1, p < 0.05$) and NE ($F(4, 45) = 9.72, p < 0.001$) in the PFC (Table 1). Haloperidol treatment did not reverse the DOI-induced DA elevation, but showed a reduction in NE ($F(4, 45) = 13.7, p < 0.05$) compared with the Saline + DOI group. The haloperidol treated DOI group (Hal + DOI) also showed increased DOPAC ($F(4, 43) = 8.70, p < 0.001$), HVA ($F(4, 44) = 16.2, p < 0.001$) and turnover rates of DA ($F(4, 45) = 12.1, p < 0.01$) and NE ($F(4, 45) = 9.72, p < 0.01$, Table 1). PS128 ingestion on the other hand, suppressed DA elevation in DOI-treated rats ($F(4, 43) = 7.39, p < 0.05$). However, PS128 + DOI group also showed increased DOPAC ($F(4, 43) = 8.70, p < 0.05$), HVA (approaching statistical significance), and DA turnover ratio ($F(4, 43) = 7.39, p < 0.01$) compared with the Saline + DOI group (Table 1). There was no significant difference in the 5-HT, 5-HIAA, and 5-HT turnover ratios among all groups in both striatum and PFC.

3.3. L. plantarum PS128 increases the level of DAT and β-arrestin and regulates DA-related signaling transduction in the striatum and PFC of rats

Since the oral gavage of PS128 mainly regulated the metabolism of DA (Table 1), we next examined changes in the DA transmission pathways. Compared with the Saline group, the DOI injection increased the levels of phosphorylated ERK (p-ERK, $F(4, 29) = 8.16, p < 0.05$) and DARPP-32 Thr34 at Thr34 (p-Thr34-DARPP-32, $F(4, 31) = 5.67, p < 0.05$), but did not affect the DA transporter (DAT), D2R, and β-arrestin expression levels in the striatum (Figs. 2A–D & A & C). Both PS128 and haloperidol treatments reversed the effects of DOI on ERK ($F(4, 29) = 8.16, p < 0.05$, p < 0.05, respectively, Fig. 4A) and DARPP-32 Thr34 phosphorylation ($F(4, 31) = 5.67, p < 0.05$, respectively, Fig. 4C). PS128 treatment also increased the DAT ($F(4, 37) = 4.76, p < 0.05$, p < 0.05, respectively, Fig. 4A) and β-arrestin levels ($F(4, 37) = 5.93, p < 0.05$, p < 0.05, respectively, Fig. 4D) in the striatum of both normal and DOI-treated rats. In the PFC, similar treatments effects of haloperidol and PS128 on the levels of DAT, β-arrestin, p-ERK, and p-Thr34-DARPP-32 were observed in the DOI-treated rats (Figs. 3 & 4 B & D). However, only PS128 administration restored DOI-induced reduction of phosphorylated DARPP32 at Thr75 (p-Thr75-DARPP-32, $F(4, 31) = 16.2, p < 0.05$) in the PFC, but not haloperidol (Fig. 4D).

3.4. L. plantarum PS128 modulates the level of peripheral 5-HT

Recent studies have revealed that gut microbiota regulates endogenous 5-HT biosynthesis in the gut, which might be an important player in the MBGA pathway (O'Mahony et al., 2015; Reigstad et al., 2015; Yano et al., 2015). In order to investigate effects of PS128 on the peripheral serotonergic system, we measured the levels of 5-HT in the ileum, colon, and serum following PS128 administration in normal and DOI-treated rats. We found that the oral gavage of PS128 clearly increased the levels of 5-HT in the ileum ($F(4, 31) = 13.5, p < 0.001$), colon ($F(4, 38) = 7.62, p < 0.001$), and serum ($F(4, 29) = 3.63, p < 0.05$) in normal rats (Fig. 5A–C). The peripheral 5-HT levels were not affected by DOI treatment in normal rats. However, PS128 administration did not increase the peripheral 5-HT level in DOI-treated rats, whereas the haloperidol apparently increased the 5-HT level in the ileum ($F(4, 31) = 13.5, p < 0.001$) but not in the colon and serum of DOI-treated rats.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Saline</th>
<th>PS128</th>
<th>DOI (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10209 ± 560</td>
<td>1027 ± 921</td>
<td>7056 ± 486***</td>
</tr>
<tr>
<td>DOPAC</td>
<td>1172 ± 86</td>
<td>1027 ± 99</td>
<td>883 ± 102</td>
</tr>
<tr>
<td>HVA</td>
<td>534 ± 52</td>
<td>568 ± 50</td>
<td>481 ± 40</td>
</tr>
<tr>
<td>5-HT</td>
<td>307 ± 20</td>
<td>342 ± 31</td>
<td>315 ± 27</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>354 ± 24</td>
<td>393 ± 35</td>
<td>441 ± 30</td>
</tr>
<tr>
<td>NE</td>
<td>241 ± 32</td>
<td>585 ± 102***</td>
<td>365 ± 51</td>
</tr>
<tr>
<td>MHPG</td>
<td>49 ± 14</td>
<td>43 ± 9</td>
<td>41 ± 7</td>
</tr>
</tbody>
</table>

Concentrations of monoamines (ng/g wet tissue), metabolites (ng/g wet tissue), and turnover ratio (%) are expressed as mean ± SEM. DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, serotonin or 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid; NE, norepinephrine; MHPG, 3-Methoxy-4-hydroxyphenylglycol; Hal, haloperidol (1 mg/kg) group. Data were analyzed by one-way ANOVA with Tukey post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001, compared to the Saline group; p < 0.05, **p < 0.01, ***p < 0.001, compared to the Saline + DOI group (n = 10/group).
rats (Fig. 5A–C).

3.5. L. plantarum PS128 modulates the cecal microbiota of rats and alleviates the DOI-induced dysbiosis

For cecal microbiota analysis, the OTUs were classified into known taxa (13 phyla, 22 classes, 35 orders, 59 families, 70 genera, and 40 species) and unclassified groups. At the phylum level, the PS128 treated group showed a significant elevation in the Proteobacteria population ($F(4, 30) = 9.59, p < 0.05$) and a significant reduction both in the Firmicutes/Bacteroidetes ratio (F/B ratio, $F(4, 27) = 3.59, p < 0.05$) and the Bacteroidetes/Proteobacteria ratio (B/P ratio, $F(4, 29) = 6.88, p < 0.05$) among all rat groups (Table 2). The PS128 + DOI group also had a lower F/B ratio compared with both the Saline and Saline + DOI groups (approaching statistical significance). The B/P ratio was also increased in the Hal + DOI group compared with the Saline + DOI group ($F(4, 29) = 6.88, p < 0.05$, Table 2).

At the family level, we observed seven predominant bacterial populations among all rat groups (Table 2 & Fig. 6A). The PS128 treated group exhibited significantly more Bacteroidaceae compared with the Saline group ($F(4, 30) = 3.96, p < 0.05$, Table 2). In the DOI treated groups, both the Hal + DOI and PS128 + DOI groups showed a significant increase in Prevotellaceae compared with Saline + DOI group ($F(4, 28) = 6.03, p < 0.001, p < 0.05$, respectively, Table 2). Furthermore, both the PS128 and PS128 + DOI groups showed a significant decrease in Lachnospiraceae compared with the Saline + DOI group ($F(4, 29) = 9.56, p < 0.001, p < 0.05$, respectively, Table 2). The proportions of these predominant families also tended to display an equal distribution only in the Saline + DOI group (Table 2 & Fig. 6A).

Based on the relative abundance of each bacterial family, we further demonstrated the principal component analysis (PCA) could decompose the data on bacterial composition into two factors that explained 81.2% of the variance. Principal component 1 (PC1) was heavily loaded with Prevotellaceae, whereas principal component 2 (PC2) was heavily loaded with Bacteroidaceae and negatively loaded with Lactobacillaceae (Fig. 6B). Samples from the Saline group and the PS128 + DOI group were distributed equally in the four quadrants of the biplot, while samples from the Saline + DOI group tended to form a cluster in the PC1-negative and PC2-negative regions. Samples from the PS128 group and the Hal + DOI group were distributed in the PC2-positive and PC2-negative regions, respectively (Fig. 6B). Further species diversity analysis showed that PS128 administration and DOI treatment led to lower and higher Alpha diversity indices respectively than saline group, although these differences did not reach a statistical significance. Both the PS128 and PS128 + DOI groups showed lower indices compared with the Saline + DOI group (Kruskal–Wallis Rank Sum Test, $p < 0.05$, $p < 0.05$, respectively), while the Hal + DOI group did not lead to a significant change (Fig. 6C). The weighted UniFrac analysis indicated that the samples from the five experimental groups showed significant differences (Fig. 6D, ANOSIM; $p < 0.05$). Differences in the microbiota communities between the rat groups could also be observed in the PCoA plots, which also showed that samples from the PS128 and PS128 + DOI groups tended to form a cluster (Fig. 6D).

4. Discussion

In the current study we used DOI induction model to assess the potential of using the prominent psychobiotic strain Lactobacillus
Plantarum PS128 to alleviate tic-like hyper-active behaviors and neurotransmissions in rat. We had reliably observed BMCs, WDSs, stereotyped behaviors and PPI deficits after two-daily DOI injection and these behaviors could be ameliorated by PS128 administration and haloperidol treatment (Fig. 1A–E). Our DOI-induction procedure could induce perseverative behaviors, regional contraction or reflex, stereotypic behaviors, and sensorimotor gating deficits in rat. Based on the previous animal studies and current results we believe that pharmacological manipulation by DOI injection could be used to mimic neurological syndromes caused by irregular neurotransmissions (Handley and Dursun, 1992; Cheer et al., 1999; Gaynor and Handley, 2001; Tizabi et al., 2001; Hayslett and Tizabi, 2003; Flaisher-Grinberg et al., 2008; Malkova et al., 2014). Therefore, the DOI-induction model could serve as a useful rodent model to study the potentials of using psychobiotic strains to ameliorate the neurological syndromes caused by aberrant neurotransmission. The major concern of using live bacteria administration to alleviate symptoms is safety and the effective dosage on normal physiology. In our dose-response trial, we found that once the PS128 ingestion dosage is $10^9$ CFU/day or over, the amelioration effects on DOI-induced behaviors and deficits became apparent. In addition, the effects of $10^9$ and $10^{10}$ CFU/day treatments were similar, suggesting that the efficacy of PS128 is not dose-dependent, but more like threshold dependent (Fig. 1F–G). We had previously demonstrated that it was safe to use PS128 at a $10^9$ CFU/day dosage, and the equivalent heat-killed PS128 did not produce any improvement effects (Liu et al., 2016a). We speculated that the novel psychotropic effects of PS128 might be originated from the effects of its symbiosis in GI tract of the host, but not mediated by its structural components such as surface exopolysaccharide. Therefore, in order to show its beneficial effects it is essential to ingest certain amount of live PS128.

The 5-HT-associated models are often used in psychopharmacological studies to explore the complex relationships between different monoamine systems (Iyer and Bradberry, 1996; Gobert and Millan, 1999; Bowers et al., 2000; Burke et al., 2014). Brain monoamine analysis in our DOI-induction model revealed that after DOI induction the DA efflux in PFC was increased whereas in the striatum the DA efflux was decrease (Table 1), suggesting both the mesocortical and nigrostriatal DA pathways were involved and regulated differently (Iyer and Bradberry, 1996; Bortolozzi et al., 2005; Pehek and Hernan, 2015). These changes might underlie the tic-like hyper-active behaviors and sensorimotor gating deficits (Sipes and Geyer, 1994; Bowers et al., 2000; Wong et al., 2008; Pehek and Hernan, 2015). On the other hand, haloperidol treatment had no significant effects on DOI-induced DA elevation or reduction but showed elevated DA metabolites in both the striatum and PFC, suggesting haloperidol exerted its effects by increasing the DA turnover rate (Table 1). In contrast, PS128-fed rats not only showed elevated DA metabolites, but also suppressed the DOI-induced DA changes, which implied that PS128-treated rats had a more stable DA system than saline-treated rats in response to DOI stimulation (Table 1); and these effects might underlie the behavioral improvement. We also found that PS128 ingestion alone could significantly increase the NE levels in both the striatum and PFC, however there was no synergistic effect on DOI-induced NE elevation in the PFC (Table 1). The DOI-induced changes in the NE system might participate in modulating the locomotor activity and sensorimotor gating deficits (Fishman et al., 1983; Oades et al., 1986; Mitchell et al., 2006) however, since PS128 could relieve excessive hyperkinetic behaviors without changing DOI-induced NE elevation, the improvement effect is probably not mediated.
by the NE system. Considering that DA is the direct precursor of NE, the increase in NE might be a consequence of DA elevation caused by DOI and PS128 ingestion through independent mechanisms (Iyer and Bradberry, 1996; Gobert and Millan, 1999; Bortolozzi et al., 2005; Liu et al., 2016a, 2016c). Although we need more studies to clarify the exact mechanisms underlying the interactions between these monoamine systems, our results nevertheless suggested that psychobiotic PS128 could assist the CNS function of the host to cope with abnormal neurotransmission and ameliorate behavioral ailments.

The expression of DAT could directly affect DA synaptic levels, therefore DAT plays a key role in regulating DA transmission. On the receiving side, the D2R is widely found in both medium spiny neurons (MSN) and DA neurons in the basal ganglia, and is an important treatment target in disorders exhibiting aberrant DA functions such as TS and schizophrenia (Minzer et al., 2004; Marsi et al., 2008; Urs et al., 2016, 2017). The signaling pathway of DRs is mainly through distinct G protein-dependent and β-arrestin-mediated pathways. The co-occurrence of these two pathways contributes to the signaling diversity of DRs, mediating several signaling regulations under different conditions and produce different period of activation (Del’guidice et al., 2011; Urs et al., 2016). Since, the DRs downstream kinases such as DARPP-32 and mitogen-activated protein kinase (MAPK) can act as integrators and coincidence detectors during the DRs signaling or the activation of other neurotransmitter systems (Svenningsson et al., 2004; Valjent et al., 2005), therefore we also examined D2R and the DRs downstream kinases in the striatum and PFC in each experimental group. PS128-fed rats with or without DOI induction all showed greater DAT expression in the striatum and PFC compared to saline-fed controls; D2R expression, on the other hand, did not show significance difference between each groups (Figs. 2A–C & 3 A–C). The DAT elevation in PFC and striatum of PS128-fed rats could explain the increased DA metabolites after DOI induction (Table 1) and no tic-like hyper-active behaviors in PS128-alone group (Figs. 1B–D, 2 B & 3 B). The higher DAT expression could also explain why in our previous

Fig. 4. DA signaling-associated kinase protein phosphorylation levels in the striatum and prefrontal cortex. The representative western blot and quantification of the ERK phosphorylation level in the striatum (A) and prefrontal cortex (B). Furthermore, the representative western blot and quantification of the DARPP-32 phosphorylation level in the striatum (C) and prefrontal cortex (D). The kinase proteins phosphorylation levels were analyzed by western blot experiments. Hal, haloperidol (1 mg/kg) group; CON, saline-fed control group. The fold-changes over the Saline group were expressed as mean ± SEM and analyzed by one-way ANOVA with Tukey’s post hoc test. *p < 0.05 compared to the Saline group; #p < 0.05 compared to the Saline + DOI group (n = 10/group).
expression and reversed the DOI-induced p-Thr75-DARPP changes in both the striatum and PFC (Figs. 2D, 3D & 4D). Besides its desensitization role, β-arrestin also serves as polyvalent GPCR-associated scaffolding proteins, having complex and multiple functions in regulating the final outcome of monoamine receptor stimulation especially for DRs (Del’guidice et al., 2011; Urs et al., 2016). It is possible that both the DAT and β-arrestins elevation might be the consequence of dopaminergic activation during the long-term PS128 ingestion. These results might imply that PS128 ingestion could help the host to establish a more flexible and adaptive DA system in response to DOI challenges.

In the MGBA, endocrine, immune, and nervous systems all have been considered as possible mediators for psychobiotics to influence the host's brain (Cryan and Dinan, 2012; Zhou and Foster, 2015; Foster et al., 2016). Our results showed that PS128 ingestion significantly increased the enteric and peripheral 5-HT level in naïve rats but not in DOI induction rats, suggesting the modulation ability of PS128 on host’s enteric serotonergic system during the different situation (Fig. 5). Gut microbiota or their metabolic products, such as short chain fatty acids (SCFAs), are essential for peripheral 5-HT biosynthesis (O’Mahony et al., 2015; Reigstad et al., 2015; Yano et al., 2015). Although peripheral serotonergic systems are separated from the CNS, they could modulate immunity, regulate energy balance, and cellular processes, and may affect CNS physiology indirectly (Flood et al., 2012; Baganz and Blakely, 2013; Namkung et al., 2015; Pawlak et al., 2017). Peripheral 5-HT may also act on the 5-HTRs on vagal afferent fibers in the GI tract and transmit information from the enteric nervous system to the CNS, forming the gut–brain neuronal circuit (Li et al., 2006; Bonaz et al., 2018). Many studies had demonstrated a close relationship between the vagus nerve and the dopaminergic system in the CNS (Ziomber et al., 2012; Surowka et al., 2015). Some psychobiotic strains like Lactobacillus rhamnosus JB-1 had lose the neurochemical and behavioral effects in vagotomized animal, demonstrating the important role of vagus nerve in the bidirectional communication of the MGBA (Bravo et al., 2011). Furthermore, a recent study provided direct evidences establishing vagal gut–brain axis as an integral component of the neuronal reward pathway, and suggesting vagal stimulation might be a possible treatments strategy in affective disorders (Han et al., 2018). In our previous study using PS128-fed GF mice, the striatal DA elevation had suggested that PS128 in GI tract of the host tract could stimulate vagus nerve and influence the mesolimbic pathway through vagal gut–brain neuronal circuit (Liu et al., 2016a). Since we observed PS128 modulated peripheral 5-HT and the central dopaminergic pathways (Table 1 & Fig. 5), the vagus nerve might be an essential pathway for PS128 to influence the DA mesolimbic system (Fig. 7). However, this hypothesis will require further studies to substantiate.

Past animal studies indicated that gut microbiota could affect brain development and modulate behaviors; changes in microbiota composition could also affect cerebral protein expression and behaviors even in mature animals (Bercik et al., 2011; Diaz Heijtz et al., 2011). DOI induction caused a dispersive cecal bacterial community as indicated by the clustering in PCA plots and the high Alpha diversity indices. However, these changes were not seen in PS128-fed DOI rats (Table 2, Fig. 6A–C). Since the rats received DOI by i.p. injection only 1 h before sacrifice, DOI might not interact with intestinal bacteria directly but might exert its effects by interfering with 5-HTRs in the GI tract to modulate intestinal secretion and motility (Gershon and Tack, 2007). Interestingly, both the PS128 and PS128 + DOI groups had lower Alpha diversity indices compared to other groups and showed a clustering tendency in their PCoA plots distribution (Fig. 6C–D). These results indicate a similar cecal microbiota community among all PS128-fed rats and the microbiota community was not significantly affected by the DOI treatment. The ratios between major bacterial phyla had been regarded as the significant relevance in composition, ecological alteration and the indication of dysbiosis of gut microbiota, whereas PS128 gavage also resulted in a decline in F/B and B/P ratios (Table 2). The F/B ratio elevation has been shown to be closely related to pathological studies we had observed PS128-fed rodents have present higher DA level but did not show hyperactive motor behaviors (Liu et al., 2016a, 2016c). Some natural chemical also showed their tics-reducing effects via regulating DAT expression which increases DA metabolism, and had potential for tics treatment (Lv et al., 2009, 2012; Wang et al., 2013; Zhang and Li, 2015). We also found enhanced p-Thr34-DARPP-32 and p-ERK after DOI induction suggesting hyper-excitation in these dopaminergic projections (Fig. 4). In addition, unlike the effects of DA antagonists haloperidol, PS128-fed rats exhibited a higher β-arrestin

![Fig. 5. Peripheral 5-HT levels in the ileum, colon, and serum of experimental rats.](image-url)

The 5-HT level of the (A) ileum, (B) colon, and (C) serum were measured using the HPLC-ECD method (n = 10/group). Data were expressed as mean ± SEM and analyzed by one-way ANOVA with Tukey post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001, compared to the Saline group; #p < 0.05, ##p < 0.01, compared to the PS128 group.
conditions such as obesity, hypertension, and aging (Mariat et al., 2009; Sanz and Moya-Perez, 2014; Yang et al., 2015). As for psychiatric conditions, some had reported an increase in *Firmicutes* or decline in *Bacteroidetes* that resulted in F/B ratio elevation in the clinical studies of major depression disorder (Naseribafrouei et al., 2014; Chen et al., 2018a, 2018b), ASD (Williams et al., 2011; Tomova et al., 2015; Strati et al., 2017), and schizophrenia (Castro-Nallar et al., 2015). However, others had reported entire opposite situations (Finegold et al., 2010; Jiang et al., 2015; Liu et al., 2016b). Although the results are still controversial, clinical studies in neuropsychiatric disorders and neurodegenerative condition increasingly reported the occurrence of gut dysbiosis (Hasegawa et al., 2012; Scheperjans et al., 2015; Vogt et al., 2017; Lubomski et al., 2019; Roy Sarkar and Banerjee, 2019). Out results suggest that PS128 administration could produce a more stable gut microbiota ecosystem to resist external stimuli, probably by influencing the enteric serotonergic system which is believed to be involved in the regulation of gastrointestinal motility and mucosal immune responses (Gross et al., 2012; Heredia et al., 2013; Reigstad et al., 2015; Yano et al., 2015). Considering the abovementioned studies and our experimental results, we believe that stabilizing the gut microbiota could be beneficial for syndrome amendment and improve the quality of life in neuropsychiatric patients.

**Table 2**

Relative abundance of bacterial composition at the phylum level, family level, and ratios of bacteria communities.

<table>
<thead>
<tr>
<th>Group</th>
<th>Saline</th>
<th>PS128</th>
<th>DOI (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phylum level (relative abundance (%))</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Firmicutes</em></td>
<td>61.5 ± 4.4</td>
<td>48.3 ± 4.1</td>
<td>63.5 ± 1.5</td>
</tr>
<tr>
<td><em>Bacteroidetes</em></td>
<td>35.1 ± 4.3</td>
<td>44.6 ± 4.0</td>
<td>32.2 ± 1.6</td>
</tr>
<tr>
<td><em>Proteobacteria</em></td>
<td>1.9 ± 0.5</td>
<td>5.5 ± 1.0 (p\lt 0.05)</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td><em>Tenericutes</em></td>
<td>0.43 ± 0.1</td>
<td>0.03 ± 0.1</td>
<td>0.77 ± 0.2</td>
</tr>
<tr>
<td><em>Cyanobacteria</em></td>
<td>0.38 ± 0.2</td>
<td>0.42 ± 0.2</td>
<td>0.49 ± 0.4</td>
</tr>
<tr>
<td><em>Actinobacteria</em></td>
<td>0.28 ± 0.2</td>
<td>0.41 ± 0.2</td>
<td>0.24 ± 0.1</td>
</tr>
<tr>
<td>Others</td>
<td>0.26 ± 0.1</td>
<td>0.68 ± 0.3</td>
<td>0.60 ± 0.2</td>
</tr>
<tr>
<td><strong>Family level (relative abundance (%))</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Prevotellaceae</em></td>
<td>21.7 ± 5.7</td>
<td>21.8 ± 5.6</td>
<td>125.3 ± 3.1</td>
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<tr>
<td><em>Lachnospiraceae</em></td>
<td>14.5 ± 1.3</td>
<td>8.0 ± 1.5 (p\lt 0.05)</td>
<td>17.8 ± 1.3</td>
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<tr>
<td><em>Lactobacillaceae</em></td>
<td>13.7 ± 3.9</td>
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<td><em>Ruminococcaceae</em></td>
<td>13.4 ± 2.2</td>
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<td>13.0 ± 1.5</td>
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<tr>
<td><em>Peptostreptococcaceae</em></td>
<td>10.0 ± 1.9</td>
<td>12.7 ± 2.4</td>
<td>10.4 ± 1.5</td>
</tr>
<tr>
<td><em>Bacteroidales</em></td>
<td>7.5 ± 3.0</td>
<td>8.0 ± 1.9</td>
<td>12.8 ± 2.1</td>
</tr>
<tr>
<td><em>Bacteroidaceae</em></td>
<td>4.1 ± 1.4</td>
<td>9.8 ± 2.4 (p\lt 0.05)</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>Others</td>
<td>15.2 ± 5.4</td>
<td>21.9 ± 7.2</td>
<td>17.9 ± 6.5</td>
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<td><strong>Bacterial communities ratios</strong></td>
<td></td>
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<tr>
<td>F/B ratio</td>
<td>2.1 ± 0.4</td>
<td>0.95 ± 0.1 (p\lt 0.05)</td>
<td>2.02 ± 0.1</td>
</tr>
<tr>
<td>B/F ratio</td>
<td>24.2 ± 4.6</td>
<td>9.5 ± 1.9 (p\lt 0.05)</td>
<td>22.6 ± 5.2</td>
</tr>
</tbody>
</table>

Cecal microbiota proportions average > 0.2% of total population at the phylum level and average > 5% of the total population at the family level were presented. *Firmicutes*; *B. Bacteroides*; *P. Proteobacteria*; Hal, haloperidol (1 mg/kg) group. Data were expressed as mean ± SEM and analyzed by one-way ANOVA with Tukey’s post hoc test; \(p\lt 0.05\) to Saline group; \(p\lt 0.05\), \(p\lt 0.001\) to Saline + DOI group. n = 7 in Saline group, n = 6 in PS128 group & PS128 + DOI group and n = 8 in Saline + DOI group & Hal + DOI group.

5. Conclusion

We demonstrated that PS128 ameliorated DOI-induced tic-like hyper-active behaviors and sensorimotor gating deficits caused by irregular DA efflux in mesocortical and nigrostriatal DA pathways. Further results suggested that PS128 stabilized cerebral dopaminergic pathways through its modulation of host’s microbiota–gut–brain axis. Our study may provide a potential new insight for psychobiotics application of improving syndromes caused by DA dysregulation in DA-related neurological disorders and movement disorders such as TS.
Relevant conflicts of interests/financial disclosures

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding agencies

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Author’s contribution statement

J.F.L., Y.F.C., S.W. and Y.C.T. conceived and organized the study. Y.F.C., W.T.L, C.C.H., S.W. and Y.C.T. contributed to experimental design. J.F.L and Y.F.C. performed the experiments. S.W.L. performed the 454 pyrosequencing data analysis. J.F.L., Y.F.C. and S.W.L. performed data statistical analysis. J.F.L. wrote the first draft of the manuscript and Y.F.C., S.W.L., C.C.W., O.J.J., S.W. and Y.C.T. contributed to writing the manuscript. All authors read, edited and approved the final manuscript.

Acknowledgments

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Fig. 6. Analysis of the cecal microbiome of experimental rats.
(A) Bar graph representing the relative community composition. (B) Principal component analysis (PCA) of bacterial abundance. The percentages of variation explained by PC1 and PC2 are indicated on the axes, and three bacterial families are indicated by arrows and family names in the PCA graph. The dash circle represents the cluster of Saline + DOI group. (C) Alpha diversities of bacterial community in individual samples in each rat group. Data were expressed as mean ± S.E.M. and analyzed by the Kruskal-Wallis Rank Sum Test. *p < 0.05 to Saline + DOI group. (D) 3D Principal coordinate analysis (PCoA) profiles, based on weighted UniFrac metric of bacterial diversity across all samples (ANOSIM; p < 0.05). The percentages of variation explained by PC1, PC2, and PC3 are indicated on the axes. The dash circle represents the cluster of PS128 group and Saline + PS128 group. n = 7 in Saline group, n = 6 in PS128 group & PS128 + DOI group, and n = 8 in Saline + DOI group & Hal + DOI group.
Fig. 7. Schematic mechanisms of PS128 on strengthening the microbiota-gut-brain axis function of the host. The symbiosis of sufficient live PS128 in GI tract of the host could influence both the enteric serotonergic system and gut microbiota. The modulation of enteric 5-HT could have an impact on the ENS, GI tract motility, mucosa function, and stimulate vagus nerve. These effects help to shape and stabilize the enteric ecosystem. In addition, PS128 in GI tract might also influence the mesocortical and nigrostriatal DA pathways to form a more efficient DA system in CNS via the vagus gut-brain neuronal circuit. The long-term ingestion of adequate amount PS128 could strengthen the MGBA function of the host to cope with gut dysbiosis and improve on abnormal neurotransmission and behavioral ailments.

service. We also thank the experimental services and animal cares from the Animal Behavioral Facility and Laboratory Animal Center at National Yang-Ming University.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.brainresbulletin.2019.07.027.

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