



Psychobiotic Supplementation of PS128™ Improves Stress, Anxiety, and Insomnia in Highly Stressed Information Technology Specialists: A Pilot Study

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Background: Information technology (IT) is an industry related to the production of computers, information processing, and telecommunications. Such industries heavily rely on the knowledge and solutions provided by IT specialists. Previous reports found that the subjective stress scores were higher in IT specialists who developed diabetes, hypertension, and depression. Specific probiotics, known as psychobiotics, may alleviate stress and mood symptoms. This study aimed to examine whether an 8-week intervention of a novel psychobiotic, *Lactobacillus plantarum* PS128™ (PS128™), improved self-perceived stress and mood symptoms among high-stress IT specialists.

Methods: This open-label, single-arm, baseline-controlled study included IT specialists from a large IT company in Northern Taiwan. Participants with a Perceived Stress Scale (PSS) 10-item version score of 27 or higher were included. Participants were asked to take two capsules containing PS128™ powder, equivalent to 20 billion colony-forming units, daily. Self-report measures, such as the Job Stress Scale, Visual Analog Scale of Stress, the Insomnia Severity Index, the State and Trait Anxiety Index, the Questionnaire for Emotional Trait and State, the Patient Health Questionnaire, the Quality of Life Enjoyment and Satisfaction Questionnaire, and Gastrointestinal Severity Index were compared at baseline and at the end of the trial period. The primary outcome was a 20% reduction in the PSS score at endpoint. Objective measures included salivary levels of stress biomarkers, including cortisol, α -amylase, immunoglobulin A, lactoferrin, and lysozymes, as well as results of the Test of Attentional Performance.

Results: Of the 90 eligible IT specialists, 36 met the inclusion criteria. After the 8-week trial period, significant improvements in self-perceived stress, overall job stress, job burden, cortisol level, general or psychological health, anxiety, depression, sleep disturbances, quality of life, and both positive and negative emotions were found.

Conclusion: Our results suggest that PS128™ has the distinct advantage of providing stress relief and can improve mental health for people with a high-stress job. Future placebo-controlled studies are warranted to explore the effect and underlying mechanisms of action of PS128™.

Clinical Trial Registration: <https://clinicaltrials.gov/> (identifier: NCT04452253-sub-project 2).

Keywords: probiotic, stress, *Lactobacillus plantarum*, PS128™, psychobiotic

INTRODUCTION

Information technology (IT) is an industry sector related to computer processing. It involves the development of computer hardware, software, semiconductors, websites, applications of statistical methods for decision-making, or telecommunications that encompass transmitting information or systems that facilitate communication through computer programs (1). In the past two decades, the number of people employed in the IT industry has grown rapidly in the US, from 400,000 people in 1990 to 1,800,000 people in 2011 (2). With an output value of 47 billion US dollars (3), the IT industry in Taiwan is ranked fourth in the world, and accounts for 3.3% of Taiwan's overall employment (4) and 92.8% of exported products. Working in the IT sector usually requires extensive knowledge and the ability to perform complex tasks to develop products or provide services and efficient solutions within limited time frames (5). Previous studies have reported that up to 54% of IT workers may have elevated stress, depression, anxiety, or sleep problems due to the high job demand and the uncertainty in their working environment (6).

When an individual is under chronic stress, their sleep and psychological well-being is often impaired (7). Research has found positive correlations between sleep disturbances and higher levels of perceived stress or job demands in high stress workers such as nurses or shift workers (8). Persistent impairment in sleep function may also augment perceived stress and work-related burnout (9). Long-term poor sleep and high stress at work may be related to the increased prevalence of anxiety and depression (10). Sleep deprivation and circadian rhythm changes might also increase cortisol levels, which in turn affects the circadian rhythm through activation of the hypothalamus-pituitary-adrenal (HPA) axis (11).

Microorganisms and their metabolites regulate the body through a series of biochemical and functional linkages. Growing evidence has demonstrated that the intestinal microbiota and their metabolites play key roles in absorption, metabolism, and maintenance of healthy immune and brain function, and may influence host behaviors (12, 13). Physiological and psychological stress may damage the intestinal microbiota and affect intestinal

permeability, leading to poor health (14). Intestinal dysbiosis was shown to be associated with dysregulation of the hippocampal serotonergic systems and the induction of anxiety behaviors in mice models (15). Psychobiotics, a class of probiotics, are defined as live microorganisms that, when ingested in adequate amounts, induces health benefits in patients with psychiatric illness (16). A previous study found small, but significant pooled effects of probiotics on depression ($d = -0.24$, $p < 0.01$) (17) and anxiety ($d = -0.10$, $p = 0.03$) (18). However, no significant differences in the levels of subjective stress or sleep qualities were found in some probiotic formulations among healthy volunteers when compared to the placebo (19, 20).

Previously, *Lactobacillus plantarum* PS128™ (PS128™), isolated from spontaneously fermented mustard greens in Taiwan (21), has been demonstrated to reduce anxiety- and depression-like behaviors (22, 23), ameliorate tic-like behaviors (24), and improve visceral hypersensitivity (25) in experimental animals. Beneficial effects of PS128™ on children with autism spectrum disorder (26), triathletes (27), and patients with major depressive disorder (28) have also been reported. PS128™ may exert its psychotropic effects by altering monoamine neurotransmitter levels (22–25), and modulating glucocorticoid (22), anti-inflammatory, and anti-oxidative responses (21, 22, 27); however, the underlying mechanisms warrant further investigation. Despite the increase in the number of IT employees, the importance of the IT industry to the economy, and the high stress due to uncertainty and workload under time pressure, no study to date has investigated whether probiotics could help alleviate stress, sleep, or mood symptoms in highly stressed IT specialists. Hence, embedded within a large ongoing clinical trial that contained two sub-projects investigating possible effects of PS128™ on high stress workers (sub-project 1 on registered nurses, and sub-project 2 on IT specialists), this study aimed to examine whether an 8-week probiotic intervention improved self-perceived stress and job-related stress, symptoms of anxiety or depression, and insomnia severity among highly stressed IT specialists. Furthermore, correlations between all psychological measures and stress biomarkers in IT specialists with high self-perceived stress were also assessed.

MATERIALS AND METHODS

Study Design and Participants

We conducted this open-label study to examine the effects of 8-week supplementation of PS128™ among highly stressed

Abbreviations: IT, Information technology; PSS, Perceived Stress Scale; STAI, State Trait Anxiety Index; JSS, Job Stress Scale; ISI, Insomnia Severity Index; PHQ, Patient Health Questionnaire; QLESQ, Quality of Life Enjoyment and Satisfaction Questionnaire; LOCF, Last observation carried forward; VAS, Visual Analog Scale; TAP, Tests of Attentional Performance; nREM, nonrapid eye movements.

information technology (IT) specialists within the framework of a larger clinical trial (NCT04452253). In the clinical registration of NCT04452253, a randomized, placebo-controlled trial targeted on registered nurses (sub-project 1); and the current open-label single arm study targeted on IT specialists (sub-project 2) were included. Although the overall trial aimed and hypothesized that the 8-weeks intervention of PS128™ may reduce perceived stress and associated mood symptoms among high stress workers including registered nurses or IT specialists, these were two separate and independent sub-projects, i.e., the study designs, outcome measures, and target participants were all different. The sub-project 1 randomized placebo-controlled trial aimed to examine the efficacies of psychobiotics PS128™, PS23™, and the PS23™ heat-treated capsule, on reducing stress and mood symptoms among high stress registered nurses, compared placebo. The sub-project 2 open-label single arm study was designed to investigate the tolerability and safety of PS128 among high-stress IT specialists. Herein, we report our findings from the sub-project 2 single arm study that focused on examining the safety and tolerability of PS128™ among IT specialists. Participants were recruited from a large IT company in Northern Taiwan. After written consent was signed, all IT specialists aged 20–60 years ($n = 90$) from this company were screened by the 10-item version of the Perceived Stress Scale (PSS-10). Those with a total PSS-10 score of 27 or higher were invited to participate in this study. Participants were excluded if they had taken or were taking antibiotics or psychotropics within the preceding month, used or were currently using any powder, capsule, or tablet probiotic products (except yogurts) within the preceding 2 weeks, had undergone surgery for any hepatobiliary gastrointestinal disorders, had any past or present inflammatory bowel disease, had a history of cancer, were allergic to lactic acid bacteria, were currently pregnant or breastfeeding, or those who smoked, consumed alcohol, or chewed betel nuts. Participants withdrew from the study if adverse reactions (such as diarrhea or bloating) occurred or if they were no longer willing to participate.

IT specialists with PSS-10 scores of ≥ 27 were further evaluated by psychological measures and tests on attentional performance at baseline (Time 1) and 8 weeks after the intervention (Time 2). Saliva was collected between 9 a.m. and 12 p.m. at baseline and at the end of 8 weeks to minimize the impact of the circadian rhythm. The subjects were asked not to drink, eat, or brush their teeth at least 1 h before the sample collection. In addition, participants were asked to not perform any kind of exercise within 4 h before saliva collection, and to not consume alcohol 12 h before sampling. Saliva was collected using a commercial device, Cortisol-Salivette® (Sarstedt, Nümbrecht, Germany). After collection, the saliva was extracted by centrifugation for 10 min at $1,000 \times g$ and frozen at -80°C until analysis. After completing the baseline evaluation, two PS128™ capsules were administered. Participants were then asked to take two capsules the next day before bedtime (Time 1), and once daily for 8 weeks. Participants were asked to return the remaining capsules at Time 2 to record the number of capsules consumed. This study was approved by the Institutional Review Board of Mackay Memorial Hospital (IRB no: 19CT013be).

Materials

The psychobiotic capsule contains 300 mg of lyophilized PS128™ powder, which is equivalent to 10 billion colony forming units of PS128™. It has been approved as a food supplement by the Taiwan Food and Drug Administration, and toxicological assessments suggest that PS128™ is safe for human consumption (29).

Perceived Stress Scale

The primary outcome was differences in the PSS score before and after consumption PS128™. The PSS is the most widely used psychological tool for measuring how perceived stress affects the participant's feelings within the past month (30). Past evidence regarding its effectiveness in research applications demonstrated that higher PSS scores were associated with failure to quit smoking (31), inability to control blood sugar levels among patients with diabetes mellitus (32), and increased susceptibility to depression or anxiety (33). A PSS-10 score between 27 and 40 indicates high perceived stress (34). The Mandarin version of the PSS was translated and was shown to have good validity, and was used in this study (35).

The State and Trait Anxiety Index

Differences in the STAI before and after the consumption of PS128™ were compared. The STAI contains two subscales to measure levels of anxiety in the current "state" or from the "trait." Higher STAI scores are correlated with higher levels of anxiety (36). The Mandarin version of STAI was found to have good reliability, and was used in this study (37).

Questionnaire of Emotional Trait and State

This scale was used to evaluate the positive and negative effects of PS128™ intervention. According to a principal component analysis, this 36-item questionnaire can be categorized into four different domains of emotions: happiness and acceptance, sadness and being scared, anger and disgust, and hopefulness. This questionnaire was reported to have good internal consistency (Cronbach's $\alpha = 0.93$) and construct validity (38).

Patient Health Questionnaire-9

The PHQ-9 was used to screen and monitor the participant's level of depression. The Chinese version of the PHQ-9 can be used to detect depression in participants with a total score of ≥ 10 , with a high sensitivity of 0.86 (39).

Insomnia Severity Index

The ISI is composed of seven questions to measure sleep difficulties. In patients under primary care, a total score of ≥ 14 indicated insomnia, with an area under receiver operating curve of 0.87, which demonstrated good validity (40).

Quality of Life Enjoyment and Satisfaction Questionnaire Short Form

The Q-LES-Q SF contains questions related to physical health, activity, general well-being, relaxation, work function, housework, school work, leisure activities, and social relations in the past week (41). The higher the score, the higher the

participant's level of satisfaction. The Mandarin version of Q-LES-Q SF was reported to have good reliability and validity (42).

Job Stress Scale

The Chinese version of the JSS (43) was translated by the Ministry of Labor in Taiwan. It contains 38 items to evaluate the degree of self-perceived stress caused by the job, satisfaction, interpersonal relationships, and sense of well-being (44). We used the JSS under the hypothesis that the job burden in the specialists' work environment did not significantly change before and after the intervention.

Visual Analog Scale of Gastrointestinal Symptoms

Differences in 10 gastrointestinal symptoms, including dry mouth, difficulty swallowing, decreased appetite, nausea or vomiting, flatulence, gastralgia, upper and lower abdominal pain, constipation, and diarrhea (45, 46), before and after the intervention were compared to determine the safety and tolerability of the intervention.

The Visual Analog Scale of Occupational Stress

The VAS was applied for assessing occupational stress. A VAS score of 7.0 has been reported to have a good sensitivity of 0.74 and specificity of 0.93 when compared to the PSS to detect high-stress in workers (47). Differences in VAS scores before and after PS128TM consumption were compared.

Tests of Attentional Performance

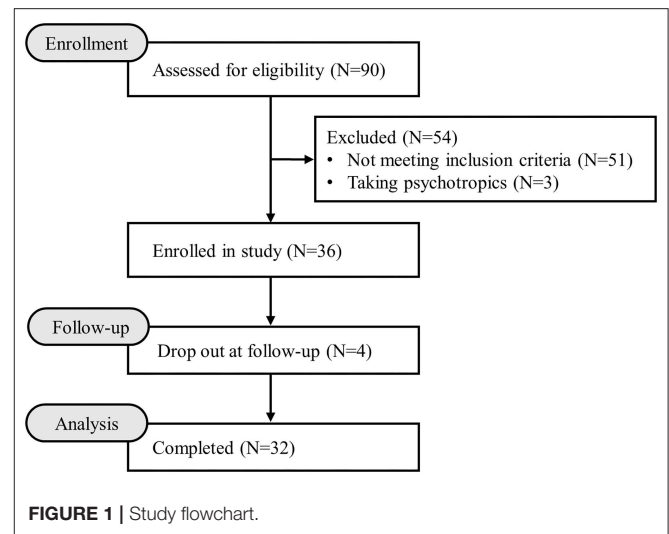
We used the windows-based TAP (including subtests of flexibility, working memory, and Go/No go) (48) as a neuropsychological test to evaluate whether PS128TM consumption would improve cognitive or attentional performance (49).

Salivary Biomarkers

We measured salivary cortisol, α -amylase (50), immunoglobulin A (IgA) (51), lactoferrin (52), and lysozyme (51) levels since they were found to be related to stress and anxiety. A recent study showed that the injection of probiotics for 12 weeks significantly reduced the level of salivary cortisol in nine fatigue subjects (53). Salivary α -amylase, IgA, lactoferrin, and lysozyme levels were measured by enzyme-linked immunosorbent assay kits (Immuno-Biological Laboratories, Inc., USA; Germany; and Assaypro LLC, USA). Salivary cortisol levels were determined using an electrochemiluminescence immunoassay kit (Elecsys Cortisol, Roche Diagnostic, Germany). All procedures were performed according to the manufacturer's instructions.

Statistical Analysis

All data were entered and error-checked by the research team and supervised by researchers experienced in clinical trials and longitudinal studies. Improvements in psychological symptoms and treatment responses were defined as a >20% change in the PSS score. A paired *t*-test was used to examine changes in continuous variables at Time 1 and 2. The missing data



from participants who dropped out of the study were treated as the "last observation carried forward." To explore possible causal relationships, we performed correlation and mediation analyses between cortisol levels and all psychological measures. A significant result was considered when two-tailed *p*-values were <0.05. SPSS version 12.0 was used to perform all analyses.

RESULTS

Demographic Information

As shown in **Figure 1**, among the 90 IT specialists who provided informed consent, 36 met the inclusion criteria. Of the 36 participants who were included and administered the PS128TM intervention, 32 completed the trial (three dropped out due to inability to return to Taiwan from China due to the pandemic; one dropped out due to self-perceived aggravated sleep condition). Comparisons of the baseline characteristics between participants who enrolled at baseline and those who completed the trial are shown in **Table 1**. Participants who completed the study had significantly longer work experience than those who enrolled at baseline ($p = 0.039$).

Subjective Outcomes

The results of the participants' perceived stress and other psychological measures at baseline and after the 8-week trial are shown in **Tables 2, 3**. Participants showed significant decreases in the levels of self-perceived stress (mean differences: 10.33, SD: 8.12, $p < 0.001$), state and trait anxiety ($p < 0.001$), overall job stress ($p = 0.003$), job burden ($p = 0.037$), insomnia severity ($p < 0.001$), depression ($p = 0.002$), negative emotions (sadness, feeling scared, anger, or disgust), some gastrointestinal symptoms (all $p < 0.01$), and improved mental or physical health ($p < 0.001$). Furthermore, participants showed significant improvements in satisfaction with their quality of life and positive emotions (happiness, acceptance, hopefulness) (all $p < 0.001$). There was no significant difference in blood pressure, but an

TABLE 1 | Comparison of baseline characteristics between participants who enrolled at baseline and those who completed the trial.

	Baseline participants (n = 36)		Trial-completed participants (n = 32)		p-value
	n	%	n	%	
Gender					0.303
Female	20	55.56	19	59.38	
Male	16	44.44	13	40.63	
Shift worker	35	97.20	35	96.88	1.000
Education					0.593
Senior high + vocational school	5	13.90	4	12.50	
College degree	23	63.90	21	65.63	
Master's degree	8	22.20	7	21.88	
	Mean	SD	Mean	SD	p-value
Age	40.14	8.05	40.34	7.59	0.096
Job experiences (years)	5.56	1.73	5.69	1.64	0.039
Years of education	16.21	1.41	16.16	1.44	0.561
Baseline systolic blood pressure	117.03	14.63	117.31	14.86	0.294
Baseline diastolic blood pressure	79.97	11.70	80.16	11.60	0.392
Baseline body mass index	26.09	7.24	25.59	4.69	0.116

TABLE 2 | Comparison of the PSS, JSC, ISI, STAI, PHQ, QLESQ, and Emotional Trait and State of participants between the baseline and endpoint.

	Baseline (n = 36)		8 weeks (n = 36; LOCF)		Mean differences (Endpoint-baseline)		p-value*
	Mean	SD	Mean	SD	Mean differences	SD	
PSS total	34.31	5.39	23.97	6.18	-10.33	8.12	<0.001
STAI							
Total	104.92	13.73	93.19	11.90	-11.72	13.29	<0.001
State	50.83	8.47	44.17	7.18	-6.67	8.08	<0.001
Trait	54.08	6.55	49.03	5.70	-5.06	6.06	<0.001
JSS							
Job stress	71.67	14.64	63.33	13.09	-8.33	15.40	0.003
Control over job	65.45	9.45	66.44	7.71	0.98	9.11	0.521
Job burden	67.48	11.06	64.29	11.17	-3.18	8.79	0.037
Interpersonal relationships	69.18	12.12	68.66	13.76	-0.52	11.75	0.792
Job satisfaction	60.56	13.93	64.44	12.75	3.89	12.48	0.070
Psychological health	45.89	15.31	55.33	13.27	9.44	14.82	0.001
Energy level	38.19	15.08	51.25	15.60	13.06	14.16	<0.001
General health	60.00	11.90	67.11	10.95	7.11	8.92	<0.001
ISI	12.83	6.07	8.94	4.50	-3.89	4.85	<0.001
PHQ	11.11	5.14	7.78	4.68	-3.33	5.84	0.002
QLESQ	46.42	7.28	50.81	6.84	4.39	6.82	<0.001
The Questionnaire of Emotional Trait and State							
Total	89.44	14.09	99.58	11.48	10.14	11.42	<0.001
Happy and acceptance	27.47	4.66	31.28	3.81	3.81	4.14	<0.001
Sad and scared	24.67	4.67	21.33	4.95	-3.33	4.25	<0.001
Angry and disgust	22.36	5.08	20.08	3.81	-2.28	3.93	0.001
Look forward to	11.11	2.03	12.33	1.77	1.22	2.09	0.001

*Changes between the baseline and endpoint were assessed by a paired t-test.

PSS, Perceived Stress Scale; STAI, State Trait Anxiety Index; JSS, Job Stress Scale; ISI, Insomnia Severity Index; PHQ, Patient Health Questionnaire; QLESQ, Quality of Life Enjoyment and Satisfaction Questionnaire; LOCF, Last observation carried forward.

TABLE 3 | Comparison of the VAS of gastrointestinal symptoms and stress of participants between the baseline and endpoint.

	Baseline (n = 36)		8 weeks (n = 36; LOCF)		Mean differences (Endpoint-baseline)		p-value*
	Mean	SD	Mean	SD	Mean differences	SD	
VAS of gastrointestinal symptoms							
Total	24.47	16.65	16.28	11.96	-8.19	12.91	0.001
Dry mouth	4.28	2.89	3.42	2.62	-0.86	2.71	0.064
Difficulty swallowing	1.17	1.77	1.08	2.09	-0.08	1.61	0.758
Decreased appetite	2.50	2.58	1.33	1.79	-1.17	2.85	0.019
Nausea or vomiting	1.08	1.65	0.75	1.34	-0.33	1.37	0.154
Flatulence	3.69	2.85	2.56	2.71	-1.14	2.77	0.019
Gastralgia	2.78	3.21	1.94	2.63	-0.83	2.29	0.036
Upper abdominal pain	1.61	2.36	0.81	1.51	-0.81	1.95	0.018
Lower abdominal pain	1.72	2.29	0.97	1.56	-0.75	2.03	0.034
Constipation	2.86	3.07	2.06	2.37	-0.86	1.79	0.010
Diarrhea	2.78	2.96	1.36	1.69	-1.42	2.57	0.002
VAS of stress	6.44	1.61	5.22	1.74	-1.22	1.84	<0.001

*Changes between the baseline and endpoint were assessed by a paired t-test.
VAS, Visual Analog Scale.

TABLE 4 | Comparison of the TAP results (seconds) of participants between the baseline and endpoint.

	Baseline (n = 36)		8 weeks (n = 36; LOCF)		Mean differences (Endpoint-baseline)		p-value*
	Mean	SD	Mean	SD	Mean differences	SD	
TAP							
Flexibility letter total	439.44	73.52	442.44	72.26	3.00	85.39	0.834
Flexibility number total	451.56	77.19	448.89	78.30	-2.67	85.49	0.853
Go/No go	406.64	66.25	409.06	60.84	2.42	48.96	0.769
Working memory	686.31	122.12	688.92	145.13	2.61	111.41	0.889

*Changes between the baseline and endpoint were assessed by a paired t-test.
TAP, Tests of Attentional Performance.

increase in body weight (mean difference: 0.64 kg, $p = 0.029$) was noted.

Objective Outcomes

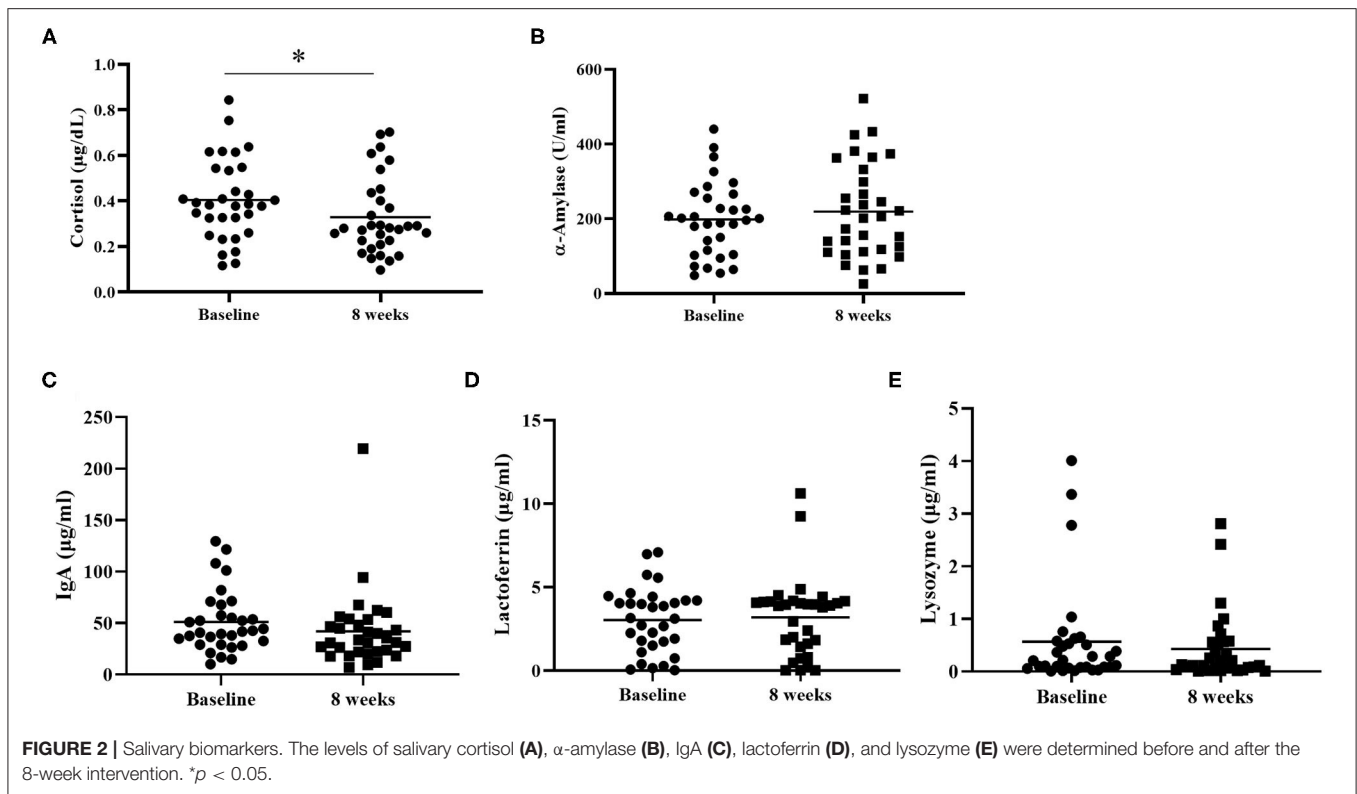
Table 4 shows the TAP results before and after the trial. No significant differences in flexibility, Go/No go, or working memory were found. Comparison of salivary stress biomarkers before and after the trial are shown in **Figure 2**. Cortisol levels were significantly decreased after the 8-week intervention period ($p < 0.05$). All other stress- and anxiety-related biomarkers were not significantly altered after the intervention.

DISCUSSION

This is the first study to evaluate the effectiveness of the psychobiotic, PS128TM, on stress and the psychological well-being of highly stressed IT specialists. Our findings revealed that PS128TM administration improved self-perceived stress, overall job-related stress, job burden, cortisol levels, general or physical health, anxiety, depression, sleep disturbances, quality of life, and both positive and negative emotions after the 8-week

PS128TM trial period. These outcomes suggest that PS128TM has the distinct advantage of providing stress relief and improving mental health in employees under high work-related stress.

The decrease in PSS scores by more than 20% compared to baseline indicated that PS128TM may play a role in sensitivity and perception of pressure in highly stressed workers. This result is in line with previous research describing a significant decrease in stress symptoms after probiotic intake (54, 55), but in contrast with another finding that there was no change in stress over time among healthy volunteers (20). This disparity may be due to the heterogeneity of the study population or differences in the probiotic strains administered. Few studies have focused on the possible effects of stress regulation by probiotics in the subclinical population of highly stressed workers, and the biological mechanisms remain to be established. It is probable that probiotics affect the central nervous system (CNS) while maintaining neuronal development through the neuroendocrine system, immune system, vagus nerves, neurotransmitters, and their receptors (13). From the findings of rodent models, we may speculate that changes in emotions and sensations in response to stress were associated with alterations in vagal



afferent signaling, or by systemic metabolic changes associated with amino acids and polysaccharides (56, 57). Elucidating the underlying mechanism still requires further investigation by assessing larger controlled samples.

The significant decrease in depressive symptoms and negative emotions observed in our study were in agreement with previous studies reporting small, but significant reductions in depression (17). Furthermore, the significant decreases in both state and trait anxiety after probiotic administration were similar to those of a previous study that revealed significant reductions in anxiety scores among pregnant and postpartum women (18), but in contrast with other reports of non-significant differences after probiotic use in healthy volunteers with anxiety symptoms (17). This inconsistency may be because this study assessed highly stressed IT specialists who may have already had some subclinical psychological burden, whereas most previous research excluded participants with psychiatric conditions that required clinical attention, and included community samples with no or only low levels of anxiety that did not reach clinical diagnosis (17). From our results, it remains to be elucidated whether the alleviation of perceived stress was secondary to positive improvements in depression, anxiety, insomnia, and positive or negative emotions, or vice versa. Of note, improvements in perceived quality of life and positive emotions of “happiness” and “hopefulness” may suggest explanations other than the aspect of resilience, which differs from the traditional disease treatment model. In particular, we found a significant correlation between the decrease in cortisol level and increase in positive emotions. It has been suggested that possible mechanisms for the positive effects of

probiotics on the CNS may include their ability to regulate mood or emotions by influencing the HPA axis, altering neural signaling pathways, or CNS neurotransmitters levels of serotonin and gamma-aminobutyric acid (GABA) (58, 59), or regulating inflammatory or immune responses by the gut microbiota (60). However, we did not find significant correlations between changes in salivary cortisol levels and changes in depression, anxiety, or perceived stress. Although this might indicate that the main mechanisms by which PS128TM alleviates depression or anxiety may not be strongly associated with the HPA axis, other possibilities of decreased intestinal permeability or anti-inflammatory effects by bacterial colonization still need to be taken into consideration (61). Further studies with larger sample sizes, placebo comparisons, and examination of inflammatory or neuroendocrinal biomarkers are needed to clarify whether the improvements may be due to adaptive immune, metabolic, or neural pathways.

Our finding that the reduction in insomnia severity was complemented by the decrease in salivary cortisol levels, which might indicate possible correlations between probiotic use and sleep improvements through the HPA axis. This finding was in concordance with studies that have shown that some psychobiotics have sleep-improving effects in mice (62) or humans (63, 64), but in contrast with another study that found that the psychobiotic strain JB-1 did not improve sleep compared to the placebo in healthy male volunteers (19). Previous studies have shown that cortisol may inhibit the syntheses of interleukin-1beta (IL-1 β) or tumor necrosis factor alpha (TNF- α) (65). Presence of these cytokines in the human blood were

found to influence the circadian rhythm and nonrapid eye movements (nREM) sleep. A weak immune response against bacterial cell wall components of PS128TM may help decrease cortisol and increase cytokine secretion (21, 22, 66). Such changes may contribute to more nREM sleep and better sleep quality. However, excessive cytokines might still disturb the sleep structure (61, 67). Other proposed explanations for the sleep-improving effects of PS128TM on the regulation of wakefulness or sleep might be related to several neurotransmitters, such as the increase in GABA (68), decrease in orexin levels or increase in inhibitory adenosine levels in the ventrolateral preoptic nucleus, striatum, hypothalamus, hippocampus, and prefrontal cortex (62, 69). Other non-invasive methods for monitoring changes in sleep stages may be required to clarify how PS128TM affects the sleep structure among highly stressed individuals.

We found that salivary cortisol levels decreased after the 8-week probiotics trial, but other biomarkers, such as α -amylase, IgA, lactoferrin, or lysozyme did not significantly deviate from baseline levels. Similarly, no significant differences were noted when comparing the cognitive performance such as attention and working memory between the baseline and endpoint. α -Amylase was found to be a surrogate marker for sympathetic activities (70). Our findings may indicate that improvements in perceived stress or mood symptoms were not specifically associated with acute adrenergic responses. The lack of a significant change in objective measures of stress, including IgA, lactoferrin, lysozyme, or attentional performance after probiotic treatment might indicate other underlying mechanisms that we were unable to determine in the current study. Explanations for our findings may be restricted by the small sample size and the open-label design, and future investigations that assess more objective biological plasma biomarkers or brain imaging studies are warranted.

The major strength of this study is the use of well-validated stress, psychological, or salivary measures to extensively assess the relevant aspects. Key limitations include the small sample size and open-label design. Further larger-scale randomized, placebo-controlled studies may be needed to demonstrate the effectiveness of PS128TM against stress. Second, although we specifically recruited participants with high perceived stress, our study sample may only represent highly stressed workers in the IT industry. Extension of the generalizability to other occupations may be restricted. Despite that our results from this single arm pilot study provided further justification on the safety and tolerability profiles of PS128TM, owing to the above limitations, sub-project 1, the four-arm randomized controlled trial among high-stress registered nurses is currently undergoing. We aimed that results from this double blind, placebo-controlled trial with larger sample size of at least 60 people in each arm may be powered to overcome these shortcomings. Third, although we assessed many self-reported psychological conditions, objective evaluations of mental health state or direct examinations of physiological or psychological responses under acute stressors may further extend our understanding of probiotics and stress-induced reactions.

Our study demonstrated that oral supplementation with PS128TM may improve perceived levels of overall or job-related

stress, as well as mental health states among highly stressed IT specialists. Good tolerability and safety profiles have also been revealed. As for the direction of future research, in addition to conducting randomized controlled trials with larger sample sizes, investigations on gut permeability, types of gastrointestinal microbiota, production rates of short chain fatty acids, brain images for functional or structural changes, and other biological markers associated with the HPA axis or inflammation such as cytokines may also help elucidate possible mechanism of action of probiotics in highly stressed specialists.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Mackay Memorial Hospital (IRB no: 19CT013be). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

S-IW, C-CW, and P-JT conceived and designed study. C-CH and I-KS assisted with the methodology. S-IW and W-LC performed the investigation. S-IW, P-YC, L-HC, T-WL, C-JK, and W-LC curated and analyzed the data. S-IW interpreted results and drafted the manuscript. S-IW, C-CW, P-YC, and C-JK reviewed and edited the manuscript. Y-CT supervised the study. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest: C-CW, P-JT, L-HC, and C-CH are employees of Bened Biomedical Co., Ltd. Y-CT owns stock in Bened Biomedical Co., Ltd. The views of this article reflect those of the authors and not necessarily those of the funder.

The remaining 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.

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