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***Bifidobacterium* and *Lactobacillus* can beneficially affect the host by producing acetic acid and lactic acid, which lower pH and thereby inhibit the growth of pathogens or allow the probiotic bacteria to compete with pathogens for epithelial adhesion sites and nutrients. The transmural migration of enteric organisms into the peritoneal cavity can cause peritonitis in peritoneal dialysis (PD) patients. We hypothesized that the composition of the intestinal microbiota with regard to *Lactobacillus* species and *Bifidobacterium* species differed between PD patients and healthy controls. The aim of the study was to investigate these differences by real-time PCR analysis of fecal samples. From 1 August 2009 to 31 March 2010, a total of 29 nondiabetic PD patients and 41 healthy controls from China Medical University Hospital were recruited after giving their informed consent. Fecal samples were collected from the PD patients and their age-matched counterparts in the morning using a standardized procedure. DNA extracted from these samples was analyzed by real-time PCR. All bifidobacteria, *Bifidobacterium catenulatum*, *B. longum*, *B. bifidum*, *Lactobacillus plantarum*, *L. paracasei*, and *Klebsiella pneumoniae* were less frequently detected in the patient samples. Dysbiosis (microbial imbalance) may impair intestinal barrier function and increase host vulnerability to pathogen invasion. Further studies are necessary to confirm our findings before clinical trials with probiotic supplementation in PD patients.**

There is a large, complex, and diverse microbial community in the human intestine. The intestinal microbiota plays an important role in digesting food, metabolizing endogenous and exogenous compounds, and producing essential vitamins. It also stimulates the immune system and prevents the colonization of the gastrointestinal tract by pathogens, and hence it influences human health (7, 9). The gastrointestinal microbiota of an adult human consists of more than 500 species, with 10^{11} to 10^{12} CFU per gram of stool (12, 25). The predominant microorganisms are non-spore-forming, obligate anaerobes, such as *Bacteroides*, *Fusobacterium*, *Eubacterium*, and *Bifidobacterium* species. Other anaerobic bacteria found in large numbers include *Lactobacillus* species, various anaerobic Gram-positive cocci, and *Clostridium* species (4). Hida et al. studied the fecal flora of hemodialysis (HD) patients and healthy controls using traditional plating methods and found quantitative and qualitative differences between the two groups (13). It is plausible to suggest that the chronic inflammatory state in dialysis patients is in part due to a microbial imbalance in the gut, resulting in alteration of proinflammatory cytokines and production of uremic toxins from proteins fermented in the large intestine (16). Moreover, impaired intestinal barrier function in peritoneal dialysis (PD) patients allows enteric organisms to enter the peritoneal cavity by transmural migration and to cause peritonitis (8, 27). Peritonitis occasionally causes death and results in significant morbidity, including catheter loss, transfer to hemodialysis, transient loss of ultrafiltration, and possible permanent membrane damage (22). *Bifidobacterium* and *Lactobacillus* can beneficially affect the host by inhibiting the growth of pathogens through production of acetic acid and lactic acid, which lower pH, or by competing with pathogens for epithelial adhesion sites and nutrients (10).

To the best of our knowledge, no study has investigated the intestinal microbiota in PD patients before. The aim of this study, therefore, was to evaluate the differences in the intestinal microbiota between PD patients and healthy controls by examining fecal samples. We focused on *Bifidobacterium* species, *Lactobacillus* species, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus* species. *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Enterococcus* species are the common bacterial pathogens derived from bowel tissue and causing peritonitis in PD patients (20).

MATERIALS AND METHODS

Study population. Between 1 August 2009 and 31 March 2010, we recruited nondiabetic PD patients and healthy controls from China Medical University Hospital, a tertiary-care medical center in central Taiwan. The inclusion criteria were PD for more than 3 months, absence of diabetes, at least 18 years of age, and an estimated glomerular filtration rate of <15 ml/min. Exclusion criteria were active infectious conditions within the previous 30 days, pregnancy, autoimmune diseases, or the consumption

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TABLE 1 Bacterial strains used in this study

Organism	Strain[a]
B. catenulatum	ATCC 27539
B. longum	ATCC 15707
B. breve	ATCC 15700
B. bifidum	NCDO 1454
L. plantarum	ATCC 14917
L. rhamnosus	ATCC 53103
L. paracasei	ATCC 11582
E. coli	ATCC 25922
K. pneumoniae	ATCC 13883
P. aeruginosa	ATCC 27853
Enterococcus species	DSM 20478
B. fragilis	ATCC 25285

[a] ATCC, American Type Culture Collection; NCDO, National Collection of Dairy Organisms; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen.

of antibiotics or yogurt within 30 days prior to sample collection. The protocol of this study was approved by the Institutional Review Board of China Medical University Hospital.

Fecal samples were collected from the PD patients and age-matched adults in the morning after they gave their informed consent to participate in the study. The samples were put immediately on ice and processed within 1 h after defecation.

DNA extraction from fecal samples. One gram of fecal sample was suspended in a solution containing 10 ml of normal saline and homogenized by vortexing for 1 min. The slurry was filtered through sterile gauze to remove any large particles and debris. One ml of filtered fecal solution was placed into a tube and centrifuged at 15,000 rpm for 2 min. The supernatants were discarded, and the pellets were treated with 20 μl of 6-mg/ml lysozyme and 30 μl of 50-U/ml mutanolysin at 37°C for 2 h, then with 0.1 ml of 10% sodium dodecyl sulfate and 80 μl of benzyl chloride at 60°C for 2 h, and finally with 80 μl of chloroform. After centrifugation at 12,500 rpm for 2 min, the supernatants were collected, and DNA was obtained by alcohol precipitation. Finally, the DNA was suspended in 50 μl of deionized water and stored at −80°C before use.

Bacterial strains. All bacterial strains are listed in Table 1.

Species-specific quantitative real-time PCR. The primers and probes for the 5′ nuclease assays were based on sequences of the 16S-23S intergenic spacer regions of the different *Bifidobacterium* species and *Lactobacillus* species according to previous studies (10, 11).

In addition, the primers and probes for *Escherichia coli* and *Bacteroides fragilis* were based on 16S rRNA gene sequences, while those for *Klebsiella pneumoniae* were based on the *phoE* gene sequence. The primers and probes for *Pseudomonas aeruginosa* and *Enterococcus* species were based on 23S rRNA gene sequences. Species-specific sequences were used to design primers and probes for *Bifidobacterium breve*, *B. bifidum*, *B. catenulatum*, *B. longum* (11), *Lactobacillus plantarum*, *L. rhamnosus*, *L. paracasei* (10), *E. coli* (14), *K. pneumoniae* (28), *P. aeruginosa* (26), *Enterococcus* species (6), and *B. fragilis* (33). All lactobacilli, all bifidobacteria, and total bacteria were also detected using previously designed primers and probes (10, 11). Table 2 shows the primers and probes used.

TaqMan minor-groove-binding probes were used to increase the specificity and the sensitivity of the assays. Extracted DNA, primers, and probes were added to TaqMan Universal PCR Mastermix (Applied Biosystems, Foster City, CA) to a final concentration of 1 μM for each primer and 250 nM for the TaqMan probes.

Real-time PCRs were performed in a Roche LightCycler 480. The temperature profile for the amplification was 10 min at 95°C, followed by 60 cycles of 20 s at 95°C, 1 min at 60°C, and 1 s at 72°C. Real-time PCR quantification of species-specific DNA was performed using a standard curve generated from the dilution series of a reference strain (Table 1).

Statistical analysis. Values are expressed as means ± standard deviations. Variables were compared using the chi-square test or Fisher's exact test for categorical variables. Independent *t* and Mann-Whitney *U* tests were used for comparison of continuous variables. All analyses were performed using SPSS statistical software (version 12.0 for Windows; SPSS, Inc.). A *P* value of <0.05 was considered significant.

RESULTS

Twenty-nine PD patients (mean age, 53.7 ± 11.7 years) and 41 healthy controls (58.2 ± 12.8 years) were enrolled ($P = 0.1$) (Table 3). There was no between-group difference in gender and mean fecal weight. The major etiology of end-stage renal disease was chronic glomerulonephritis, accounting for 82.8% of cases. The mean duration of PD was 49.7 ± 35.4 months.

Table 4 shows the detection limits. The detection limits ranged from 0.05 to 2.53 CFU/ml, which are similar to those in a previous report (10). Table 5 presents the mean colony numbers in samples from colonized patients and the percentages of real-time-PCR-positive fecal samples. Fecal samples from PD patients were less likely to be positive for all bifidobacteria ($P < 0.001$), *B. catenulatum* ($P = 0.003$), *B. longum* ($P < 0.001$), *B. bifidum* ($P = 0.001$), *L. plantarum* ($P = 0.001$), *L. paracasei* ($P < 0.001$), and *K. pneumoniae* ($P = 0.004$). However, fecal samples from both groups were equally likely to contain *B. breve*, all *Lactobacillus* spp., *E. coli*, *P. aeruginosa*, *Enterococcus* species, and *B. fragilis*. For PD patients, the mean colony count in samples from colonized patients was lower for all bacteria ($P < 0.001$), *L. plantarum* ($P = 0.014$), *K. pneumoniae* ($P = 0.005$), and *Enterococcus* species ($P < 0.001$) and higher for *P. aeruginosa* ($P = 0.029$). In addition, *L. rhamnosus* was not detectable in either group. Figure 1 shows the distribution of bacterial counts in samples from both groups.

DISCUSSION

To the best of our knowledge, this study is the first to analyze intestinal microbiotas by real-time PCR in PD patients. The natural intestinal microbiota is disrupted in uremic patients. In 1978, Simenhoff et al. reported increased numbers of both aerobic (10^6 bacteria/ml) and anaerobic (10^7 bacteria/ml) organisms in the duodenum and jejunum of patients with chronic kidney disease but very low numbers or no bacterial colonies in healthy controls (29). Hida et al. found no significant difference in the total number of bacteria in the colon of hemodialysis (HD) patients and healthy controls using traditional plating methods to analyze fecal samples (13). However, the HD group had increased numbers of intestinal aerobic bacteria (*E. coli*, *K. pneumoniae*, and *Enterococcus*) and intestinal anaerobic *Clostridium perfringens* and decreased numbers of intestinal anaerobic bacteria (*Bifidobacterium* species).

In our study, fecal samples from PD patients were less likely to contain all *Bifidobacterium* species, *B. catenulatum*, *B. longum*, *B. bifidum*, *L. plantarum*, *L. paracasei*, and *K. pneumoniae*. In adults, *B. catenulatum*, *B. longum*, and *B. bifidum* are common intestinal *Bifidobacterium* species (17, 18), whereas *L. plantarum* and *L. paracasei* are common intestinal *Lactobacillus* species (1, 35). In contrast to Hida's study, our study found no significant between-group differences in the rate of *E. coli* detection. Moreover, the detection rate and bacterial counts for *K. pneumoniae* were lower in PD patients.

Several possible mechanisms may account for changes in the intestinal microbiota in uremic patients. Kalantar-Zadeh et al. showed that HD patients eat less dietary fiber than healthy controls (12.4 ± 5.8 g/day versus 17.9 ± 10.6 g/day; $P < 0.05$) (15).

TABLE 2 Primers and probes used in the real-time PCR

Microorganism	Primer or probe	Sequence (5′→3′)	GenBank accession no.	Reference
All bacteria	Forward	TCCTACGGGAGGCAGCAGT	GQ893232.1	11
	Reverse	GGACTACCAGGGTATCTAATCCTGTT		
	Probe	CGTATTACCGCGGCTGCTGGCAC		
All *Bifidobacteria*	Forward	GGGATGCTGGTGTGGAAGAGA	DQ298393.1	11
	Reverse	TGCTCGCGTCCACTATCCAGT		
	Probe	TCAAACCACCACGCGCCA		
B. catenulatum	Forward	GTGGACGCGAGCAATGC	U09522.1	11
	Reverse	AATAGAGCCTGGCGAAATCG		
	Probe	AAGCAAACGATGACATCA		
B. longum	Forward	TGGAAGACGTCGTTGGCTTT	AP010890.1	11
	Reverse	ATCGCGCCAGGCAAAA		
	Probe	CGCACCCACCGCA		
B. breve	Forward	GTGGTGGCTTGAGAACTGGATAG	AJ245850.1	11
	Reverse	CAAAACGATCGAAACAAACACTAAA		
	Probe	TGATTCCTCGTTCTTGCTGT		
B. bifidum	Forward	GTTGATTTCGCCGGACTCTTC	U09517.1	11
	Reverse	GCAAGCCTATCGCGCA		
	Probe	ACTCCGCTGGCAACACAAATCATCA		
All *Lactobacillus* spp.	Forward	TGGATGCCTTGGCACTAGGA	AY365115.1	10
	Reverse	AAATCTCCGGATCAAAGCTTACTTAT		
	Probe	TATTAGTTCCGTCCTTCATC		
L. plantarum	Forward	TGGATCACCTCCTTTCTAAGGAAT	AB362387.1	10
	Reverse	TGTTCTCGGTTTCATTATGAAAAAATA		
	Probe	ACATTCTTCGAAACTTTGT		
L. rhamnosus	Forward	CGGCTGGATCACCTCCTTT	AP011548.1	10
	Reverse	GCTTGAGGGTAATCCCCTCAA		
	Probe	CCTGCACACACGAAA		
L. paracasei	Forward	ACATCAGTGTATTGCTTGTCAGTGAATAC	AJ542563.1	10
	Reverse	CCTGCGGGTACTGAGATGTTTC		
	Probe	TGCCGCCGGCCAG		
E. coli	Forward	CATGCCGCGTGTATGAAGAA	JF412031.1	14
	Reverse	CGGGTAACGTCAATGAGCAAA		
	Probe	TATTAACTTTACTCCCTTCCTCCCCGCTGAA		
K. pneumoniae	Forward	CCTGGATCTGACCCTGCAGTA	AF009172.1	28
	Reverse	CCGTCGCCGTTCTGTTTC		
	Probe	CAGGGTAAAAACGAAGGC		
P. aeruginosa	Forward	TCCAAGTTTAAGGTGGTAGGCTG	AJ549386.1	26
	Reverse	CTTTTCTTGGAAGCATGGCATC		
	Probe	AGGTAAATCCGGGGTTTCAAGGCC		
All *Enterococcus* spp.	Forward	AGAAATTCCAAACGAACTTG	HM007611.1	6
	Reverse	CAGTGCTCTACCTCCATCATT		
	Probe	TGGTTCTCTCCGAAATAGCTTTAGGGCTA		
B. fragilis	Forward	TCRGGAAGAAAGCTTGCT	AB618793.1	33
	Reverse	CATCCTTTACCGGAATCCT		
	Probe	ACACGTATCCAACCTGCCCTTTACTCG		

TABLE 3 Characteristics of the PD patients ($n = 29$) and healthy controls ($n = 41$)

Characteristic	PD patients	Controls	*P* value
Age (yr)	53.7 ± 11.7[a]	58.2 ± 12.8[a]	0.13
Gender (F/M)	19/10	26/15	0.86[b]
No (%) with ESRD[c] etiology			
Chronic glomerulonephritis	24 (82.8)		
Tubulointerstitial nephritis	3 (10.3)		
Hypertension	2 (6.9)		
Duration of dialysis (mo) (range)	49.7 ± 35.4[a] (3–135)		
Fecal wt (grams)	0.9 ± 0.6[a]	1.1 ± 0.9[a]	0.28

[a] Mean ± standard deviation.
[b] Chi-square test.
[c] End-stage renal disease.

Although there was no significant between-group difference in protein intake, potassium restriction compels uremic patients to reduce vegetable and fruit intake and thereby fiber intake. In addition, colon transit times are longer in uremic patients. The prevalence of constipation is as high as 63% in HD patients and 29% in PD patients, compared to 10 to 20% in healthy controls (36). Inactivity, phosphate binders, potassium absorbents, water restriction diet, lack of fiber intake, and comorbidities such as diabetes may play roles in the high prevalence of constipation in uremic patients. Because bacterial growth is promoted by peristalsis, which mixes fibers with bacteria widely, increased colon transit time is correlated with reduced bacterial cell mass in stool (3, 30). The populations of lactobacilli and bifidobacteria are reduced among adults with chronic constipation (23). In our study, the rate of constipation was 31.0%. The lower counts of all bacteria and *K. pneumoniae* in PD patients might be due to a longer colon transit time. We excluded diabetic patients to eliminate the confounding effect of diabetes. Moreover, markedly increased concentrations of urea, creatinine, and other nitrogen metabolites accumulate in the blood and diffuse into the gut, where they become accessible to microbial metabolism. Fecal ammonia is mainly derived from bacterially mediated hydrolysis of urea. Thus, a high level of ammonia is responsible for elevation of luminal and fecal pH, which might lead to changes in the intestinal microbiota in uremic patients (4).

TABLE 4 Detection limits of target organisms

Target organism	Detection limit (CFU/ml)
All bacteria	0.54
Bifidobacterium spp.	1.31
B. catenulatum	0.48
B. longum	1.44
B. breve	0.96
B. bifidum	0.05
Lactobacillus spp.	1.20
L. plantarum	1.96
L. paracasei	1.35
E. coli	1.30
K. pneumoniae	2.53
P. aeruginosa	1.92
Enterococcus spp.	0.37
B. fragilis	1.02

TABLE 5 Comparison of the fecal microbiotas of PD patients and controls

Fecal flora	PD patients ($n = 29$)		Controls ($n = 41$)	
	Log_{10} CFU/gram stool[a]	No. of positive samples (%)	Log_{10} CFU/gram stool[a]	No. of positive samples (%)
All bacteria	8.9 ± 1.8[a]	29 (100)	10.7 ± 1.1	41 (100)
Bifidobacterium spp.	7.0 ± 3.5	16 (55.2)[b]	8.3 ± 1.8	41 (100)
B. catenulatum	6.0 ± 3.6	8 (27.6)[c]	6.9 ± 2.5	26 (63.4)
B. longum	8.1 ± 1.8	4 (13.8)[b]	9.5 ± 0.9	23 (56.1)
B. breve	4.7	1 (3.4)	7.8 ± 1.7	3 (7.3)
B. bifidum	5.2 ± 3.0	8 (27.6)[c]	6.5 ± 1.8	28 (68.3)
Lactobacillus spp.	4.8 ± 1.2	28 (96.6)	5.2 ± 1.1	41 (100)
L. plantarum	2.7 ± 1.7[d]	3 (10.3)[c]	5.1 ± 0.8	19 (46.3)
L. paracasei	4.9 ± 1.4	17 (58.6)[b]	5.7 ± 1.1	39 (95.1)
E. coli	5.7 ± 1.9	29 (100)	5.5 ± 2.0	41 (100)
K. pneumoniae	5.2 ± 1.3[d]	19 (65.5)[c]	6.3 ± 1.5	38 (92.7)
P. aeruginosa	4.8 ± 0.6[d]	7 (24.1)	3.9 ± 0.8	8 (19.5)
Enterococcus spp.	4.2 ± 1.7[a]	22 (75.9)	6.1 ± 1.8	35 (85.4)
B. fragilis	7.4 ± 1.4	17 (58.6)	6.9 ± 1.3	20 (48.8)

[a] $P < 0.001$ versus controls (Mann-Whitney *U* test).
[b] $P < 0.001$ versus controls (chi-square test).
[c] $P < 0.01$ versus controls (chi-square test).
[d] $P < 0.05$ versus controls (Mann-Whitney *U* test).

Probiotics containing *Bifidobacterium* species and *Lactobacillus* species could beneficially affect the host by suppressing the growth or epithelial invasion of pathogenic bacteria, improving intestinal barrier function, and modulating immune system activity (2, 5, 19, 24). Few studies have assessed the possible benefit of probiotics for patients with chronic kidney disease. The demonstrated benefits in HD patients include reduction of plasma levels of uremic toxins, especially those of indoxyl sulfate and *p*-cresol, inhibition of overgrowth of aerobic bacteria (13, 21, 31), decrease in pre-HD serum levels of homocysteine (probably as a result of folate produced by *B. longum* in the intestine), and normalization of bowel habits (21, 32). Ranganathan et al. (23a) reported that probiotics containing *L. acidophilus*, *B. longum*, and *S. thermophilus* significantly reduced blood urea nitrogen and improved the quality of life without serious side effects in patients with stage 3 and 4 chronic kidney disease. Our scientific evidence justifies proceeding with clinical trials to evaluate the efficacy of oral probiotics in PD patients.

There are several limitations to our study. The small number of patients may have led to the chance occurrence of statistically significant results. In addition, *L. rhamnosus*, a common *Lactobacillus* species, was not detectable in either group. *L. rhamnosus* can

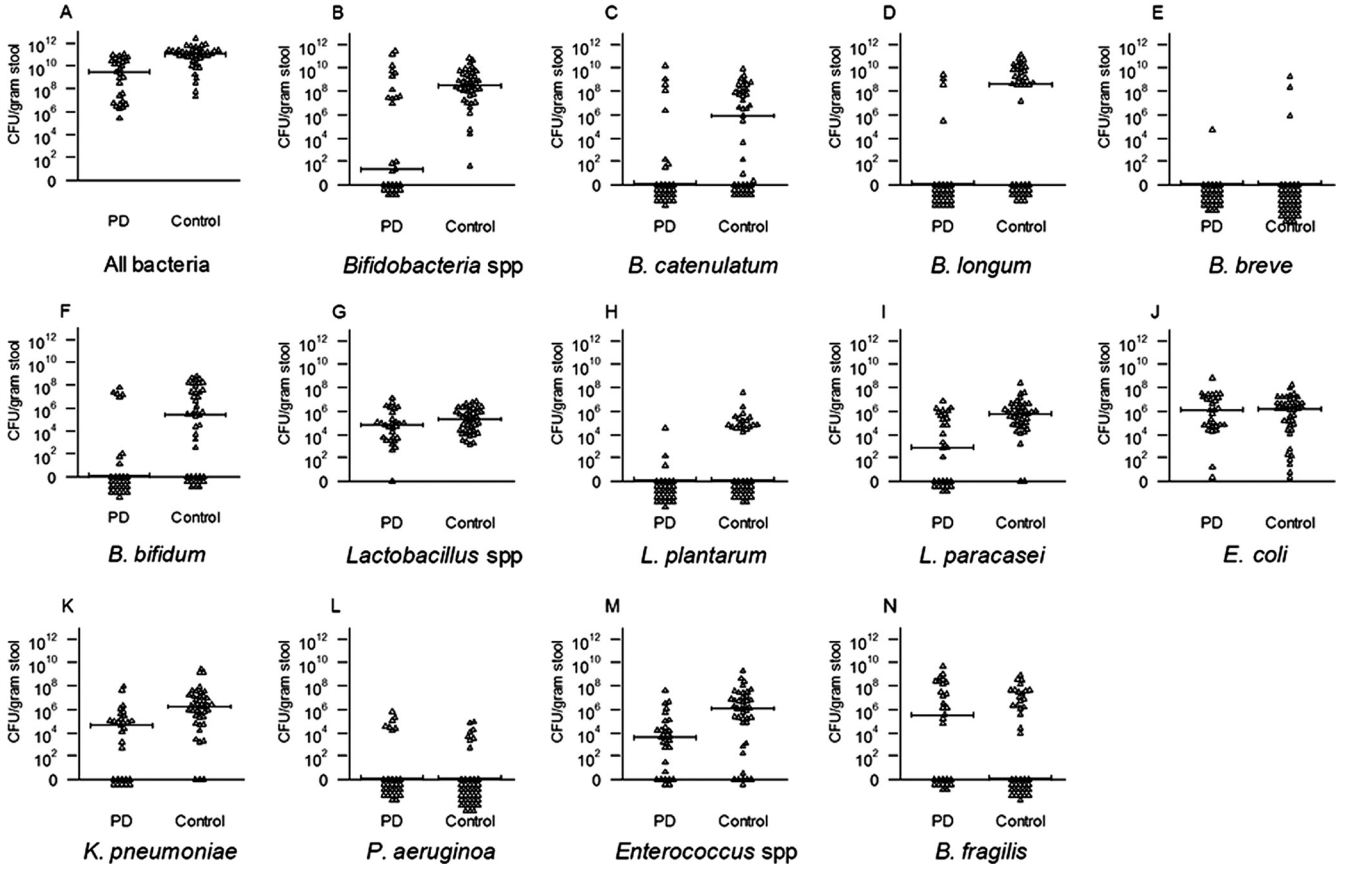

FIG 1 Distribution of bacterial counts in feces from the PD and control groups. Horizontal lines represent median values.

be detected in the feces of infants in Taiwan (34). Dietary change may make it undetectable in adults. Another concern relates to the lack of standardization of diet in both groups. Selective carbohydrate ingestion may alter the gastrointestinal environment (e.g., by changing the pH). Moreover, because the duration of dialysis ranged from 3 to 135 months, differences in gut microbiota may either be due to renal disease or be an effect of PD.

In conclusion, *Bifidobacterium* species, *B. catenulatum*, *B. longum*, *B. bifidum*, *L. plantarum*, and *L. paracasei* were detected at lower rates in PD patients. Dysbiosis might result in impaired intestinal barrier function and increased host vulnerability to pathogen invasion. Further studies are necessary to confirm our findings before clinical trials with probiotics supplementation in PD patients.

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