

Research Article

Alterations of Microorganisms in Tongue Coating of Gastric Precancerous Lesion Patients with a Damp Phlegm Pattern

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Objective. In the research, the microbial changes in the tongue coating of patients with a damp phlegm pattern of gastric precancerous lesion (GPL) were investigated. **Methods.** This was a case-control study, in which 80 tongue coating samples were collected including 40 patients with a damp phlegm pattern of GPL, 20 patients with a nondamp phlegm pattern of GPL, and 20 healthy control people. The 16S rRNA microbiome technology was used to analyze the alterations of microorganisms in tongue coating of GPL patients with a damp phlegm pattern. **Results.** Microorganisms in the genus level were analyzed. Compared with the healthy control group, the relative abundance of 4 microorganisms (Solobacterium, Rothia, Oribacterium, and Alloprevotella) in the GPL group was significantly higher ($P < 0.05$). The relative abundance of 10 microorganisms (Terrisporobacter, Solobacterium, Porphyromonas, Parvimonas, Lactobacillus, Johnsonella, Gemella, Fusibacter, Azoarcus, and Acidothermus) in the GPL damp phlegm pattern group was significantly lower than that in the GPL nondamp phlegm pattern group ($P < 0.05$). In the comparison of phenotype “forms biofilms,” the relative abundance of microorganisms in the GPL group was significantly higher than that in the healthy control group ($P < 0.05$). In the comparison of phenotype “contains mobile elements,” the relative abundance of microorganisms in the GPL damp phlegm pattern group was significantly lower than that in the GPL nondamp phlegm pattern group ($P < 0.05$). In the comparison of microbial metabolic functions, the abundance ratio of “infectious diseases: bacterial” in the GPL group was significantly lower than that in the healthy control group ($P < 0.05$). The abundance ratio of the “excretory system” and “folding, sorting, and degradation” in the GPL group was significantly higher than that in the healthy control group ($P < 0.05$). **Conclusions.** Solobacterium may be a marker microorganism of the GPL damp phlegm pattern. The differential phenotype of microorganisms in tongue coating of the GPL damp tongue pattern is mainly reflected in “forms biofilms” and “contains mobile elements.”

1. Introduction

Gastric cancer is the 3rd most common malignant tumor in the whole world in terms of incidence and mortality and is a serious threat to human health [1]. According to the global cancer statistics in 2018, new gastric cancer accounts for 5.7% of new cancer cases every year, of which more than

40% are in China. From normal tissue to gastric cancer, it usually goes through several stages: chronic gastritis, atrophy, gastrointestinal epithelial metaplasia, and dysplasia, and finally develops into gastric cancer [2]. Gastric precancerous lesions (GPL) are a kind of histopathological changes of gastric mucosa, mainly including intestinal metaplasia and dysplasia whose developments are considered reversible

[3, 4]. Therefore, early identification and active prevention and treatment of GPL can reduce the incidence rate of gastric cancer with a certain probability. However, modern medicine still lacks ideal treatment for GPL. Numerous clinical reports have confirmed that traditional Chinese medicine (TCM) treatment can reduce and remove some intestinal epithelial metaplasia and dysplasia and has certain curative effect on both symptom improvement and pathological reversal [5].

According to the theory of TCM, distinguishing the TCM patterns of GPL correctly is fundamental to treat the disease effectively by using Chinese medicine. One of the common TCM patterns of GPL is damp phlegm pattern. And the change of tongue coating appearance is one of the most significant diagnostic criteria of the GPL damp phlegm pattern. There are many pathogenic commensal bacteria in the human body. In a healthy state, these microorganisms exist in the form of symbiosis. It plays an important role in human's nutrition absorption, energy metabolism, immune function, and other physiological activities. The diversity and abundance of these microorganisms will also change relatively under unhealthy conditions, leading to the formation and development of multiple diseases, such as gastric cancer and other tumor diseases [6]. Oral microorganism is an important part to change the balance between oral and systemic health and disease. In the oral cavity, the morphological structure of the tongue surface allows the formation of a unique bacterial biofilm. Therefore, tongue coating has been considered the most complex ecological biofilm niche in the mouth [7]. In the previous study, it was found that there were different microorganisms in the tongue coating between GPL patients and healthy people, and the metabolites in the tongue coating of GPL patients with a damp phlegm pattern were significantly different from those of the nondamp phlegm pattern [8, 9]. Therefore, whether there are some special microorganisms in the tongue coating of GPL patients with a damp phlegm pattern may affect the formation of metabolites, which is a problem worthy of study.

16S ribosomal RNA (16S rRNA) gene sequencing has become the preferred method to study the composition and distribution of microbial communities [10]. In recent years, this technology has been widely used in the study of microbial diversity and relative abundance in the human body. Therefore, the 16S rRNA microbiome technology is adopted in this study; the changes of microorganisms in tongue coating of GPL patients with a damp phlegm pattern are explored. The significant fluctuations in the species and abundance of these microorganisms may help us better understand the formation and development mechanism of the GPL damp phlegm pattern from various aspects.

2. Materials and Methods

2.1. Samples. From December 2018 to October 2019, 60 patients with GPL voluntarily enrolled in Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine were selected as the GPL group, including 40 cases of the damp phlegm pattern group and 20 cases of the nondamp phlegm pattern group. There were 20 people in the

healthy control group who currently had no stomach discomfort and no history of stomach disease. Their routine physical examination indicators were normal. These indicators included blood cell analysis, liver and renal function, blood lipid, blood glucose, blood pressure, carcinoembryonic antigen, alpha-fetoprotein, color Doppler ultrasound of neck and abdomen, chest computed tomography, and X-ray barium meal. After the tongue coating samples of GPL patients were collected, the gastric mucosae were immediately examined by gastroscopy and pathology.

Table 1 summarizes the demographic and clinical information of all participants. It can be seen from this table that the numbers of GPL patients with a damp phlegm pattern with only mild, moderate, and severe intestinal metaplasia were 31, 7, and 1, respectively. The numbers of GPL patients with a nondamp phlegm pattern with only mild, moderate, and severe intestinal metaplasia were 13, 3, and 4, respectively. In addition, one GPL patient with a damp phlegm pattern had mild intestinal metaplasia and dysplasia at the same time. Among GPL patients with *Helicobacter pylori* (Hp) infection, there were 9 cases of a damp phlegm pattern and 1 case of a nondamp phlegm pattern.

In terms of treatment, there were 8, 10, 17, and 5 GPL patients with a damp phlegm pattern who were not treated, only treated with Western medicine, only treated with traditional Chinese medicine, and treated with integrated traditional and Western medicine, respectively, while there were 2, 5, 5, and 8 GPL patients with a nondamp phlegm pattern corresponding to the above four treatments.

2.2. Ethics Approval. In the study, all subjects gave written informed consent before collecting samples and the study was conducted in accordance with the Declaration of Helsinki. In addition, this study was approved by the Ethics Committee of Shanghai University of TCM in December 2018.

2.3. Criteria. The diagnostic criteria of GPL were as follows:

When the doctor used the endoscope to examine the patient's stomach, a small amount of gastric mucosa was removed from the suspected lesion sites (such as gastric antrum, gastric horn, gastric body, or cardia) for histopathological evaluation, which was conducted by two experienced pathologists according to the clinical guidelines of the "updated Sydney system" [11]. When the pathological evaluation of gastric mucosa showed atrophy with intestinal metaplasia or/and dysplasia, the patient was diagnosed with GPL [12].

The diagnostic criteria of the GPL damp phlegm pattern according to the "Diagnostics of Traditional Chinese Medicine" [13] were as follows:

GPL patients felt full stomach, even nausea, and/or vomiting, and their appetite decreases, accompanied by an unformed stool and greasy tongue coating.

The inclusion criteria were as follows:

- (1) Patients that meet the above diagnostic criteria of the GPL damp phlegm pattern were included

TABLE 1: Summary of demographics and clinical information of the participants.

Demographics and clinical information	Damp phlegm pattern group	Nondamp phlegm pattern group	Healthy control group
Sample number	40	20	20
Ratio of male to female	1:0.82	1:1.22	1:1.86
Average age (year)	43.28 ± 14.73	42.9 ± 16.1	30.95 ± 11.68
Number (percentage) of samples diagnosed for less than 10 years	30 (75.00%)	15 (75.00%)	N/A
Number (percentage) of samples diagnosed for 10–20 years	6 (15.00%)	2 (10.00%)	N/A
Number (percentage) of samples diagnosed for 20–30 years	2 (5.00%)	1 (5.00%)	N/A
Number (percentage) of samples diagnosed for 30–40 years	2 (5.00%)	2 (10.00%)	N/A
Number (percentage) of samples only with intestinal metaplasia (mild)	31 (77.50%)	13 (65.00%)	N/A
Number (percentage) of samples only with intestinal metaplasia (moderate)	7 (17.50%)	3 (15.00%)	N/A
Number (percentage) of samples only with intestinal metaplasia (severe)	1 (2.50%)	4 (20.00%)	N/A
Number (percentage) of samples with intestinal metaplasia (mild) and dysplasia (mild)	1 (2.50%)	0 (0.00%)	N/A
Number (percentage) of samples with <i>Helicobacter pylori</i> infection	9 (22.50%)	1 (5.00%)	N/A
Number (percentage) of samples untreated	8 (20.00%)	2 (10.00%)	N/A
Number (percentage) of samples only taking Western medicine	10 (25.00%)	5 (25.00%)	N/A
Number (percentage) of samples only taking traditional Chinese medicine	17 (42.50%)	5 (25.00%)	N/A
Number (percentage) of samples taking Western medicine and traditional Chinese medicine	5 (12.50%)	8 (40.00%)	N/A

(2) The healthy controls were not found to have systemic organic diseases in routine physical examination

(3) The age range is 20–70 years old

(4) No antibiotics or probiotics were taken before collection

(5) All signed informed consent

The exclusion criteria were as follows:

(1) The patients had other digestive system diseases except gastritis

(2) Patients that are suffering from major organ diseases such as nervous system, circulatory system, and respiratory system were excluded

(3) Patients that are suffering from mental illness were excluded

(4) The female subjects were pregnant or lactating

(5) There were lesions in oral mucosa

(6) The subjects took antibiotics within half a year or probiotics as well as foods containing probiotics within one month before the sample was collected

(7) The subjects who smoke or drink alcohol were excluded

(8) The body mass index (BMI) exceeds 28 [14]

2.4. *Sample Collection and Experimental Methods.* We followed the sample collection and experimental methods of our previous research [8].

Tongue coating samples were collected in the morning, and all the subjects need not eat breakfast before being sampled. When collecting, first let the person to be collected gargle with sterile normal saline for 3 times to ensure that the residue in the mouth is removed as much as possible. Then, we used sterile sample collection swabs (CY-98000, iClean, Huachenyang Technology Co. Ltd., CN) to scrape tongue coating samples five times in the area with thick tongue coating. Finally, the swab head with a tongue coating sample was put into a sterile centrifuge tube and stored in an ultralow temperature refrigerator at -80°C . All tongue coating samples were scraped by the same person to ensure that the force used when scraping tongue coating was as consistent as possible. The patient underwent gastroscopy after the tongue coating sample was collected.

The Power Soil DNA Isolation Kit (MO BIO Laboratories) was used to extract microbial DNA from tongue coating samples. The quality and quantity of DNA were evaluated according to the ratio of 260 nm/280 nm and 260 nm/230 nm. First, the V3-V4 region of the microbial 16S rRNA gene was amplified by combining the adapter sequence and bar code sequence with common primer pairs (forward primer, 5'-ACTCCTACGGGAGGCAGCA-3'; reverse primer, 5'-GGACTACHVGGGTWTCTAAT-3'). Then, PCR amplification was performed. The initial denaturation lasted for 5 minutes at 95°C , followed by lasting for 1 minute cycles at 95°C (15 cycles), 1 minute at 50°C , 1 minute at 72°C , and finally 7 minutes at 72°C . The above is the first round of PCR. In this process, PCR products are

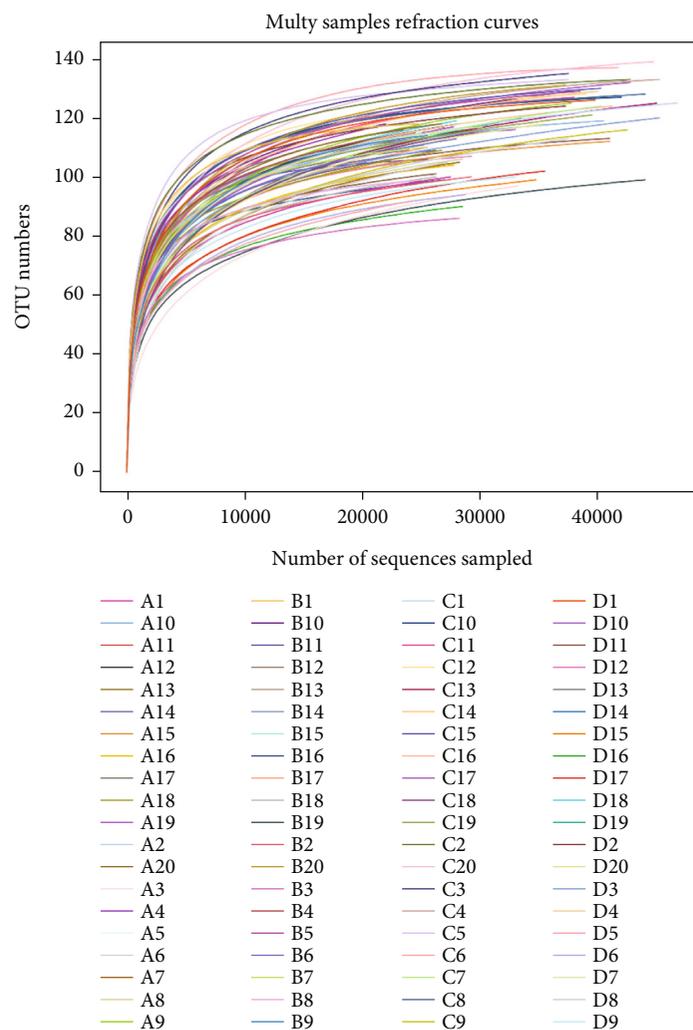


FIGURE 1: Rarefaction curve of each sample. The abscissa was the number of randomly selected sequencing strips, and the ordinate was the number of OTUs obtained by clustering based on the number of sequencing strips. Each curve represented a sample and was marked with different colors. The figure reflected the rate of emergence of new OTUs (new species) under continuous sampling: within a certain range, with the increase of the sequencing number, when the curve showed a sharp rise, it meant that a large number of species were found in the community. When the curve tended to be flat, it indicated that the species in this environment would not increase significantly with the increase of sequencing quantity.

purified by VAHTSTM DNA Clean Beads. Then, the second round of PCR was performed in the 40 μ L reaction. The initial denaturation lasted for half a minute at 98°C, followed by lasting for 10 second at 98°C (10 cycles), half a minute at 65°C, half a minute at 72°C, and finally 5 minutes at 72°C. Finally, Quant-iT™ dsDNA HS Reagent was used to quantify and mix all PCR products. The Illumina Hiseq 2500 platform (2 \times 250 paired ends) was used to perform high-throughput sequencing analysis of microbial rRNA genes on the purified mixed samples.

3. Statistical Analysis

The operational taxonomic unit (OTU) was analyzed by Trimmomatic (version 0.33), UCHIME (version 8.1), and USEARCH (version 10.0). Alpha diversity was calculated by mothur (version v.1.30). The Shannon index was used to measure the diversity of microorganisms. Beta diversity

was calculated by QIIME. The unweighted algorithms named unweighted UniFrac was used to calculate the distance between samples to obtain the beta value. Microbial relative abundance between samples was compared using the Mann–Whitney *U* test. BugBase algorithm was used to predict the biological level coverage and biointerpretable phenotype of functional pathways between the two groups. Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to analyze the differences in metabolic pathways of functional genes between the two groups of microbial communities. The *P* value was corrected by the false discovery rate (FDR) of the rank sum test ($P < 0.05$).

4. Results

4.1. OTU Analysis. Through 16S rRNA gene sequencing of 80 tongue coating samples and subsequent splicing, filtering, and evaluation of tags, we finally obtained 151 OTUs.

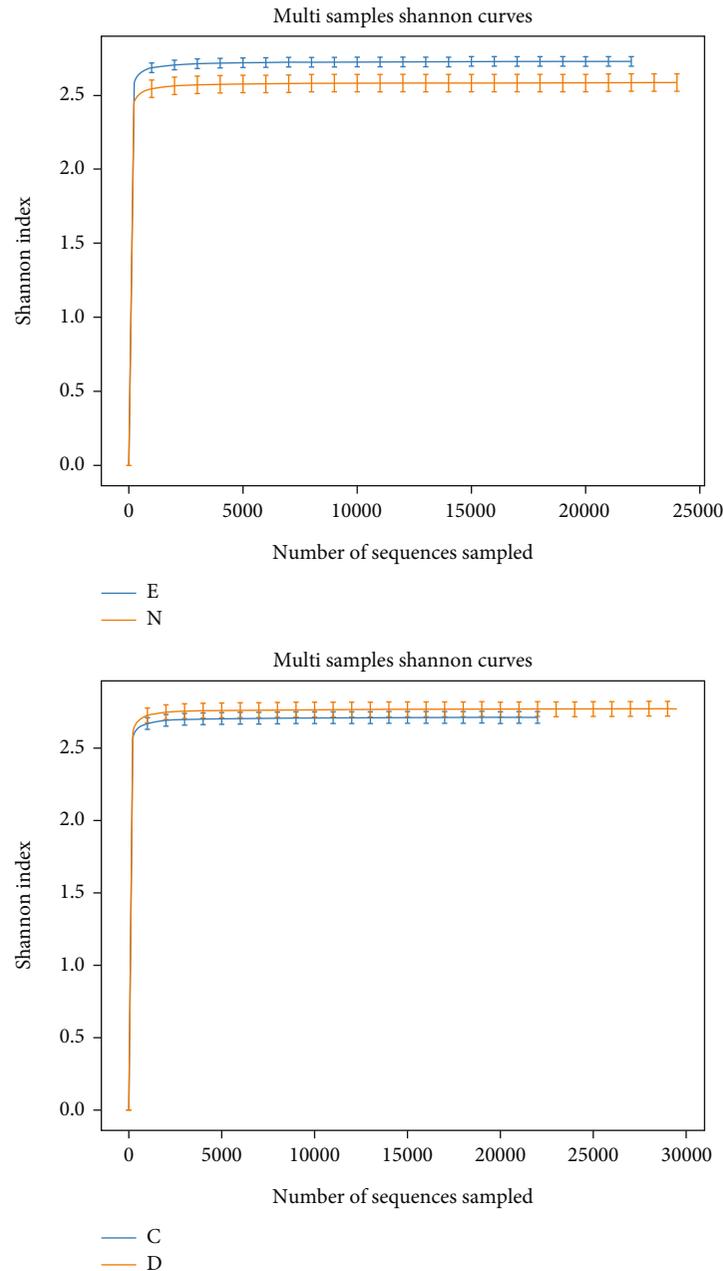
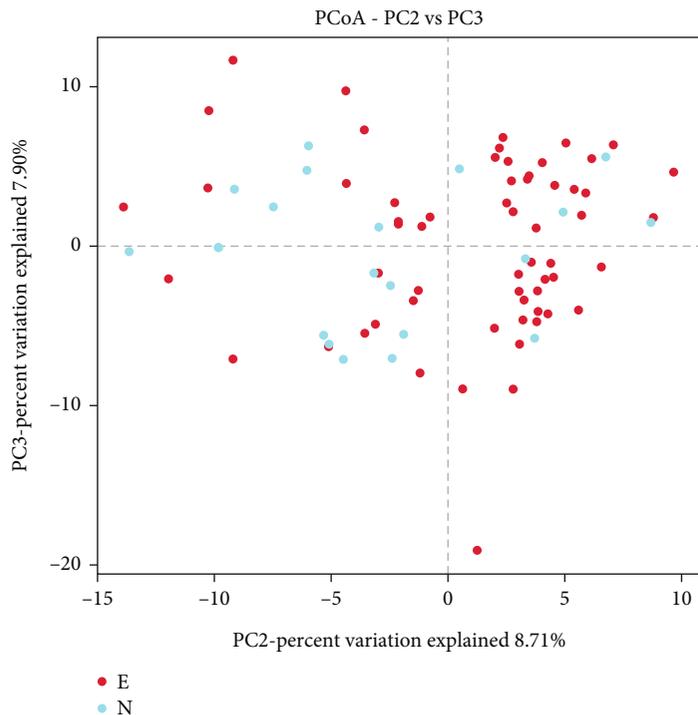


FIGURE 2: Shannon index curve of each group (E: GPL group, N: healthy control group, C: GPL damp phlegm pattern group, and D: GPL non-damp phlegm pattern group). The abscissa was the number of sequencing strips randomly extracted from samples, and the ordinate was the Shannon index. With the increase of sequencing quantity, more species were found. Until the species were saturated, increasing the number of sampling strips could not find new OTUs (new species). The microbial diversity of tongue coating in the GPL group was significantly higher than that in the healthy control group. There was no significant difference in microbial diversity of tongue coating between the GPL damp phlegm pattern group and GPL nondamp phlegm pattern group.

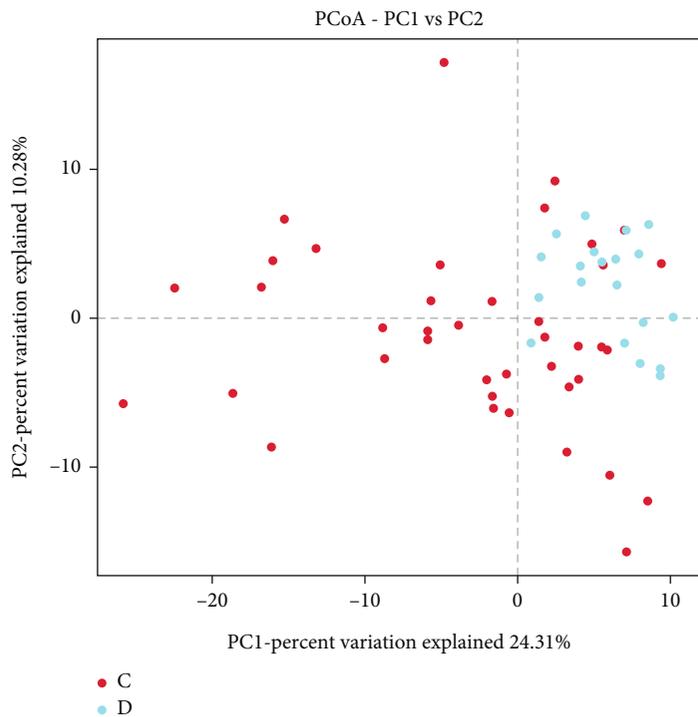
4.2. *Alpha Diversity Analysis.* The rarefaction curve was plotted before analyzing the microbial diversity of tongue coating. The rarefaction curve was formed by randomly sampling a certain number of sequences from the samples, counting the number of species represented by these sequences, and constructing the sequence number and the number of species. The curve was used to verify whether the amount of sequencing data was sufficient to reflect the species diversity in the samples and indirectly reflect the

richness of species in the samples [15]. As shown in Figure 1, the curve representing each sample gradually tended to be gentle, indicating that the sequencing amount of each sample was sufficient and the data diversity analysis can be conducted.

In the alpha diversity analysis, the Shannon index was used to analyze the diversity of the microbiota between the two groups. The Shannon index was affected by species abundance and community evenness in samples. Under

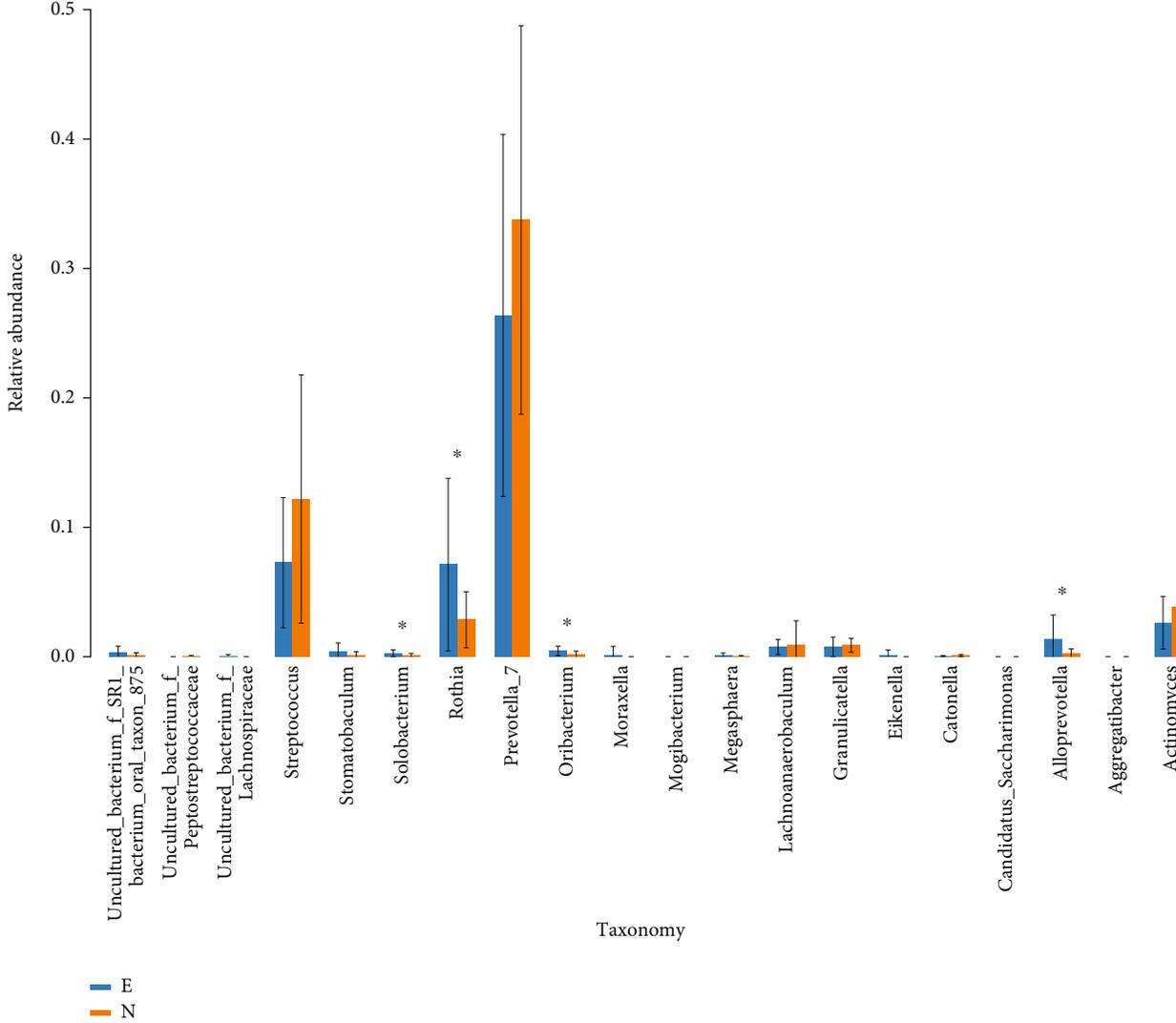


(a)



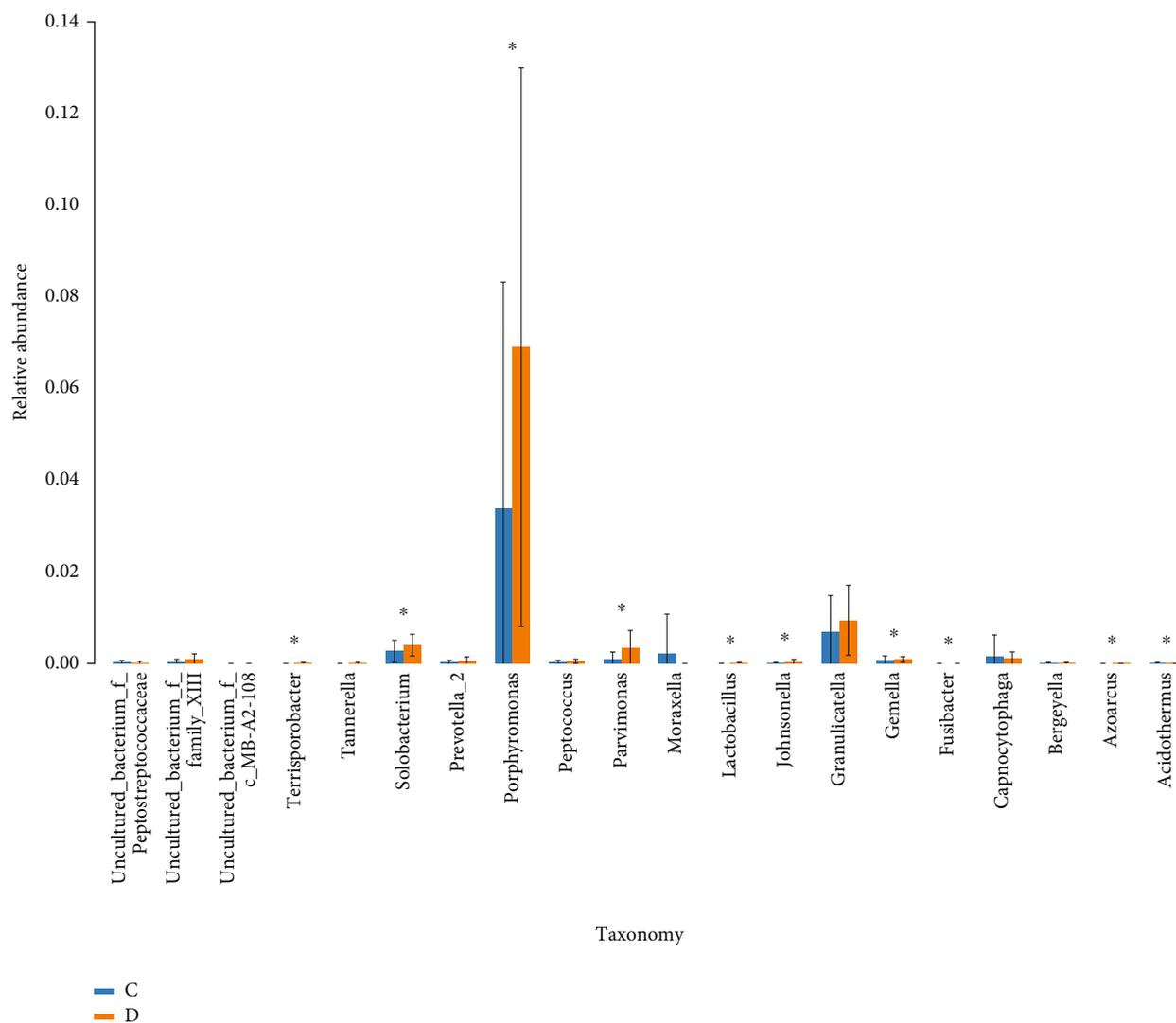
(b)

FIGURE 3: (a) PCoA diagram of the GPL group compared with the healthy control group (E: GPL group, N: healthy control group). (b) PCoA diagram of the GPL damp phlegm pattern group compared with the GPL non-damp phlegm pattern group. (C: GPL damp phlegm pattern group, D: GPL nondamp phlegm pattern group). The dots represented each sample. The abscissa and ordinate were the two characteristic values that lead to the largest difference between samples, and the main influence degree was expressed in the form of percentage. (a) The GPL group and healthy control group had a certain degree of differentiation. (b) The GPL damp phlegm pattern group and GPL non-damp phlegm pattern group had obvious differentiation.



(a)

FIGURE 4: Continued.



(b)

FIGURE 4: (a) Column figure of differential microorganism of the GPL group compared with the healthy control group (E: GPL group, N: healthy control group). (b) Column figure of differential microorganism of the GPL damp phlegm pattern group compared with the GPL nondamp phlegm pattern group (C: GPL damp phlegm pattern group, D: GPL non-damp phlegm pattern group). The abscissa represented the species of microorganisms (showing the top 20 species with the lowest P value), the ordinate represented the relative abundance of species, the columns with different colors represented each sample, and the “*” on the column represented significant difference ($P < 0.05$). (a) The microorganisms named Solobacterium, Rothia, Oribacterium, and Alloprevotella with significant differences in the relative abundance between the GPL and healthy control groups at the genus level. Compared to healthy controls, the relative abundances of four microorganisms were significantly higher in the GPL group ($P < 0.05$). (b) The microorganisms named Terrisporobacter, Solobacterium, Porphyromonas, Parvimonas, Lactobacillus, Johnsonella, Gemella, Fusibacter, Azoarcus, and Acidothermus with significant differences in the relative abundance between the GPL damp phlegm pattern group and the GPL nondamp phlegm pattern group at the genus level. The relative abundances of ten microorganisms were significantly lower in the GPL damp phlegm pattern group compared to those in the GPL nondamp phlegm pattern group ($P < 0.05$).

the same species abundance, the greater the evenness of each species in the community, the greater the diversity of the community. The larger the Shannon index value, the higher the species diversity of the samples [16]. According to the Shannon index (2.73 ± 0.25 vs. 2.59 ± 0.26 , $P = 0.03$), there was significant difference in microbiota diversity between the GPL group and healthy control group, with higher diversity being present in the GPL group. However, the Shannon index (2.71 ± 0.26 vs. 2.77 ± 0.23 , $P = 0.41$) between the

GPL damp phlegm pattern group and GPL nondamp phlegm pattern group indicated that there was no significant difference between the two groups (Figure 2).

4.3. Beta Diversity Analysis. In the beta diversity analysis, principal coordinates analysis (PCoA) was used to analyze the diversity of the microbiota between the two groups. PCoA was a dimension reduction sorting method. By assuming that there was data to measure the difference or

TABLE 2: Microorganisms with significant differences in the relative abundance between the GPL and healthy control groups at the genus level.

Microorganism	GPL group	Healthy control group	<i>P</i> corrected
Solobacterium	$3.19E-03 \pm 2.43E-03$	$1.36E-03 \pm 1.53E-03$	$5.13E-03$
Rothia	$7.16E-02 \pm 6.66E-02$	$2.93E-02 \pm 2.16E-02$	$4.85E-02$
Oribacterium	$4.61E-03 \pm 3.58E-03$	$2.22E-03 \pm 2.73E-03$	$6.47E-03$
Alloprevotella	$1.36E-02 \pm 1.89E-02$	$2.63E-03 \pm 3.56E-03$	$1.08E-03$

TABLE 3: Microorganisms with significant differences in the relative abundance between the GPL damp phlegm pattern and nondamp phlegm pattern groups at the genus level.

Microorganism	Damp phlegm pattern group	Nondamp phlegm pattern group	<i>P</i> corrected
Terrisporobacter	$1.85E-05 \pm 5.30E-05$	$1.16E-04 \pm 1.20E-04$	$8.71E-04$
Solobacterium	$2.74E-03 \pm 2.36E-03$	$4.09E-03 \pm 2.38E-03$	$2.47E-02$
Porphyromonas	$3.38E-02 \pm 4.95E-02$	$6.91E-02 \pm 6.09E-02$	$2.24E-02$
Parvimonas	$9.25E-04 \pm 1.70E-03$	$3.25E-03 \pm 4.07E-03$	$1.11E-02$
Lactobacillus	$3.51E-05 \pm 1.38E-04$	$1.08E-04 \pm 1.73E-04$	$5.33E-03$
Johnsonella	$8.68E-05 \pm 2.47E-04$	$3.61E-04 \pm 6.93E-04$	$1.18E-02$
Gemella	$6.88E-04 \pm 9.99E-04$	$9.85E-04 \pm 5.77E-04$	$2.40E-02$
Fusibacter	$1.74E-05 \pm 5.49E-05$	$4.29E-05 \pm 4.92E-05$	$1.03E-02$
Azoarcus	$2.65E-05 \pm 8.36E-05$	$6.27E-05 \pm 6.58E-05$	$1.33E-02$
Acidothermus	$6.24E-05 \pm 1.91E-04$	$1.02E-04 \pm 7.06E-05$	$2.49E-03$

distance between the samples, a rectangular coordinate system could be found to represent the samples as points and make the square of the Euclidean distance between the points equal to the original difference data, so as to realize the quantitative conversion of the qualitative data and extract the most important elements and structures from the multidimensional data. Through the principal coordinate analysis, the classification of multiple samples can be realized to further display the species diversity differences among the samples.

Figure 3(a) was the PCoA diagram of the GPL group compared with healthy control group. This figure showed that the samples (red dots) of the GPL group were relatively concentrated on the right side, while the samples (blue dots) of the healthy control group were relatively scattered on the left side. The two groups had a certain degree of differentiation. This result told that there were some differences in the microbial diversity of tongue coating between the GPL group and healthy control group.

Figure 3(b) was the NMDS diagram of the GPL damp phlegm pattern group compared with the GPL nondamp phlegm pattern group. From this figure, we can see that the samples (red dots) of the damp phlegm pattern group and the samples (blue dots) of the nondamp phlegm pattern group were concentrated in the lower left and upper right of the figure, respectively. The two groups had obvious differentiation. The results showed that there were significant differences in microbial diversity of tongue coating between the

GPL damp phlegm pattern group and GPL nondamp phlegm pattern group.

4.4. Microbial Relative Abundance Analysis. In the comparison of the differences of microbial community abundance in the tongue coating samples between GPL patients and healthy controls, there were significant differences in the abundance of four kinds of microorganisms at the genus level. In Figure 4(a) and Table 2, the relative abundances of Solobacterium, Rothia, Oribacterium, and Alloprevotella were significantly higher in the GPL group compared to those in the healthy control group ($P < 0.05$).

In the comparison of the differences of microbial community abundance in the tongue coating samples between GPL damp phlegm pattern group and GPL nondamp phlegm pattern group, there were significant differences in the abundance of ten kinds of microorganisms at the genus level. In Figure 4(b) and Table 3, the relative abundances of Terrisporobacter, Solobacterium, Porphyromonas, Parvimonas, Lactobacillus, Johnsonella, Gemella, Fusibacter, Azoarcus, and Acidothermus were significantly lower in the damp phlegm pattern group compared to those in the nondamp phlegm pattern group ($P < 0.05$).

It can be seen from the above results that, compared with that of the healthy control group, the overall level of the relative abundance of microorganisms named Solobacterium was increased in the tongue coating of GPL patients, but it was at a relatively low level in GPL patients with a damp

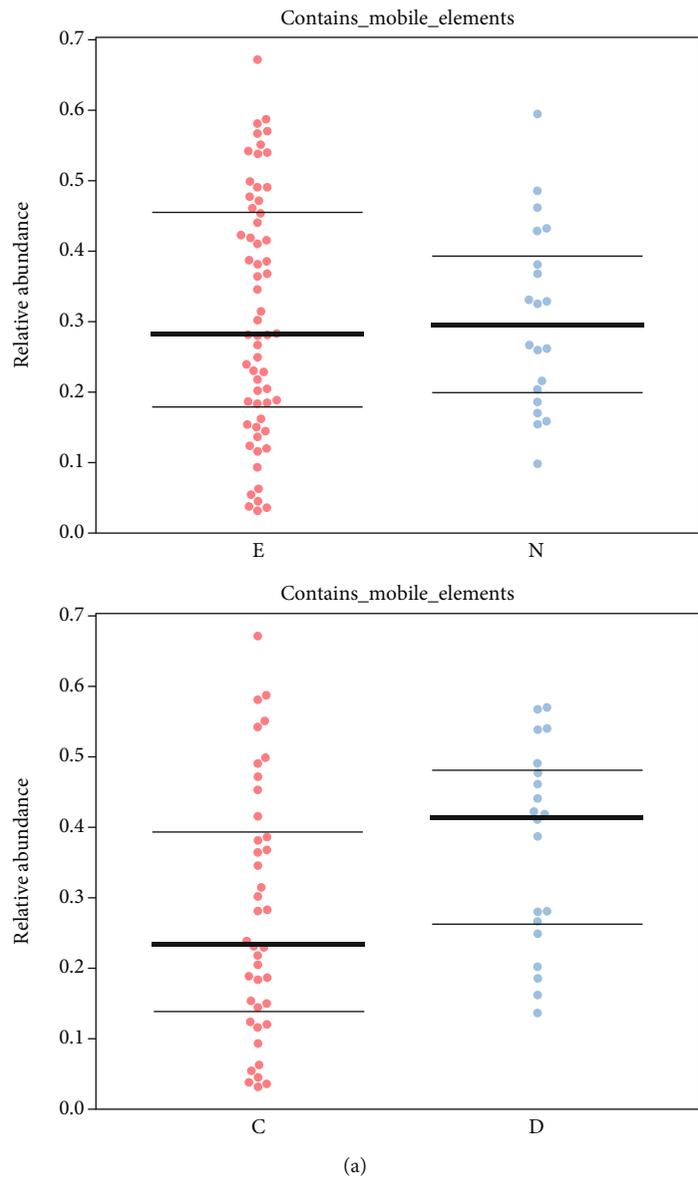


FIGURE 5: Continued.

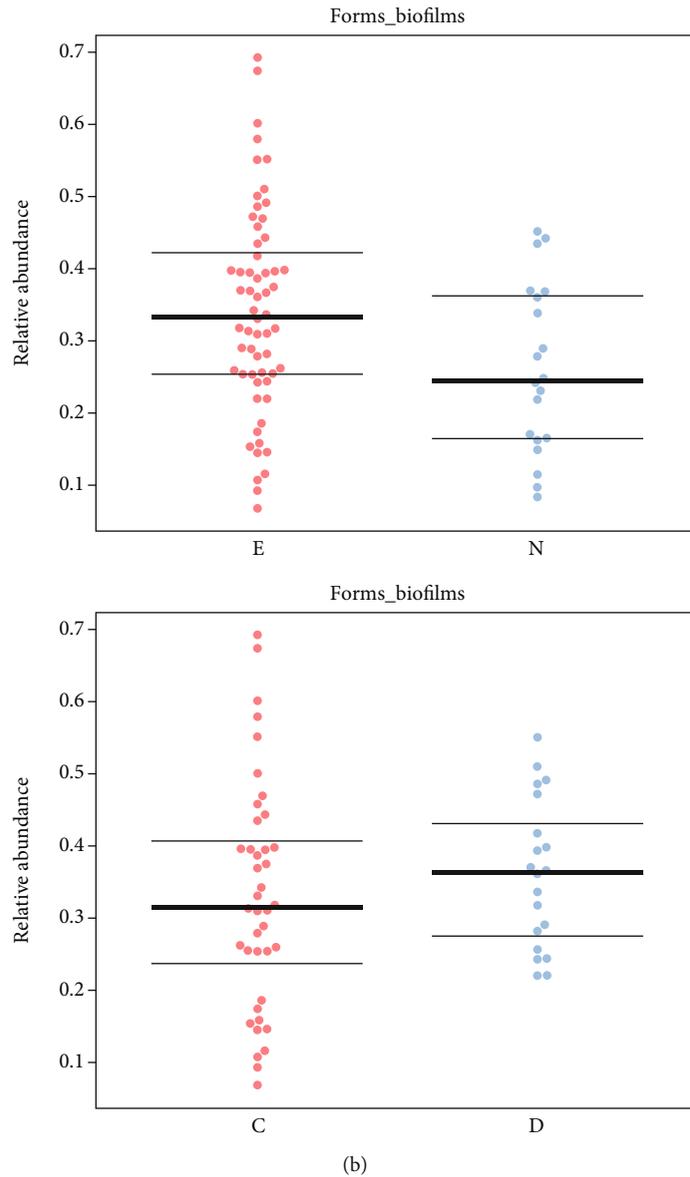


FIGURE 5: (a) BugBase phenotype “contains mobile elements” prediction diagram (E: GPL group, N: healthy control group). (b) BugBase phenotype “forms biofilms” prediction diagram (C: GPL damp phlegm pattern group, D: GPL non-damp phlegm pattern group). The abscissa was the group name, the ordinate was the relative abundance percentage, and the three lines from bottom to top were the lower quartile, the average, and the upper quartile. (a) In the comparison of phenotype “contains mobile elements,” the relative abundance of microorganisms in the GPL damp phlegm pattern group was significantly lower than that in the GPL nondamp phlegm pattern group ($P < 0.05$). (b) In the comparison of phenotype “forms biofilms,” the relative abundance of microorganisms in the GPL group was significantly higher than that in the healthy control group ($P < 0.05$).

TABLE 4: Comparison of relative abundance of microorganisms between the GPL and healthy control groups with different phenotypes.

Phenotype	GPL group	Healthy control group	P corrected
Contains mobile elements	0.31 ± 0.17	0.31 ± 0.13	0.99
Forms biofilms	0.34 ± 0.14	0.26 ± 0.12	0.02

phlegm pattern. The results suggested that *Solobacterium* may be used as a microbial marker to identify the GPL damp phlegm pattern.

4.5. *Microbial Phenotype Prediction.* In the prediction and analysis of microbial phenotype of tongue coating, as shown in Figure 5 and Tables 4 and 5, in the comparison of phenotype “contains mobile elements,” there was no significant difference between the GPL group and the healthy control group; however, the relative abundance of microorganisms in the GPL damp phlegm pattern group was significantly lower than that in the GPL nondamp phlegm pattern group

TABLE 5: Comparison of relative abundance of microorganisms between the GPL damp phlegm pattern and nondamp phlegm pattern groups with different phenotypes.

Phenotype	Damp phlegm pattern group	Nondamp phlegm pattern group	<i>P</i> corrected
Contains mobile elements	0.28 ± 0.18	0.37 ± 0.14	0.04
Forms biofilms	0.33 ± 0.16	0.36 ± 0.10	0.39

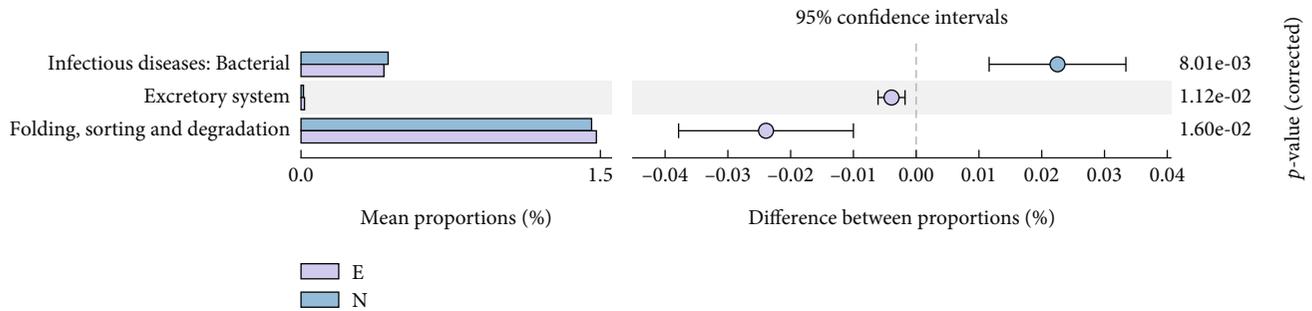


FIGURE 6: KEGG metabolic pathway difference analysis diagram (E: GPL group, N: healthy control group). The left part showed the abundance ratio of different metabolic functions in the two groups of tongue coating microorganisms, the middle figure showed the difference ratio of functional abundance within the 95% confidence interval, and the rightmost value was the *P* value. The abundance ratio of metabolic function “infectious diseases: bacterial” in the GPL group was significantly lower than that in the healthy control group ($P < 0.05$). The abundance ratio of the metabolic function “excretory system” and “folding, sorting, and degradation” in the GPL group was significantly higher than that in the healthy control group ($P < 0.05$).

TABLE 6: Comparison of abundance ratio (%) between the GPL and healthy control groups with different metabolic functions.

Metabolic function	GPL group	Healthy control group	<i>P</i> corrected
Infectious diseases: bacterial	0.41 ± 0.02	0.43 ± 0.02	8.01E – 03
Excretory system	0.02 ± 0.01	0.01 ± 0.05E – 02	1.12E – 02
Folding, sorting, and degradation	1.48 ± 0.04	1.45 ± 0.02	1.60E – 02

($P < 0.05$). In the comparison of phenotype “forms biofilms,” the relative abundance of microorganisms in the GPL group was significantly higher than that in healthy control group ($P < 0.05$), but there was no significant difference between GPL damp phlegm pattern group and nondamp phlegm pattern group.

4.6. Microbial Metabolic Function Prediction. In the prediction and analysis of microbial metabolic function of tongue coating, as shown in Figure 6 and Table 6, the abundance ratio of metabolic function “infectious diseases: bacterial” in the GPL group was significantly lower than that in the healthy control group ($P < 0.05$). The abundance ratio of metabolic function “excretory system” and “folding, sorting, and degradation” in the GPL group was significantly higher than that in the healthy control group ($P < 0.05$). However, the significant difference in the metabolic pathway of the functional genes of the tongue coating microorganisms between the GPL damp phlegm pattern group and the GPL nondamp phlegm pattern group was not found.

5. Discussion

GPL is an important stage in the development of chronic gastritis into gastric cancer. The damp phlegm pattern is

one of the most common TCM patterns of GPL. The appearance change of tongue coating is one of the important criteria for TCM doctors to diagnose the GPL damp phlegm pattern, but the related research on tongue coating microorganisms of GPL and its patients with damp phlegm pattern is rarely reported.

In this study, we used 16S rRNA technology to detect microbial changes in patients’ tongue coating. From the results of alpha and beta diversity analysis, it can be seen that there are differences in the microbial diversity of tongue coating between the GPL group and healthy control group as well as the GPL damp phlegm pattern group and GPL nondamp phlegm pattern group. In the further comparison of the relative abundance of microorganisms in each group, we found that there were significant differences in the relative abundance of 4 microorganisms between the GPL group and the healthy control group, which were *Solobacterium*, *Rothia*, *Oribacterium*, and *Alloprevotella*. The relative abundance of these four microorganisms in the GPL group was significantly higher than that in the healthy control group. There are significant differences in the relative abundance of 10 microorganisms between the GPL damp phlegm pattern group and GPL nondamp phlegm pattern group, including *Terrisporobacter*, *Solobacterium*, *Porphyromonas*, *Parvimonas*, *Lactobacillus*, *Johnsonella*, *Gemella*, *Fusibacter*,

Azoarcus, and Acidothermus. The relative abundance of these 10 microorganisms in the GPL damp phlegm pattern group was significantly lower than that in the GPL nondamp phlegm pattern group.

Among these differential microorganisms, *Solobacterium* deserves our attention. Its relative abundance in the tongue coating of GPL patients increased, and its relative abundance in GPL damp phlegm pattern patients was significantly higher than that in GPL nondamp phlegm pattern patients. Studies have shown that *Solobacterium* is a Gram-positive, non-spore-forming obligate anaerobic bacterium from human feces [17]. This bacterium can cause halitosis and affect the development of digestive tract cancer, and the malodor causing cancer is hydrogen sulfide and acetaldehyde produced by *Solobacterium* [18]. Among the other three microorganisms whose relative abundance in the GPL group is significantly higher than that in the healthy control group, *Rothia* is a member of Gram-positive cocci of the *Micrococcus* family, which is considered to be an opportunistic pathogen, mainly affecting people with low immune function [19]. However, there is no report showing that this bacterium is found in patients with chronic gastritis or gastric cancer. *Oribacterium* has been found in saliva samples from patients with reflux esophagitis that its abundance is higher than that of healthy people [20]. *Alloprevotella* is also rarely found in patients with chronic gastritis or gastric cancer, but it was found to be increased in stool samples of ulcerative colitis or canceration [21].

Among the other 9 microorganisms with different relative abundance expression found in the comparison between the GPL damp phlegm pattern group and nondamp phlegm pattern group, *Terrisporobacter* has not been found in patients with stomach disease, but its relative abundance is different from that of healthy people in the feces of irritable bowel syndrome patients [22]. *Porphyromonas*, as an anaerobic bacterium, not only has local effects on its natural oral cavity but also has systemic tumorigenic effects, which may be related to GPL. *Porphyromonas gingivalis* can promote distant metastasis of cancer cells and resistance to anticancer drugs. This mechanism is mainly through affecting the gene expression of defensins, peptidyl arginine deaminase, and noncanonical activation of β -catenin. In addition, the microorganism can also convert ethanol into acetaldehyde, which is a carcinogenic intermediate [23]. *Parvimonas*, as an aerobic bacterium, is also related to the occurrence of tumors [24]. A study claimed that the presence of this microorganism in gastric mucosa could be used as one of the biomarkers to distinguish superficial gastritis from gastric cancer [25]. *Lactobacillus* has been confirmed in many studies that its relative abundance will increase in the development of gastric cancer [26]. *Johnsonella* was found in the oral cavity of patients with gastric internal metaplasia, and its enrichment degree was significantly higher than that of healthy people, which was related to the regulation of inflammation-related pathways [27]. *Gemella* has a high degree of centrality in the progression of precancerous lesions of gastric cancer [28]. *Fusibacter*, *Azoarcus*, and *Acidothermus* have not been reported in the study of digestive system diseases.

In the prediction and analysis of the microbial phenotype and metabolic function, we found that there was significant difference between the GPL group and the healthy control group in terms of phenotype “forms biofilms” and there was significant difference between the GPL damp phlegm pattern group and the GPL nondamp phlegm pattern group in terms of phenotype “contains mobile elements.” In addition, there were significant differences between the GPL damp phlegm pattern group and the GPL nondamp phlegm pattern group in the abundance ratio of metabolic function “infectious diseases: bacterial,” “excretory system,” and “folding, sorting, and degradation.” Although there is no relevant research on gastric diseases of the microbial phenotype and metabolic function which we found, our research results also provide some evidence for the microbial characteristics of GPL and its tongue coating of dampness syndrome and will guide us to further explore.

However, there are still some deficiencies in our research results. A total of 10 cases of Hp infection were distributed in the GPL damp phlegm pattern group and nondamp phlegm pattern group. In the current study, whether Hp affects oral microbiota is still controversial [29]. And we previously analyzed the differential microorganisms in the tongue coating of 60 patients with GPL in this study compared with 15 healthy people. The inclusion of these patients with Hp infection does not affect our final screening of differential microorganisms [8]. Even so, in future research, we will still carefully consider the factors of Hp infection. In addition, we will also expand the number of samples and use cohort research methods, metagenomics methods, and multiomics methods to further explore the formation mechanism of GPL and its damp phlegm pattern.

Data Availability

The 16S rRNA data used to support the findings of this study have been deposited in the OMIX repository (file ID: OMIX001727-01).

Disclosure

Xiangqun Xiao and Renling Zhang share the first authorship.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yiming Hao designed the study. Xiangqun Xiao and Yiming Hao wrote the manuscript. Renling Zhang, Yiming Hao, Junhong Lu, and Yifeng Xu helped with the clinical sample collection. Zhujing Zhu helped with the experimentation. Yiqin Wang and Yaxiang Shi helped with the ideas of the study. All authors of this study agreed to be accountable for all aspects of the work.

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