Analysis of Microbial Community Diversity in the Rhizosphere Soil of Peach Trees

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Abstract [**Objectives**] To analyze the microbial community structure and diversity in the rhizosphere soil of peach trees in the Tangshan area of Hebei Province, identify the dominant microbial groups, and explore their potential ecological functions. [**Methods**] Amplification sequencing analysis of bacterial and fungal communities in the rhizosphere soil of a peach orchard in Qian'an County, Tangshan City, Hebei Province, was performed using Illumina MiSeq high-throughput sequencing technology. [**Results**] The indices of Sobs, Chao, ACE, and Shannon for soil bacteria in the rhizosphere soil of peach trees were all higher than those for fungi, indicating a more uniform and diverse bacterial community structure. At the phylum level, the bacteria with relatively high abundance included Pseudomonadota (28.29%), Acidobacteriota (18.10%), Bacillota (12.17%), and Actinomycetota (11.73%). In contrast, the fungi with relatively high abundance were Ascomycota (64.64%), Basidiomycota (14.22%), and Mortierellomycota (14.09%). At the genus level, the bacteria with relatively high abundance comprised *Sphingomonas* (5.00%), *Priestia* (3.38%), *Nitrospira* (2.05%), *etc.* The fungi with relatively high abundance included *Fusarium* (13.13%), *Mortierella* (12.86%), *Tausonia* (6.97%), *Neocosmospora* (4.77%), *etc.* [**Conclusions**] This study offers a foundational dataset and theoretical reference for the regulation of rhizosphere microecology and the management of soil health in peach orchards in Tangshan.

Key words Rhizosphere soil, Peach tree, Microbial diversity, Amplicon high-throughput sequencing, 16S rRNA/ITS, Microecological regulation

1 Introduction

The peach tree [*Prunus persica* (L.) Batsch] is a member of the genus *Prunus* within the Rosaceae family. It is a significant stone fruit species globally and is indigenous to China[1]. Peach trees exhibit strong adaptability, allowing cultivation in temperate open fields as well as subtropical controlled environments. Currently, various cultivars have been developed, including fresh peaches, processed peaches, and ornamental varieties. In China, peach trees rank among the leading deciduous fruit trees in terms of both cultivation area and production, establishing themselves as an economically important fruit crop, particularly in northern regions[2].

The rhizosphere constitutes a critical zone for the exchange of materials and information between plant roots and the surrounding soil. The microbial community within the rhizosphere plays a central role in nutrient transformation, organic matter decomposition, elemental cycling, and the maintenance of ecological processes in this environment[3]. Influenced by root exudates and the selective pressures of the microenvironment, rhizosphere microorganisms exhibit highly specialized characteristics. These microorganisms can enhance plant growth through mechanisms such as nitrogen fixation, solubilization of phosphorus and potassium, production of plant hormones, and improvement of nutrient use efficiency. Furthermore, they influence plant community succession and ecosystem functions by affecting ecological niches and community stability[4-5]. Studies have demonstrated that when plants are infected or subjected to stress by pathogens, they can alter the composition of root exudates through systemic signal regulation. This process selectively recruits beneficial microorganisms with growth-promoting and disease-resistant properties, thereby reshaping the rhizosphere community structure. Consequently, this enhances the adaptability and survival prospects of plants and their progeny within the same soil environment[6]. Therefore, understanding the response of rhizosphere microorganisms to external stress, as well as the dynamic changes in their structure and function, is fundamental to elucidating plant-soil interactions and rhizosphere ecological processes. In recent years, advancements in high-throughput sequencing and bioinformatics have facilitated the widespread use of bacterial 16S rRNA and fungal ITS amplicon sequencing as a standard approach for analyzing the diversity, dominant taxa, and community assembly patterns of rhizosphere microorganisms. This methodology serves as a crucial tool for elucidating the microecological characteristics of fruit tree rhizospheres across various regions and management practices[7].

The Tangshan region in Hebei Province is situated in the eastern part of the North China Plain. It experiences a warm temperate semi-humid monsoon climate, with heat and moisture conditions that are conducive to the cultivation of fruit trees. In recent years, leveraging its geographical location and market advantages, the area has actively developed a distinctive fruit industry primarily focused on fresh early- and mid-ripening peaches, progressively establishing large-scale, intensive peach orchards. This industry has become a significant contributor to enhancing agricultural productivity and increasing farmers' incomes. Due to variations in re-

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gional climate, soil parent material, and cultivation management practices, the rhizosphere microbial communities of peach trees may exhibit distinct regional characteristics. However, systematic research on rhizosphere microorganisms in Tangshan peach orchards remains relatively limited at present. This study selected a peach orchard in Qian'an City as the research site and employed the Illumina MiSeq platform to perform high-throughput sequencing of the bacterial 16S rRNA gene and the fungal ITS region. The objective was to analyze the compositional characteristics and diversity patterns of the microbial community in the rhizosphere soil of peach trees, identify dominant taxa, and explore their potential ecological functions. The findings aim to provide a theoretical foundation for the regulation of soil microecology and the healthy cultivation management of peach orchards in the Tangshan area.

2 Materials and methods

2.1 Collection and processing of soil samples from the rhizosphere of peach trees The experimental soil samples were collected in May 2025 from a peach orchard located in Qian'an City, Tangshan, Hebei Province (118°37′ – 118°55′ E, 39°51′ – 40°15′ N). The physicochemical properties of the soil at the collection site were as follows: nitrogen (N) 18.69 mg/kg, phosphorus (P) 22.61 mg/kg, potassium (K) 216.55 mg/kg, electrical conductivity (EC) 468.33 μS/cm, and pH 7.76. Soil adhering to the root systems of peach trees was collected from the peach orchard. Approximately 2 mm of rhizosphere soil from the root surface was carefully sampled onto sterile paper and promptly transferred into pre-labeled sterile cryotubes. The samples were then transported to the laboratory and preserved under dry ice refrigeration conditions.

2.2 DNA extraction of the sample Total DNA extraction from microbial communities was performed following the protocol provided with the E. Z. N. A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The quality of the extracted DNA was assessed by 1% agarose gel electrophoresis. DNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

2.3 PCR amplification and sequencing library construction Utilizing the previously extracted DNA as a template, primers 27F and 1492R with barcodes, or ITS1F and ITS4R, were employed. PCR amplification targeted the full length of the 16S rRNA gene or the entire ITS region. The PCR reaction mixture consisted of 4 μL of 5 × FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of upstream primer (5 μM), 0.8 μL of downstream primer (5 μM), 0.4 μL of FastPfu polymerase, 0.2 μL of BSA, and 10 ng of template DNA, with the final volume adjusted to 20 μL. Each sample was prepared in triplicate. The amplification protocol was conducted as follows: an initial denaturation at 95 ℃ for 3 min; 27 cycles consisting of denaturation at 95 ℃ for 30 sec, annealing at 60 ℃ for 30 sec, and extension at 72 ℃ for 30 sec; followed by a final extension at 72 ℃ for 10 min; and subsequent storage at 4 ℃. The PCR was performed using a T100 Thermal Cycler (Bio-Rad, USA). Following amplification, products were subjected to 2% agarose gel electrophoresis, after which magnetic bead purification was carried out. The purified products were then quantified using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA). Subsequently, the samples were combined in proportions corresponding to their respective sequencing volume requirements. Library construction was performed using the SMRTbell Prep Kit 3.0, which involved DNA damage repair, end repair, and adapter ligation. Sequencing was carried out using the PacBio Sequel IIe System (Shanghai Majorbio Technology Co., Ltd.). HiFi reads were generated from the sequenced subreads utilizing the CCS mode of SMRT-Link version 11.0 for subsequent data analysis.

2.4 Sequencing data analysis The data for each sample were differentiated based on the barcode sequence. Length filtering and direction correction were subsequently performed, retaining sequences of 1 000 – 1 800 bp for bacteria and 300 – 900 bp for fungi. Using default parameters, the DADA2 or Deblur plugin within the Qiime 2 process was employed to conduct noise reduction on the quality-controlled and merged sequences. The sequences produced by the DADA2 or Deblur denoising process are referred to as amplicon sequence variants (ASVs). All samples annotated as sequences of chloroplast and mitochondrial origin were excluded. To minimize the influence of sequencing depth on subsequent Alpha and Beta diversity analyses, all sample sequence numbers were set to 6 000 through random sampling. Following this, the average sequence coverage (Good's coverage) for each sample remained at 99.09%. Taxonomic analysis of bacterial ASVs was conducted using the Naive Bayes classifier within Qiime 2, based on the SILVA 16S rRNA gene database (version 138). Functional prediction of the 16S rRNA gene was performed using PICRUSt2 software (version 2.2.0).

2.5 Statistical analysis All data analyses were conducted using the Majorbio Cloud Platform (https://cloud.majorbio.com). Alpha diversity indicators, including Chao1 and Shannon indices, were calculated with the mothur software (http://www.mothur.org/wiki/Calculators). Dilution curves were generated using R software, with Sobs dilution data computed via mothur. In these curves, the horizontal axis represented the number of randomly selected sequencing reads, while the vertical axis corresponded to the observed Sobs index. The inter-group differences in Alpha diversity were analyzed using the Wilcoxon rank-sum test. Principal Coordinate Analysis (PCoA), based on the Bray-Curtis distance metric, was employed to assess the similarity of microbial community structures among samples. A PERMANOVA non-parametric test was conducted to determine whether the differences in microbial community structures among sample groups were statistically significant. Linear discriminant analysis effect size (LEfSe) (http://huttenhower.sph.harvard.edu/LEfSe) with a threshold

of LDA > 2 and $P < 0.05$ was employed to identify bacterial taxa exhibiting significant differences in relative abundance from the phylum to genus levels across different groups. Distance-based redundancy analysis (db-RDA) was utilized to investigate the impact of soil physicochemical properties on the structure of soil bacterial communities. Linear regression analysis was employed to assess the impact of key soil physicochemical parameters identified through db-RDA analysis on the microbial Alpha diversity index. Additionally, species exhibiting a Spearman correlation coefficient with an absolute value greater than 0.6 ($|r| > 0.6$) and a significance level of $P < 0.05$ were selected for correlation network graph analysis.

3 Results and analysis

3.1 Overview of high-throughput sequencing of soil microorganisms The dilution curve serves to assess whether the sequencing data obtained are sufficient and appropriate, illustrating the trend in the number of detected species as sequencing depth increases. Fig. 1 presents the dilution curves for both the fungal and bacterial communities. Based on the shapes of these curves, both were approaching a plateau phase, indicating that the current sequencing depth adequately captured the majority of species present in the samples. Consequently, the sequencing data were essentially saturated and exhibited high coverage, rendering them suitable for subsequent analyses of diversity and community structure.

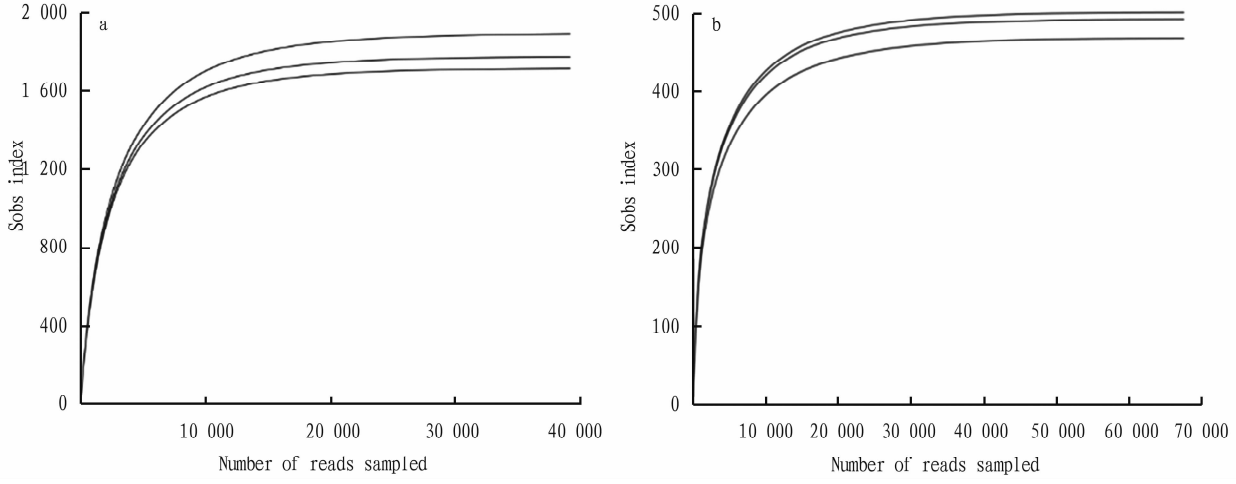

Fig. 1 Dilution curves of bacteria (a) and fungi (b)

Following the optimization of the original bacterial sequencing data, a total of 39 259 sequences were obtained, from which 8 922 ASVs were identified through cluster analysis. Similarly, after optimizing the sequencing data of soil fungi in the samples, 67 563 sequences were acquired, yielding 1 967 ASVs through cluster analysis.

As shown in Table 1, the Sobs, ACE, Chao, and Shannon indices of the bacterial community in the rhizosphere soil of peach trees were significantly higher than those of the fungal community. Conversely, the Simpson index for bacteria was 0.002, which was significantly lower than the fungal value of 0.028. The Coverage index for both bacterial and fungal communities was approximately 0.99, indicating high sequencing coverage and reliable results.

Table 1 Sample Alpha diversity index results

Sample	ACE	Chao	Shannon	Simpson	Coverage	Sobs
Fungi	486.82	486.70	4.42	0.028	0.99	486.67
Bacteria	1 791.22	1 791.00	6.88	0.002	0.99	1 789.00

3.2 Composition of bacterial and fungal communities in the rhizosphere soil The rhizosphere soil of peach trees was analyzed at the phylum level (Fig. 2). Based on relative abundance from highest to lowest, the dominant phyla were Pseudomonadota (28.29%), Acidobacteriota (18.10%), Bacillota (12.17%), and Actinomycetota (11.73%), followed by Chloroflexota (8.09%), Gemmatimonadota (5.36%), Bacteroidota (5.31%), Myxococcota (2.31%), Nitrospirota (2.05%), Thermodesulfobacteriota (1.56%), Verrucomicrobiota (0.97%), and Patescibacteria (0.88%). Other phyla, including Methylomirabilota and other low-abundance groups, were present at relatively low proportions.

The bacterial community composition in the rhizosphere soil of peach trees was analyzed at the genus level (Fig. 3). The genera were ranked according to their relative abundance, with *Sphingomonas* (5.00%), *Priestia* (3.38%), and *Nitrospira* (2.05%) being the most prevalent. Other genera, including *Steroidobacter*, *Microvirga*, *Bryobacter*, *Sporosarcina*, *Paenibacillus*, and *MND1*, exhibited relative abundances ranging approximately from 0.7% to 1.9%.

The fungal community composition in the rhizosphere soil of peach trees was examined at the phylum level (Fig. 4). Based on relative abundance, the dominant phyla were Ascomycota (64.64%), Basidiomycota (14.22%), and Mortierellomycota (14.09%). Other phyla exhibited comparatively lower abundances, including Chytridiomycota (0.50%), Rozellomycota (0.11%), Blastocladiomycota (0.07%), Kickxellomycota (0.06%), and Zoopagomycota (0.03%).

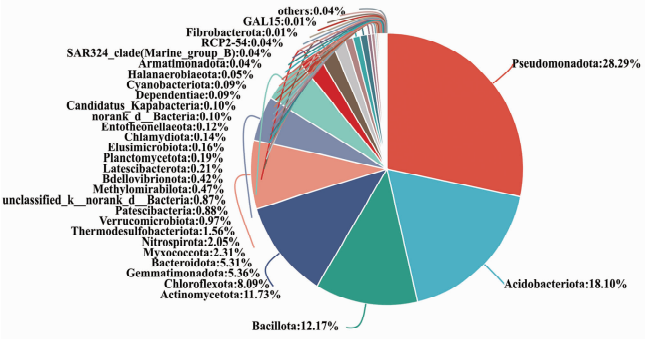

Fig. 2 Community analysis pieplot of soil bacteria at the phylum level

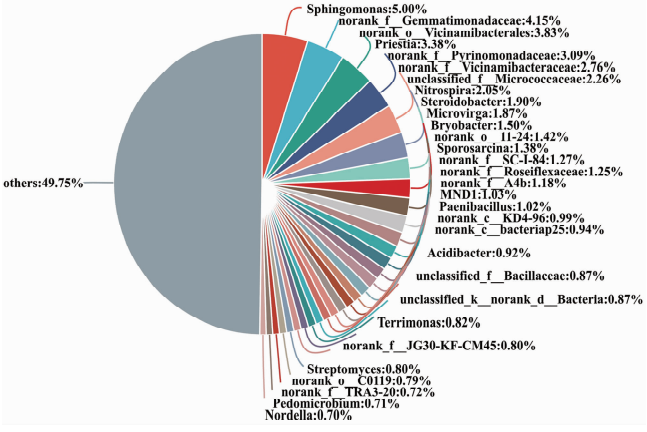

Fig. 3 Community analysis pieplot of soil bacteria at the genus level

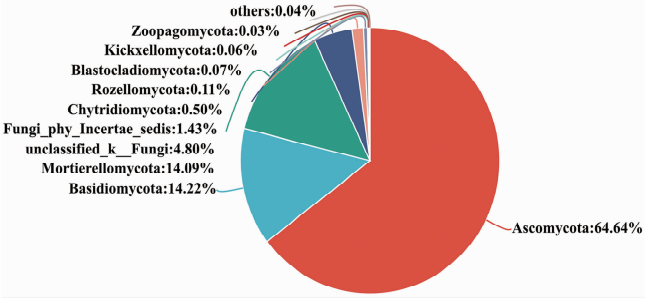

Fig. 4 Community analysis pieplot of soil fungi at the phylum level

The fungal community composition in the rhizosphere soil of peach trees was analyzed at the genus level, and the relative abundance of each taxonomic unit was determined. The results are presented in Fig. 5. *Fusarium* was identified as the dominant genus, comprising 13.13% of the fungal community, followed closely by *Mortierella* at 12.86%. Additionally, *Tausonia* represented a substantial proportion at 6.97%. Other notable taxa included *unclassified_k__Fungi* (4.80%), *Neocosmospora* (4.77%), *Pseudogymnoascus* (3.85%), and *unclassified_f__Pyronemataceae* (3.38%). The remaining genera exhibited moderate to low abundance, such as *Solicoccozyma* (2.46%), *Sporormia* (2.06%), and *Stachybotrys* (2.03%). Collectively, *Fusarium*, *Mortierella*, and *Tausonia* were identified as the key genera shaping the fungal community structure at the genus level.

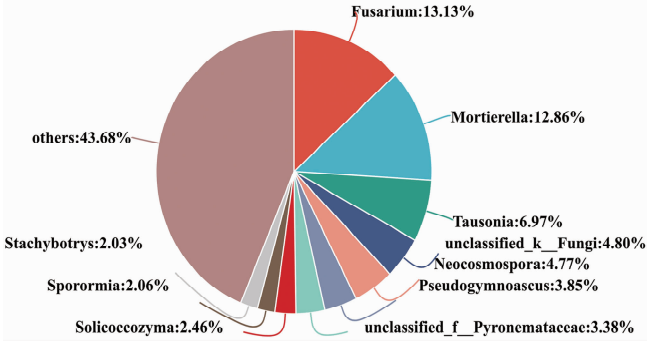

Fig. 5 Community analysis pieplot of soil fungi at the genus level

4 Discussion

A comprehensive analysis of the potential ecological functions of bacterial communities was performed at both the phylum and genus taxonomic levels. The findings revealed that the bacterial community collectively exhibited potential ecological functions, including organic matter decomposition, nutrient mineralization, nitrogen cycle transformation, and regulation of microbial interactions. Furthermore, the rhizosphere was found to be enriched with key functional units associated with organic matter transformation, plant growth promotion, and nitrification[8]. Regarding carbon source utilization and organic matter decomposition, Pseudomonadota and Actinomycetota, as metabolically active groups, were extensively involved in organic carbon mineralization and the degradation of diverse organic substrates. These groups also included numerous members exhibiting strong rhizosphere colonization capabilities[9]. In contrast, Acidobacteriota and Chloroflexota were primarily associated with the degradation of complex organic matter and carbon conversion processes, thereby playing a significant role in the soil carbon cycle[10]. Regarding plant growth promotion and stress resistance, the bacterial phyla Pseudomonadota and Bacillota were enriched with various groups capable of producing plant hormone-like substances, solubilizing phosphorus and potassium, or inducing systemic resistance in plants. For instance, *Pseudomonas fluorescens* WCS417 could induce induced systemic resistance (ISR) in plants following colonization of the rhizosphere. Regarding functions associated with the nitrogen cycle, *Nitrospira*, a key group involved in nitrification, participated in the oxidation of nitrite and ammonia and served as a crucial driver of soil nitrogen transformation. Additionally, certain members of the genus *Microvirga* contributed to symbiotic nitrogen fixation and exhibited potential for promoting plant growth[11]. Overall, the bacterial community within the rhizosphere primarily focuses on the decomposition of organic matter and the transformation of carbon and nitrogen compounds, functioning synergistically to enhance plant growth and improve stress resistance.

A comprehensive analysis of the potential ecological functions of fungal communities was performed at two taxonomic levels: phylum and genus. The results indicated that these fungal communities primarily engaged in saprophytic decomposition and organic matter turnover. They included multiple active decomposer fungi

and yeast-like groups, along with a notable proportion of environmentally adaptive fungi. Ascomycota and Basidiomycota, as predominant fungal groups, played a crucial role in the decomposition of organic matter and the carbon cycle. They were extensively involved in the degradation of plant residues, organic matter, and lignocellulose, particularly lignin, thereby serving as key drivers of the soil carbon cycle. Additionally, Chytridiomycota functioned as saprophytic decomposers that contributed to the breakdown of particulate organic matter and served an integrative role in the material cycle. In terms of plant interactions and growth-promoting functions, the phylum Ascomycota encompassed a substantial diversity of endophytic groups. Mortierellomycota, particularly its predominant genus *Mortierella*, not only contributed to the decomposition of organic matter but also possessed the capacity to solubilize phosphorus, thereby enhancing phosphorus availability and promoting plant growth[12]. Additionally, taxa such as Rozellomycota and Neocosmospora displayed a range of ecological strategies, including parasitic, saprophytic, and endophytic lifestyles, and exhibited complex functional roles within the rhizosphere environment[13]. From a genus-level perspective, genera such as *Fusarium*, *Pseudogymnoascus*, and *Tausonia* exhibited potential for multi-substrate utilization and decomposition. Notably, *Tausonia* was characterized by an abundance of various extracellular enzymes and carbohydrate-active enzymes, which may play a critical role in the conversion of complex carbon sources[14-16].

The comprehensive sequencing analysis of bacterial and fungal amplicons reveals a well-defined soil microbial community structure within the rhizosphere of peach trees in the Qian'an peach orchard, characterized by a distinct functional orientation. Under conditions of mild alkalinity and low salinity, the microbial community exhibited microecological features dominated by organic matter decomposition and nutrient cycling, with the coexistence of multiple functional groups. Based on the physicochemical properties of the soil, subsequent research and regulatory efforts can concentrate on the coupled cycling of carbon, nitrogen, and phosphorus. By measuring indicators such as dissolved organic carbon, root exudate characteristics, key enzyme activities (including β-glucosidase, cellulase, urease, and phosphatase), functional genes, as well as inorganic nitrogen and available phosphorus, the roles of key microbial groups, such as *Nitrospira* and *Mortierella*, in nutrient transformation were elucidated[17]. Building upon this foundation, further investigation should be conducted into functional microbial resources and the development of a minimal functional microbial community. Priority should be given to the screening of dominant taxa, including *Sphingomonas*, *Priestia*, *Microvirga*, and *Mortierella*. Pot experiments should be performed to verify their roles in rhizosphere colonization, nutrient transformation, and organic matter degradation. Concurrently, strategies such as phased fertilization, the selection of appropriate organic amendment materials, and the optimization of soil aggregate structure should be employed to regulate the physicochemical environment. These interventions can stabilize the rhizosphere microenvironment, thereby enhancing the decomposition and mineralization of organic matter, improving nutrient use efficiency, and achieving targeted regulation of the soil microecology in peach orchards.

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