

Identification of bacterial fossils in marine source rocks in South China

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Abstract Based on the results of conventional geochemistry analysis including thin sections and SEM observations, different shapes of bacterial fossils, with size ranging from dozens of nanometers to several microns, were discovered in the low-mature marine source rocks and coal seams in South China, of which the Permian source rocks were dominated by the bacterial fossils derived from symbiotic sulphur bacteria with gypsum, and the Chengkou section in the Cambrian strata were occupied by abundant nanoscale bacterial fossils with rod and bar shapes. In contrast, a large quantity of possible bacterial fossils found in the high-mature Permian, Silurian, and Cambrian source rocks using SEM need to be further explored. Despite this, this study has indicated that bacterial fossils were prevalent in the source rocks, such as mudstone, siliceous rock and gypsum-bearing coal seams in South China, which has been ignored before. It also suggests that the bacterial fossils may play an important role in the formation and accumulation of shale gas in the geological history.

Keywords South China · Excellent marine source rocks · Bacterial fossil · Sedimentary environment

1 Introduction

Bacteria, one of the earliest life forms on earth, evolved from single-celled creatures four billion years ago. From then on, bacteria and archaea dominated the biosphere for three billion years (Schopf 1994; Delong and Pace 2001). Bacterial fossil record, traced back to Archean and Proterozoic (Soudry and Champetier 1983; Glenn and Arthur 1988; Rao and Nai 1988; Soudry and Lewy 1988; Reimers et al. 1996; Westall 1999; Bailey et al. 2007; Berndmeyer et al. 2012; Arning et al. 2009; Eric et al. 2015), were very meaningful for exploring extraterrestrial life (Westall 1999). Some scholars suggested that bacterial fossils were usually widespread at the time when the global marine microorganisms exploded and the marine environment was reductive, and the high paleoproductivity of microorganisms and hypoxic environment created favorable conditions for the formation of marine source rocks (Yang et al. 2007; Wang et al. 2007). Particularly worth mentioning is the bacteria that is a critical source of sedimentary organic matter, and also a key type of parent materials in the process of hydrocarbon generation (Zhang and Lu 1993; Wang and Tao 2005).

The bacterial fossils were generally diagnosed in respect of morphology, size and texture, through the use of organic petrology, environmental scanning electron microscopy (ESEM) and transmission electron microscopy (TEM). Though Xie et al. (2013) and Zhang et al. (2015) had done some work on bacterial fossils in the Cambrian Niutitang Formation source rocks and sediments in Guizhou, research on bacterial fossils in the marine source rocks in South China remains rare, and this paper will focus on the identification of bacterial fossils in these excellent marine source rocks in different strata, potentially giving some hints on the genesis of the bacterial fossils.

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2 Samples and experiments

2.1 Geological settings and samples

Sichuan basin and adjacent areas is one of the regions rich in natural gas. The explored geologic reserves in marine strata account for 70% of all geologic reserves in this region (Liu et al. 2015). Weiyuan gas field and Puguang gas field were discovered in 1953 and 2001 (Ma 2007; Ma et al. 2010). With the breakthrough of shale gas exploration and development in Fuling area, it revealed a good exploration prospect of Paleozoic shale gas (Guo et al. 2014), due to the abundant materials in this region—as one of most important factors. Current research results showed that there were 4 series of regional main hydrocarbon source rocks in the Sichuan basin and adjacent areas, including Lower Cambrian, Lower Silurian, Lower Permian and Upper Permian (Liang et al. 2008).

The samples were collected from 5 profiles and 5 wells in South China (Fig. 1), mainly in the Permian strata, Lower Silurian Longmaxi Formation and Lower Cambrian strata (Table 1). All selected samples were excellent source rocks with total organic carbon (TOC) $\geq 2\%$ and Ro $> 1.3\%$. In addition, the source rocks from Lower

Silurian Longmaxi Formation were with higher maturity than other samples.

2.2 Methods

In order to get rid of the weathering, samples were prepared for observations as soon as they were obtained. Each sample was made into thin section and polished section for transmitted light and secondary electron observations respectively. Considering contamination, all the specimens were made from the fresh cuts. For thin section preparation, distilled water was used and specimens were placed in sterile dryer. As polished section, specimens with preparatory grinding by Leica EM TXP target preparation device were highly polished by Leica EM RES102 ion beam milling system. Each specimen was observed in 8 h since its preparation was completed.

Thin sections were observed using transmitted light in Leica DM4500 P microscope with various magnitude objective lenses. 50 \times magnitude objective lens was selected for image capturing. FEI helios nanolab 650 high-resolution scanning electron microscopy was adopted for polished sections, which can obtain nanometer characterization of organic fossils. Morphological characterization of

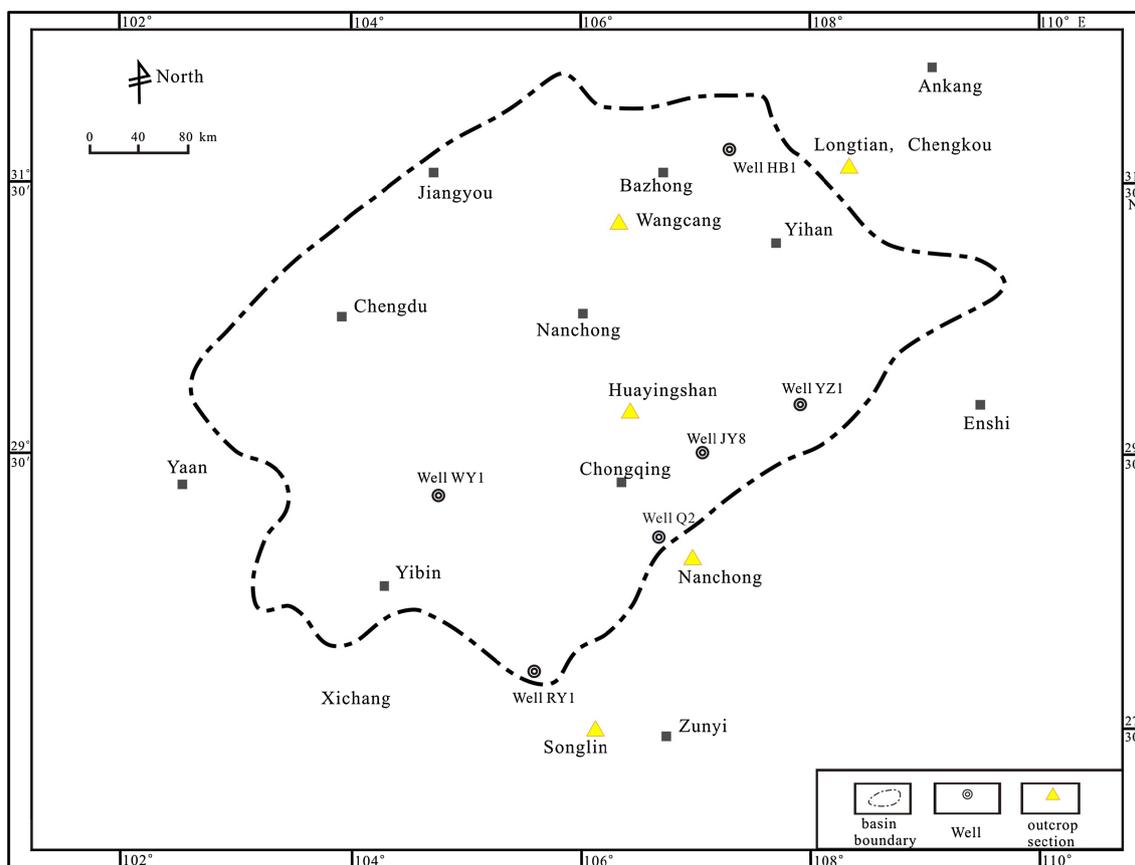


Fig. 1 The sample location of outcrop sections and wells in Sichuan basin and adjacent areas

Table 1 Geochemical characteristics of samples studied

Outcrop section/Well	Name	Formation	Sample type	TOC (%)	Ro (%)	Thin section	SEM
Outcrop section	Huayingshan	Upper Permian Longtan Formation	Source rock	11.18	1.67	✓	✓
	Nanchuan	Upper Permian Longtan Formation	Siliceous limestone	3.65	1.53	✓	✓
	Chengkou	Lower Cambrian Niutitang Formation	Carbonaceous shale	6.24	1.35	✓	✓
	Songlin	Lower Cambrian Niutitang Formation	Source rock	4.73	3.17	✓	✓
	Huayingshan	Lower Silurian Longmaxi Formation	Source rock	5.57	2.89	✓	✓
Well	Heba1	Upper Permian Dalong Formation	Source rock	4.21	1.62	✓	✓
	Qian2	Upper Permian Longtan Formation	Siliceous limestone	3.15	1.73	✓	✓
	Yanzhi1	Lower Silurian Longmaxi Formation	Source rock	2.18	2.63	✓	✓
	Jiaoye8	Lower Silurian Longmaxi Formation	Source rock	3.54	2.43	✓	✓
	Renye1	Lower Silurian Longmaxi Formation	Source rock	3.46	2.5	✓	✓
	Weiyel	Lower Silurian Longmaxi Formation	Source rock	3.62	2.72	✓	✓

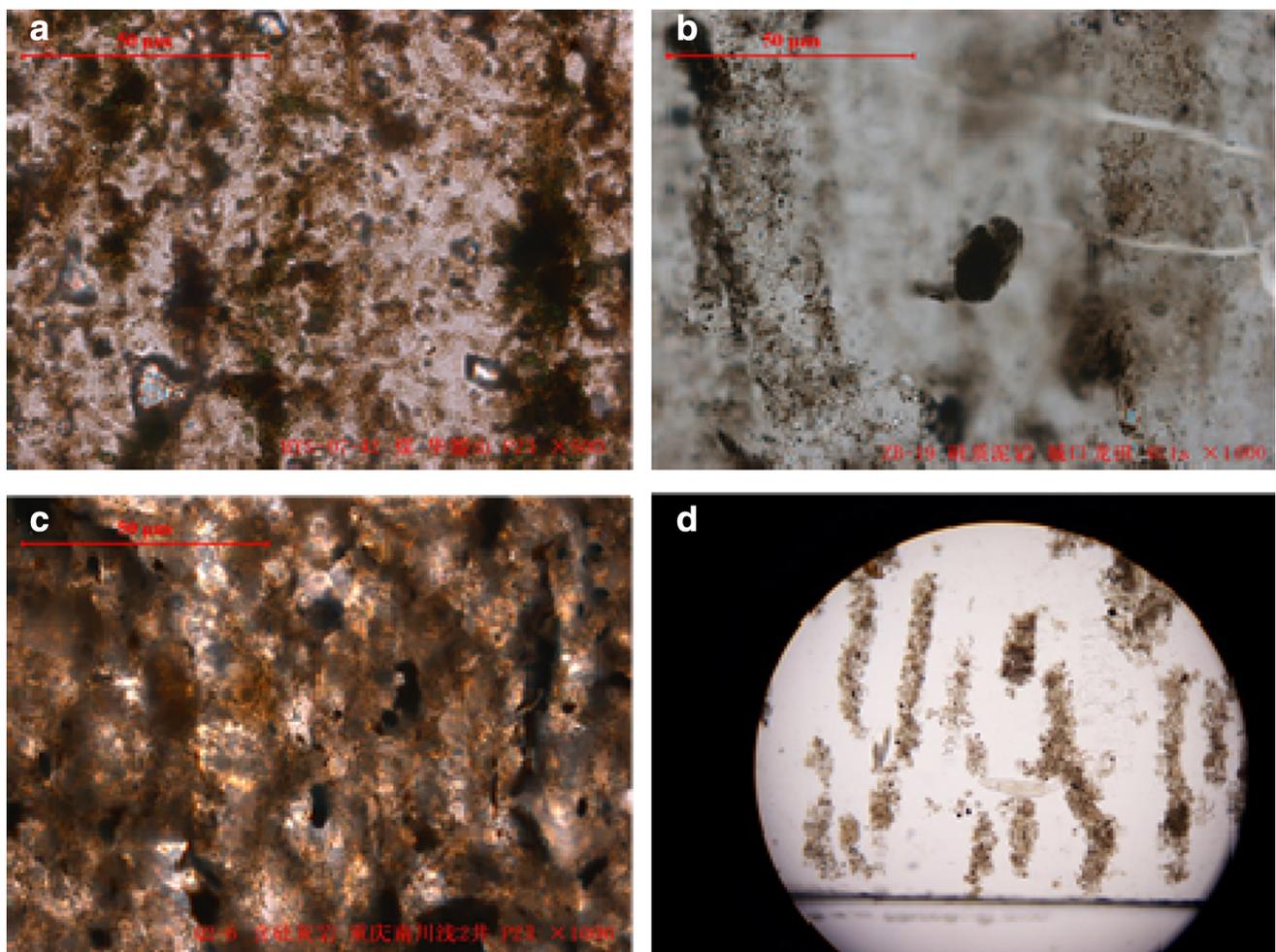


Fig. 2 Zoogloea fossils in source rocks collected from South China. **a** Shale in Longtan Formation in Huayingshan; **b** siliceous limestone in Well Qian 2, Longtan Formation; **c** Lower Cambrian ZB19 carbonaceous shale in Chengkou, Sichuan; **d** modern activated sludge

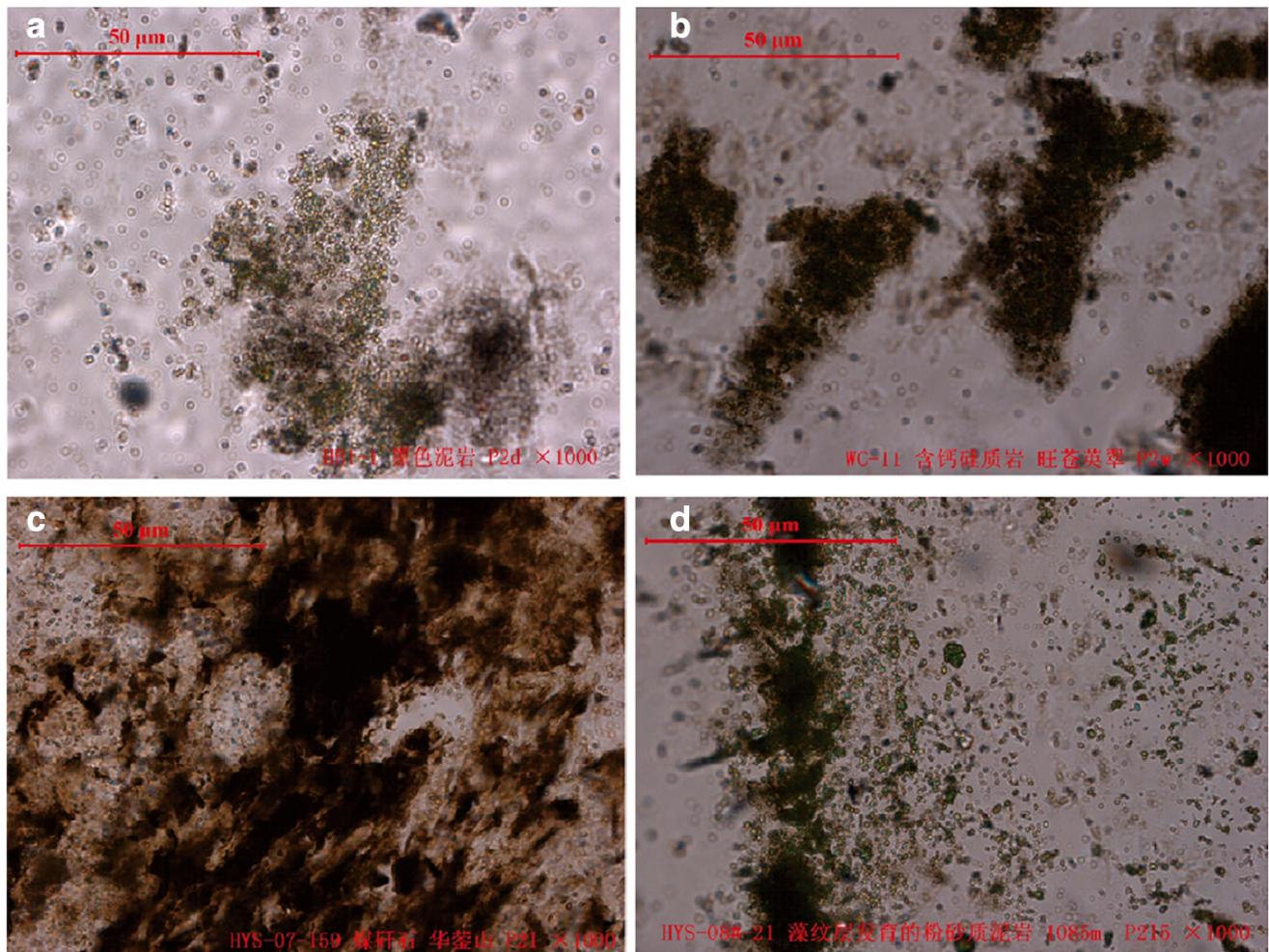


Fig. 3 Zoogloea fossils in the Permian strata in South China. **a** Black shale in Well Heba 1 (P₂d); **b** calciferous siliceous rock from Cangwang section, Sichuan (P₂w); **c** gangue from Huayingshan section (P₂l); **d** silty mudstone from Huayingshan section (P₂l)

bacterial fossils was recorded as secondary electron images. All the experimental observations were implemented in Wuxi Research Institute of Petroleum Geology.

3 Results

In consideration of above mentioned specimen preparation process, contamination is regarded as non-existent in our specimens. Thus, bacterial fossils discussed in this paper were inherited from geological times. For the sake of prudence, we divided the observed bacterial fossils into confirmed and possible bacterial fossils. The confirmed bacterial fossils are characterized by: (1) suspected zoogloea identified on the thin sections of the source rocks; (2) bacterial fossil communities observed under SEM; (3) bacterial biomarkers detected by using GC–MS. We also found a number of microfossils comparable with bacteria in morphology and size, which were deemed as possible

bacterial fossils due to only SEM data obtained in those high-mature source rock samples.

3.1 Thin sections

Due to genetic characteristics, some bacterial cells arranged in a special way, and were embedded with an extracellular matrix, so that they were well preserved and protected from devouring by other micro-animals. Generally, the suspected zoogloea fossils observed in low-mature source rocks ($R_o < 1.5\%$) exist in the aggregated form with blurry bacterial cells (Fig. 2), due to low-resolution of light microscopes. The zoogloea fossils found in the Permian source rock are spherical, mushroom-like, oval, branched, filamentous or irregular (Fig. 2a, b), similar to those found in modern activated sludge (Fig. 2d). The zoogloea were also in the Permian mudstone and coal seams (Fig. 3). Additionally, the zoogloea fossils were found in the Lower Cambrian carbonaceous shales in Chengkou, Sichuan

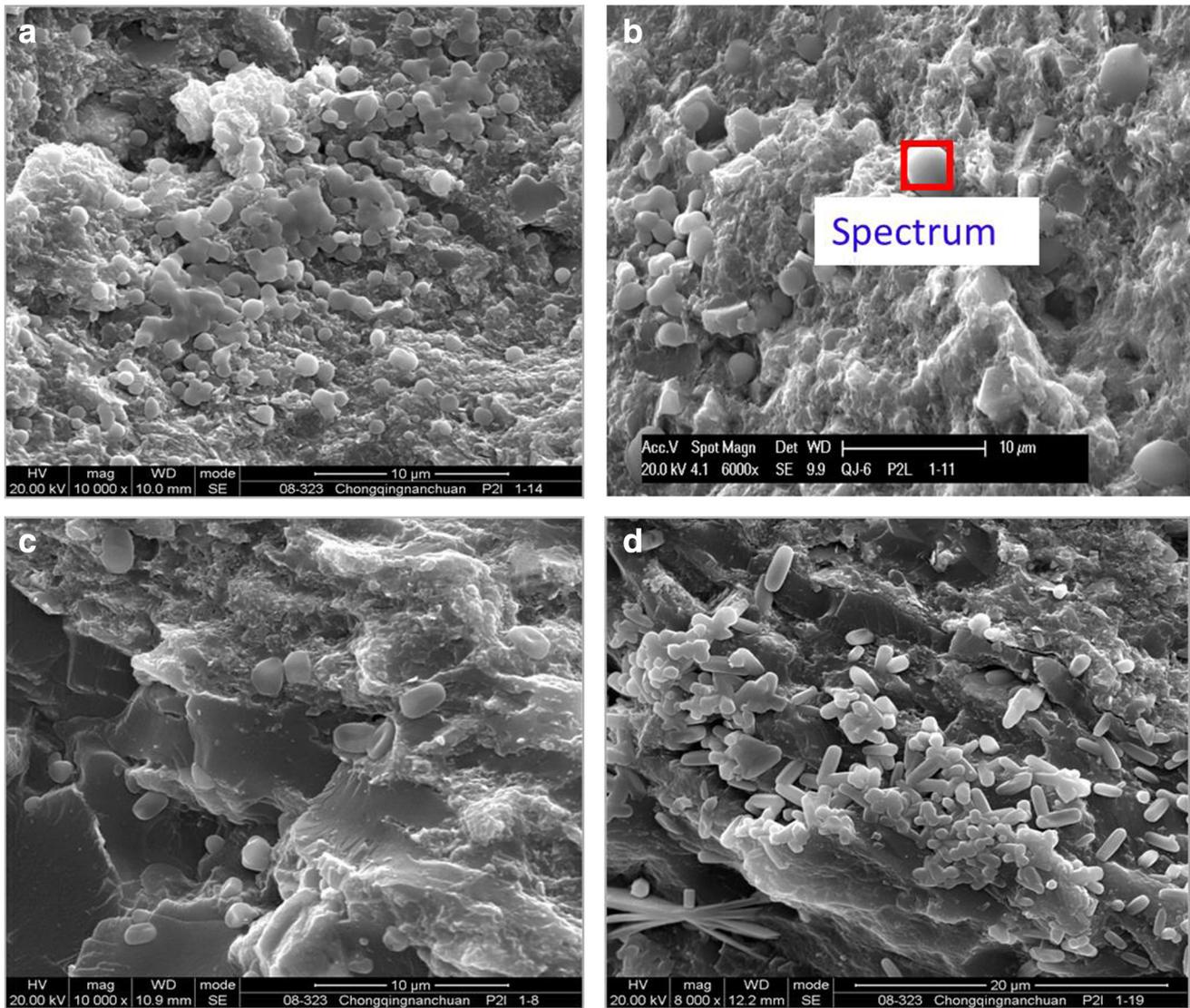


Fig. 4 Bacterial fossils in the Permian Longtan Formation, Nanchuan, Chongqing

Table 2 Characteristics of element compositions in Spectrum 1 (%)

Spectrum	C	O	Na	Mg	Al	Si	S	K	Ca	Fe	Total	Organic carbon
Spectrum1	51.71	25.54	1.34	0.12	4.4	4.16	6.82	1.46	3.6	0.85	100	50.63

(Fig. 2c). However, no zoogloea fossils were observed in the Lower Cambrian Niutitang Formation in Songlin or the Longmaxi Formation in Huayingshan. This may be the result that the source rocks are highly mature so that the zoogloea disappeared in the process of hydrocarbon generation.

3.2 SEM

In order to prove that the bacterial fossils found are original rather than contaminants or minerals, SEM was also used to further observe the microstructures of the source rocks.

Moreover, energy spectrum and biomarker compositions of some samples were also analyzed.

3.2.1 The Permian source rocks

Abundant bacterial fossils were found in the thin sections of the siliceous limestone from Well Qian 2 (the Upper Permian Longtan Formation) (Fig. 2b), Nanchuan, Chongqing (Fig. 4). The bacteria fossil communities were distributed in a variety of forms. Some were oblate (Fig. 4c), and others were spherical, rod- or bar-shaped, with flattened characteristics and distinguished concave

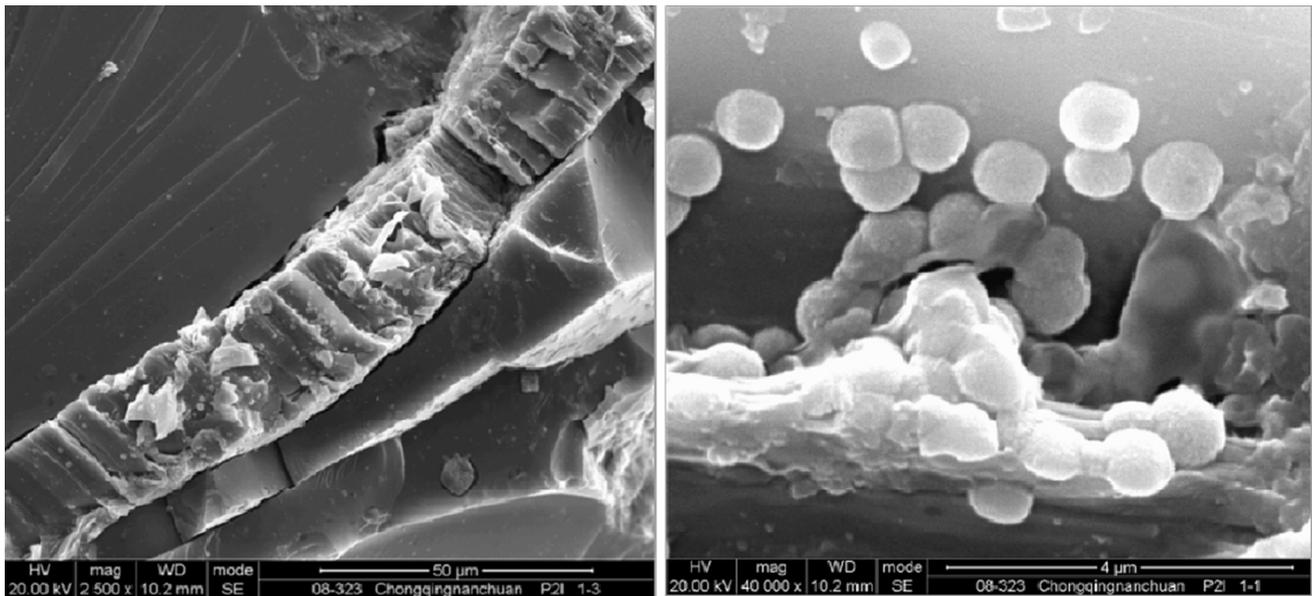


Fig. 5 Possible bacterial fossils in the interspersed siliceous vein from the Permian Longtan Formation, Nanchuan, Chongqing

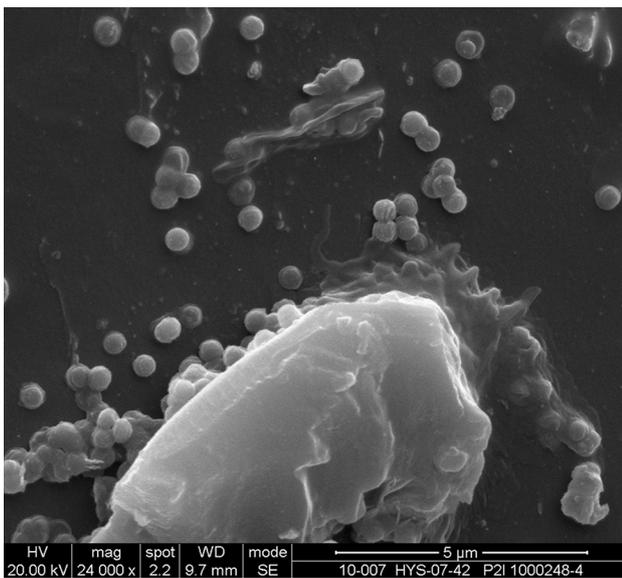


Fig. 6 Nanoscale bacterial fossils in the rock sample of Huayingshan Longtan Formation, Chongqing

and convex sides on some communities (Fig. 4d). These bacteria occurring in different parts of the rocks may present different bacterial species, or variations of the same bacterial species. Some bacterial fossils in local Longtan Formation, Nanchuan, were symbiotic with gypsum crystals (Fig. 4a), indicating that the formation of gypsum might have a certain relationship with bacterial fossils, and the symbiotic bacteria might belong to sulfur bacteria (Edwards et al. 2006), which was confirmed through

further spectrum analysis (Fig. 4b; Table 2). In general, chemoorganotrophic sulfur bacteria exist in aerobic environment with poor organic matter. However, the bacterial fossils found in this study were preserved in the source rocks with high organic matter abundance. Therefore, we suggested that the symbiotic bacteria may be anaerobic photoorganotrophic sulfur bacteria, mostly inhabiting in hydrogen sulfide-containing anaerobic waters and using the hydrogen from sulfide hydrogen as an electron donor to reduce carbon dioxide.

Additionally, spherical bacterial fossil with individual diameter below 1 μm were observed and some were in a split reproductive state in the interspersed siliceous vein from the Permian Longtan Formation, Nanchuan, and Chongqing (Fig. 5). Moreover, possible nanoscale bacterial fossils were found in the Huayingshan section (Fig. 6), which however were greatly different in size from that in the Longtan Formation (Figs. 5, 6).

3.2.2 The Silurian source rocks

Possible bacterial fossils were also found in the kerogen of the Silurian Longmaxi Formation in Huayingshan, with length of 1.2–1.5 μm and diameter of $\sim 0.6 \mu\text{m}$, and located in the common size of bacteria (Fig. 7). In addition, many rod-like bacterial particles were found in the samples, which were tightly bound to the kerogen and were not easily filtered during the treatment. In terms of the preserved morphology, these bacteria was fossilized and very different from the observation results of modern live bacteria under SEM.

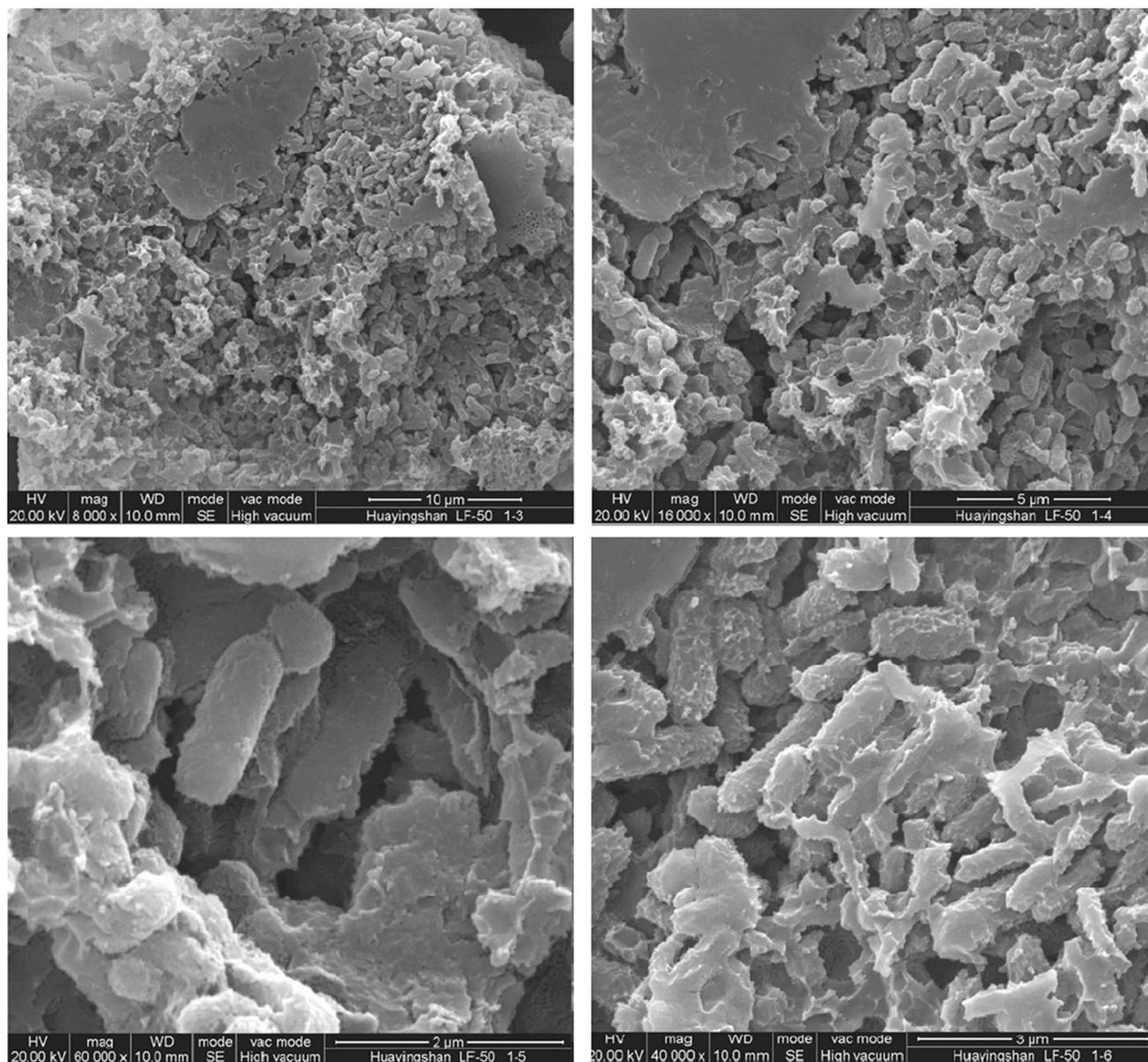


Fig. 7 Possible bacterial fossils in the kerogen of the Lower Silurian Longmaxi Formation, Huayingshan, Chongqing

3.2.3 The Cambrian source rocks

Evident bacterial polymorphism was found in Lower Cambrian ZB19 carbonaceous shale in Longtian, Chenkou (Figs. 2c, 8). The bacteria fossil communities were well preserved in rod or bar, and irregular shapes. Some bacteria were symbiotic with residues of linear leafed plants (Fig. 9), which can be confirmed by the presence of biomarker-tricyclic diterpene (Fig. 10). However, the higher plants have not been observed in the Cambrian system, and the reason that why tricyclic diterpene can be detected needs to be further studied. Moreover, hopanoids were also

detected (Fig. 10), indicating bacterial fossil present in the Cambrian carbonaceous shale.

Micro-phosphate nodules ($\sim 30 \mu\text{m}$) were found in the Cambrian source rocks in Songlin, Guizhou. Filamentous bacteria ($\sim 1 \mu\text{m}$) were located in these phosphate nodules and preserved in a growing state (Fig. 11). Moreover, spore-like fossils were observed (Fig. 12). Though these spore-like fossils were found elsewhere and not contaminated products (Lu et al. 2001), they were considered to be possible bacterial fossils due to lack of other relative evidence (such as rock section photo and biomarker data).

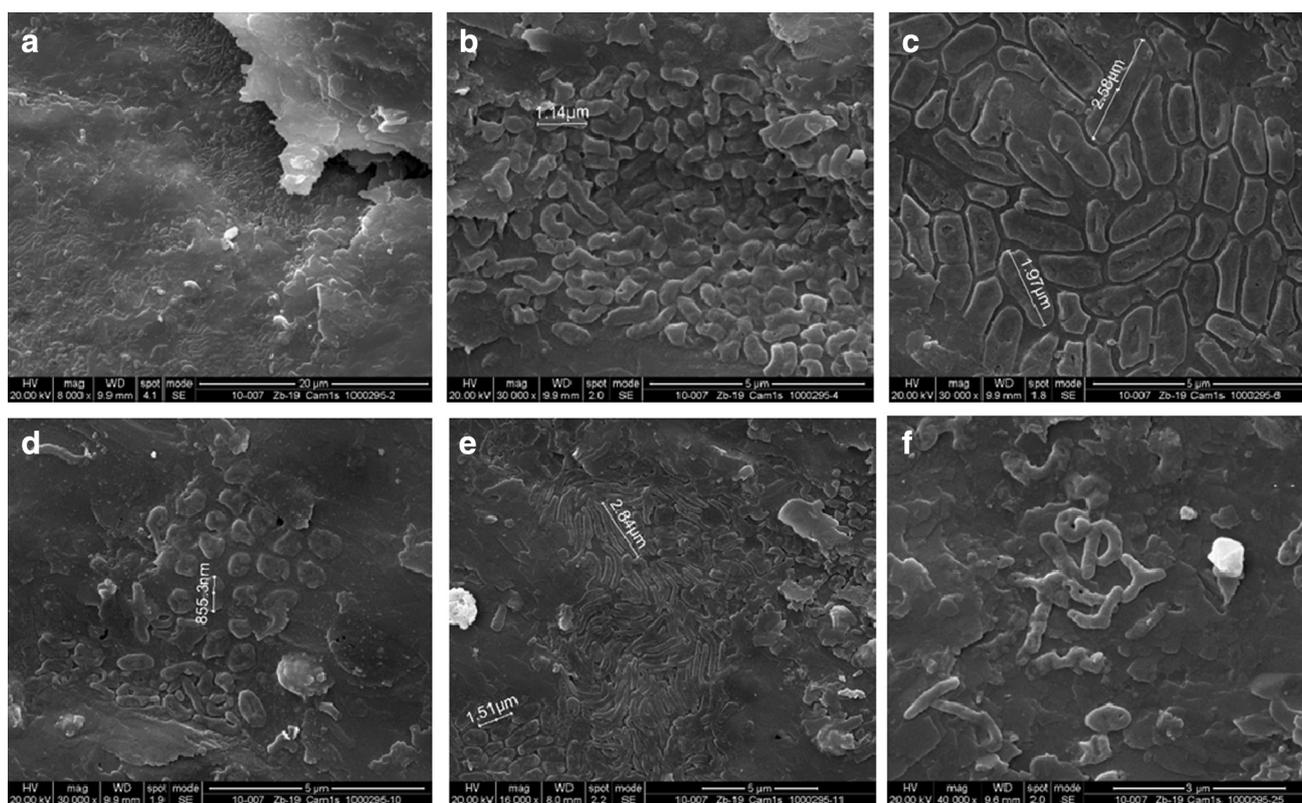


Fig. 8 Bacterial fossils in the Lower Cambrian carbonaceous shale of ZB19, Longtian, Chengkou

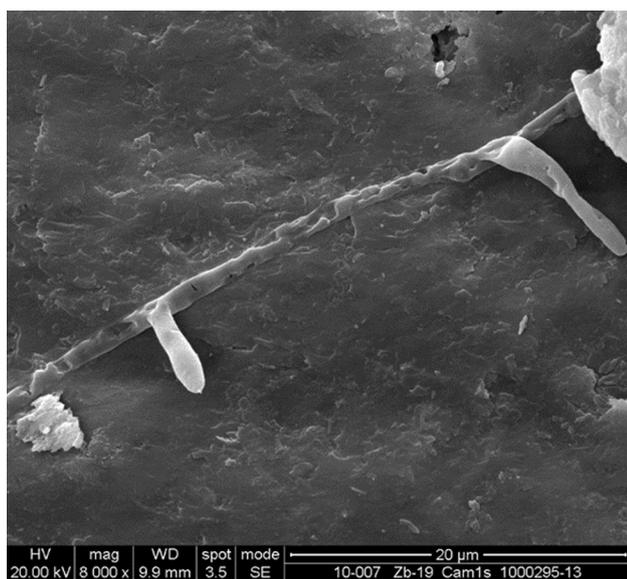


Fig. 9 Symbiotic bacteria with the residues of linear leafed plants

4 Discussion

Previous studies have shown that bacterial fossils can be found in the Cambrian–Permian source rocks (Lei et al. 1994; Edoardo and Maurice 2007; Wu et al. 2007; Xie et al. 2013; Zhao 2007; He et al. 2010; Zheng 2013; Zhang

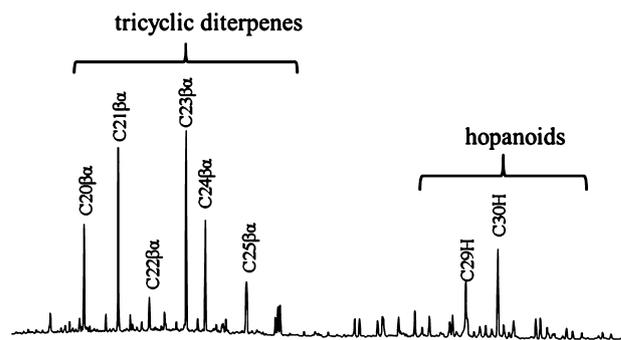


Fig. 10 Gas chromatograms (m/z 191) showing tricyclic diterpenes and hopanoids in ZB19

et al. 2015), especially in organic-rich source rocks and coal seams, which were confirmed by zoogloea observed on thin sections, SEM data and biomarker compounds. Possible bacterial fossils were identified only through SEM but without other evidences, due to the high maturity characteristics of samples. However, some specialists considered that the zoogloea was more like degraded organic matter, which we cannot tell what it was before. Though bacterial fossil communities were observed under SEM, the biggest problem is that the SEM pictures cannot be related with the ones by microscope and the age of these fossils shown in SEM pictures couldn't be measured. These

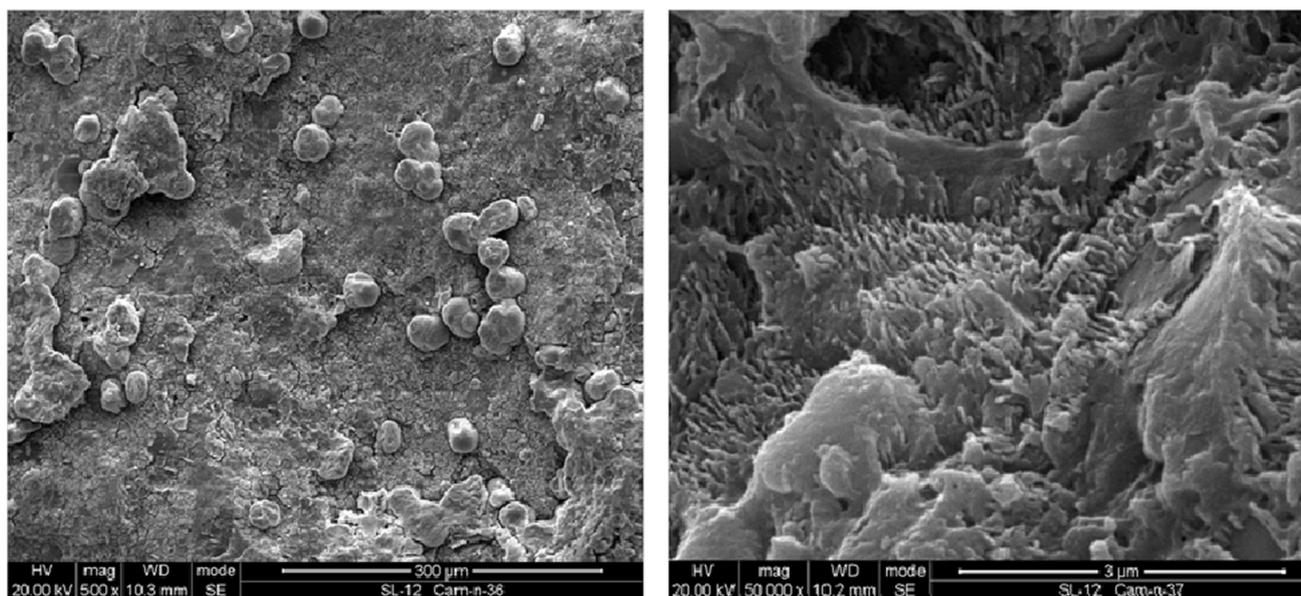


Fig. 11 Phosphate nodules and inner filamentous bacteria in the Lower Cambrian rocks from Songlin

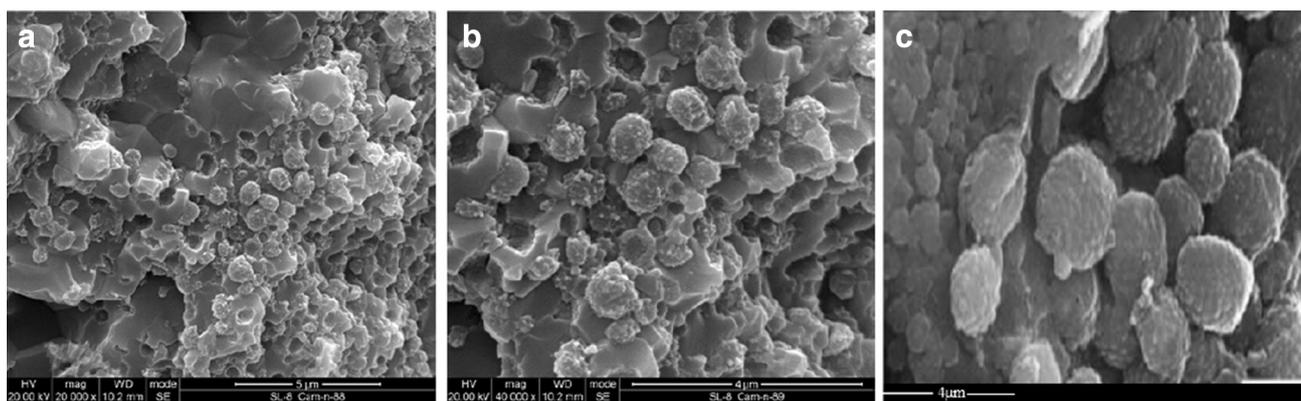


Fig. 12 Bacterial spore-like fossils in the Cambrian rocks from Songlin, Guizhou: **a, b** The No. 8 sample from Songlin, and **c** from Ref. Wang and Tao (2005)

fossils could be formed from any time after the rock was deposited. The study of determining the formation time of fossils in the SEM pictures would be focused in the future, but even so, previous studies have shown their relationship with the formation of hydrocarbon.

Bacterial fossils are common in the Permian strata, where many scholars have found a large number of bacterial fossils (Edoardo and Maurice 2007; Wu et al. 2007; He et al. 2010). In South China, organic sulfur is prevalent in the Permian coal seams (Zheng 2013), in which the distribution characteristics and genesis of sulfur was also studied by Zhao (2007) and Lei et al. (1994) also observed bacterial fossils in the high-sulfur coal in Guiding Coal Mine, Guizhou Province as we did in Longtan Formation, Nanchuan. It is suggested that bacteria is not accidentally present in high-sulfur environment. However, the

formation mechanism and environment of sulfur bacteria are still unknown and should be studied in details in the future.

There are few reports about bacterial fossils found in the Silurian strata, where samples were of high maturity. Edwards et al. (2006) performed a detailed analysis of the bacterial structure in kerogen samples, which could be reliable, due to any possible contamination avoided in the process of sample processing. Possible bacterial fossils were found in the kerogen of the Lower Silurian Longmaxi Formation, Huayingshan, Chongqing, indicating that bacterial fossils might be also possibly widespread in the Silurian strata.

Pleomorphic bacterial fossils were found in the Cambrian strata, reflecting the diverse micro-environment in different micro-sections. Irregular bacterial community

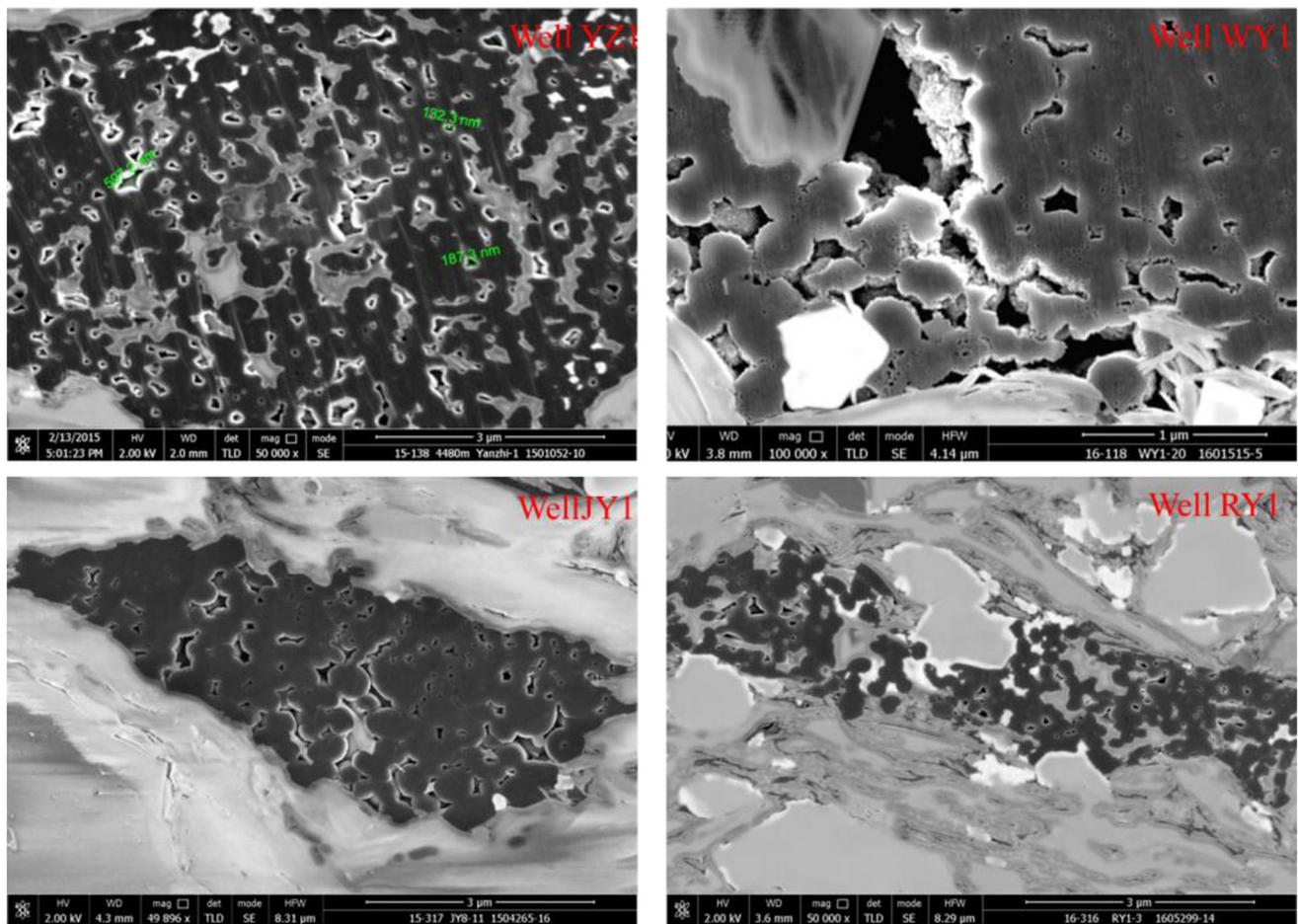


Fig. 13 Pore formed by bacterial fossils in the Longmaxi Formation source rocks in South China

might be caused by their polymorphism, representing different species. Scholars found that many phenomena observed in phosphate nodules were related to the activities of microorganisms, the significant agent in the deposition of phosphorus. This provided strong evidence for the biomineralization of phosphate nodules (Lei et al. 2000). Therefore, the formation of the phosphate nodules found in the Cambrian rock samples taken from Songlin, Guizhou, was possibly related to the activity of bacteria (Fig. 11). In addition, the bacterial fossil found on the Songlin section (Fig. 12) was similar to that was described by Edwards et al. (2006), with similar size, morphology and spore-like fossils.

The discovery of bacterial fossils in source rocks is important to the study on the formation and preservation of natural gas. First of all, it is significant to understand hydrocarbon generation. Havey et al. (1995) believed that bacterial degradation had an important impact on hydrocarbon generation through experimental simulation, even as important as the composition of original organic matter. Ding et al. (1997) studied the organic petrology of source rocks in the Junggar Basin and found a lot of amorphous

bodies degraded by bacteria, which were the products of degradation of organic matter by bacteria and disproportionation reaction. Because bacteria participated in the transformation of organic matter, hydrocarbon generation potential of the source rocks was improved, and a lot of oil and gas would be produced in low mature stage ($R_o = 0.5\%–0.7\%$) (Wang et al. 1995). Zhang et al. (1996) investigated the characteristics of gas, oil and kerogen through *Chlorella* simulation before and after the degradation of bacteria, indicating that the pyrolysis rate of *Chlorella* increased by 2.09 times after the degradation of bacteria. Since bacterial fossils were commonly found in the source rocks in South China, they could have a great contribution to the formation of oil and gas at the early stage of hydrocarbon generation. We have also found the contribution of bacterial fossils to reservoir space (Fig. 13). Based on previous analysis of the morphology and size of bacterial fossils, pore development in the bacterial fossils was observed using Argon ion laser and SEM. The nanoscale pores developed in these bacterial fossils were generally greater than 100 nm and locally connectable, providing primary space to shale gas.

In summary, we suggest that bacteria is important for shale gas generation and preservation, and different types of bacterial fossils might indicate different microbial sources, sedimentary environments and preservation mechanisms. However, a lot of future work on the relationship between bacterial fossils and the formation of source rocks, favorable sedimentary environment and the mineralization mechanism of bacterial fossils, needs to be done. By virtue of study on bacterial fossils, source rock evaluation will be triggered from micro to macro scale. Further understanding of the mysterious microscope world will provide theoretical support to the progress in exploration and development theory.

5 Conclusions

1. Confirmed and/or possible bacterial fossils are commonly found in marine source rocks in South China, and they have a symbiotic relationship with the source rocks. Bacterial fossils have been confirmed in low-mature samples taken from the Upper Permian Longtan Formation in Huayingshan and the Lower Cambrian Niutitang Formation in Chengkou, using thin sections, SEM and GC/MS analysis. The possible bacterial fossils may exist in the high-mature Lower Cambrian Niutitang Formation in Songlin and the Lower Silurian Longtan Formation in Huayingshan.
2. Bacteria in different marine source rocks have different characteristics, such as sulfur bacteria in coal seams and symbiotic with gypsum playing an important role in the formation of the gypsum. Bacterial fossils might also play an important role in the generation and preservation of hydrocarbons, which can improve hydrocarbon generation rate and provide reservoir space.

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