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Revisiting amorphous organic matter in Kimmeridgian laminites: what is the role of the vulcanization process in the amorphization of organic matter?

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ABSTRACT

Kimmeridgian bituminous laminites from Orbagnoux (France) contain abundant amorphous organic matter (AOM). Previous studies have shown that the vulcanization pathway was the dominant preservation mechanism of AOM in these laminites, and led to its structureless aspect (a process called amorphization) at the nanoscale. In contrast, new observations in scanning electron microscopy and transmission electron microscopy demonstrate that this AOM exhibits typical cyanobacterial structures (exopolymeric substances, filamentous and coccoid bacteria) and ultralaminae. This identification is supported by a comparison with a recent cyanobacterial biofilm considered as an analogue. Moreover, this comparison demonstrates that ultralaminae in the Orbagnoux environment cannot solely be attributed to microalgal cell walls, but also to constituents of cyanobacteria. The microscopic identification of a ubiquitous cyanobacterial imprint demonstrates that the selective preservation pathway has been largely underestimated in Orbagnoux AOM and/or that the vulcanization process does not lead to the amorphization of organic matter automatically.

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Introduction

Organic geochemistry and palynofacies are standard methods used to study sedimentary organic matter in ancient sediments. Organic matter (OM) in organic-rich rocks is mainly constituted of amorphous organic matter (AOM), which is commonly observed at the scale of light microscopy. In the studied Upper Kimmeridgian bituminous laminites at Orbagnoux (France), deposited in a lagoonal, backreef environment, most of the OM is of the AOM type. This AOM has been considered as a good example for the preservation of OM through vulcanization of lipids rather than the selective preservation pathway (Mongenot *et al*., 1997). The former pathway is a process that can be revealed only through organic geochemical methods (Sinninghe Damsté *et al*., 1988, 1989; Kohnen *et al*., 1991; Adam *et al*., 1993), whereas selective preservation can also be identified through microscopical observations (Goth *et al*., 1988; Largeau *et al*., 1990; Derenne *et al*., 1991). So far, previous studies have claimed that petrographical observations support vulcanization as the dominant preservation pathway leading to an amorphization of the preserved OM at the nanoscale (Mongenot *et al*., 1997, 2000).

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This study presents a new optical investigation of this fossil AOM down to the nanoscale using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The aim was to re-evaluate petrographically the role of vulcanization in the amorphization of OM. Observed structures were compared with those from a recent cyanobacterial analogue (biofilm).

Geological and palaeoenvironmental settings

The Upper Jurassic bituminous laminites at Orbagnoux were deposited in a carbonate platform environment, which displays a shallowing-up trend from pelagic to paralic deposition (Figs 1 and 2). Organic-rich laminites correspond to cyanobacterial mats deposited in a lagoon sheltered from the opened platform by a well-developed barrier reef (Bernier, 1984). Recent sediments in Pacific atolls containing cyanobacteria, and called 'kopara' deposits, have been proposed as a recent sedimentological analogue (Tribovillard *et al*., 2000).

Many authors have studied these sediments because of their great potential as petroleum source rocks (Riche, 1904; Gubler and Louis, 1956; Bernier and Courtinat, 1979; Bernier, 1984; Courtinat, 1989; Gorin *et al*., 1989; Tribovillard *et al*., 1992, 1994, 1999; Mongenot *et al*., 1997; Tribovillard, 1998). Five facies have been identified, originating from the development of cyanobacterial benthic mats (light-coloured undulating laminae), coccolith blooms (massive limestones) and the interaction between benthic, cyanobacterial activity and coccolith settling (dark-coloured undulating laminae, dark or light-coloured parallel laminae).

Extensive studies have been carried out on the chemical structure of the kerogen, source organisms and formation pathways in the dark-coloured laminae (Fig. 2). Bulk sediment Rock-Eval pyrolysis data from the latter indicate high hydrogen indices (780–960 mg HC per g TOC) and a TOC content averaging 7.2% (Mongenot *et al*., 1997). The kerogen from these laminites belongs to type I–II OM (Gorin *et al*., 1989). These organic-rich sediments are characterized by sulphur-rich OM. The vulcanization pathway, which is associated with iron-poor organic-rich sediments, is usually known in pelagic deposits, although it can also take place in

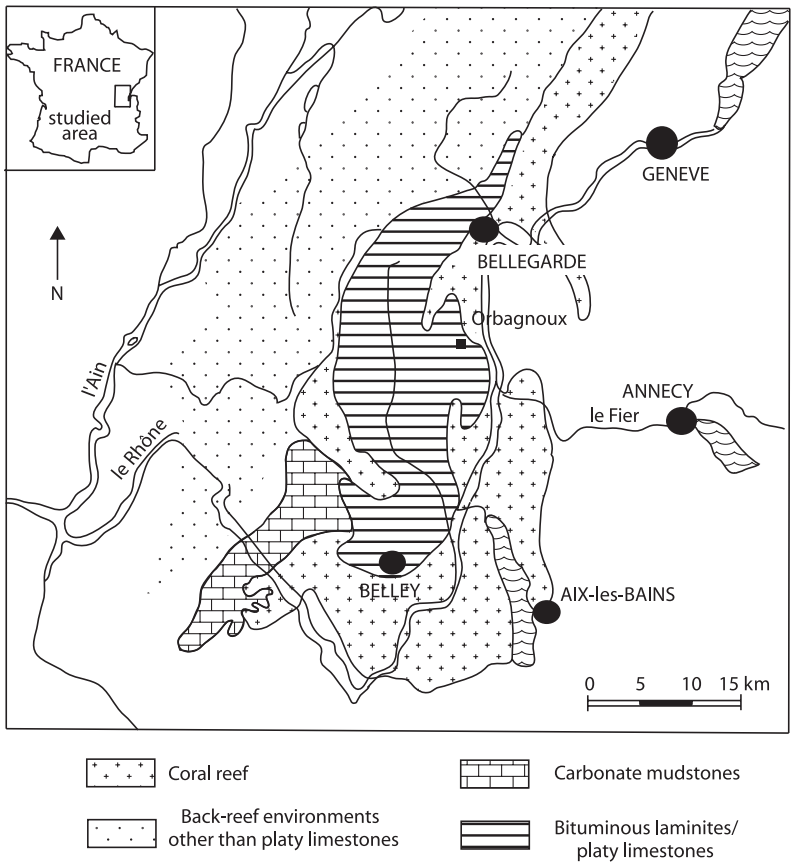

Fig. 1 Geological setting of the Upper Jurassic bituminous laminites at Orbagnoux (modified after Bernier, 1984).

shallow peritidal environments where bacterial activity is intense (Tribovillard *et al.*, 2000). In the Orbagnoux kerogen, thiophenes are the dominant sulphur organic component (Sarret *et al.*, 2002).

Methods

The studied fossil AOM originates from the dark-coloured parallel laminae (Fig. 2), whereas the recent bacterial analogue comes from a biofilm developed in the laboratory from a microbial mat sampled in a Brazilian lagoon (Lagoa Vermehla located about 100 km east of Rio de Janeiro city; Vasconcelos, 1994). Fossil and recent OM were studied microscopically after crushing of rock samples, dissolution of the mineral fraction through acids (32% HCl and 70% HF), followed by heavy liquid separation ($ZnCl_2$ at a specific gravity of 2.0) of heavy minerals (standard palynological preparation technique, e.g. Steffen and Gorin, 1993). In this study, the effect of acid treatment has been evaluated on recent bacteria, and no noticeable artefact has been shown to be generated on the organic material. Subsequently, two microscopical techniques have been used to analyse the morphology and structure of OM at the nanoscale: SEM (Jeol JSM 6400, France) on gold-coated samples and TEM (Philips EM 208, France) on ultrathin sections. SEM provides surface morphological information down to micrometre scale. Prior to microscopical study, the recent bacterial material was dried using the critical point drying method. In TEM, 70-nm thick ultrathin sections were coloured with uranyl acetate for 15 min and permitted the investigation of internal textures and structures at a scale of 100 μm down to 10 nm. Many ultrathin sections were investigated in each sample so as to provide a representative overview of the general organic fabrics.

Results

Kimmeridgian laminites

In standard light microscopy, AOM particles appear as grumose (*sensu* Combaz, 1980) kerogen without any structure (Fig. 3). Under the SEM, AOM is characterized by an alveolar network. Alveoli are holes which show a large size range in both SEM and TEM (Figs 4a,b and 5). Their size ranges from 5 μm to 150 nm. Imprints of shells are sometimes observed within this network (Fig. 6).

Fossil bacteria are well preserved with coccoid and filamentous forms within the size range of bacteria (respectively 1–5 μm in diameter and 10–50 μm in length, Fig. 7). In ultrathin sections, filamentous bacteria showing a characteristic cell wall with a double membrane can be recognized (Fig. 8). Several 1- to 2-μm thick laminated bodies (Fig. 9) showing a specific internal organiza-

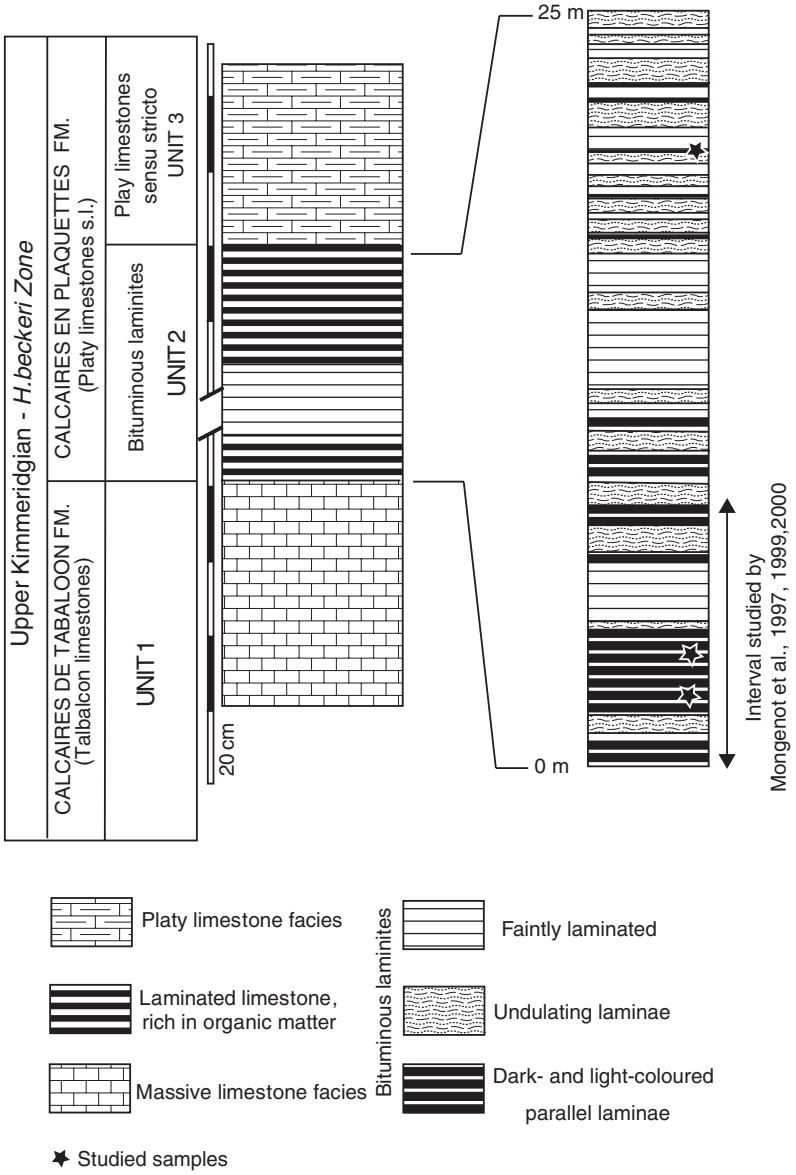

Fig. 2 Schematic lithological column of the bituminous laminites at Orbagnoux (modified after Tribovillard *et al.*, 2000).

tion can also be observed. They comprise a succession of light- (125 nm thick) and dark-coloured (~300 nm thick) laminae, referred to as ultralaminae (cf. Derenne *et al.*, 1991).

Recent cyanobacterial biofilm

Prior to acid etching, the recent cyanobacterial biofilm (Fig. 10) shows different bacterial communities which include coccoid bacteria (2–4 μm in diameter) and filamentous bacteria (20–50 μm in length). Microorganisms are surrounded by the exopolymeric substances (EPS) they secrete, the whole being attached to either an inert or living surface (Madigan *et al.*, 2003). In the studied biofilm, the structure resulting from the mixing of EPS and bacteria displays depressions and an alveolar network perpendicular to the biofilm surface (Fig. 11). Cyanobacteria can easily be recognized because they contain typical components such as cytoplasmic inclusions and 60- to 100-nm wide thylakoids, responsible for photosynthesis (Fig. 12).

After acid etching, bacterial internal structures remain recognizable. Thylakoids associated with cytoplasmic inclusions are well preserved with sizes similar to those in the unetched biofilm (Fig. 13). They often appear on their own following the break-up of the bacterial cell wall. Moreover, in TEM, EPS form a typical, well-defined, alveolar network with different orders of magnitude varying in size from 5 μm to 150 nm (Figs 14 and 15).

Discussion

The presence of cyanobacteria in sedimentary rocks has been already iden-

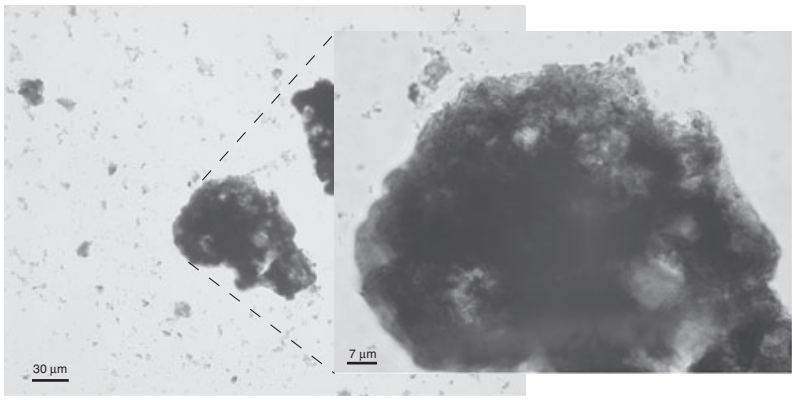

Fig. 3 Palynofacies slide: fossil amorphous organic matter (AOM) in transmitted light microscopy.

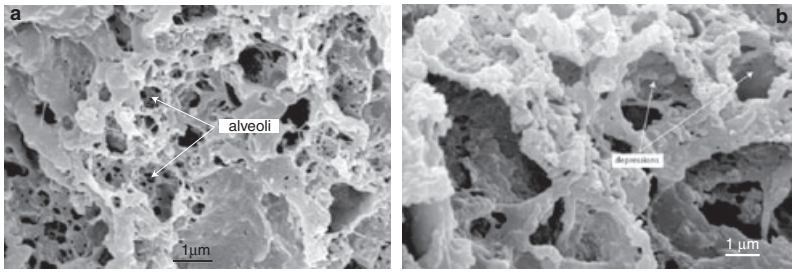

Fig. 4 (a) Fossil amorphous organic matter (AOM) in scanning electron microscopy (SEM) characterized by alveoli with different orders of magnitude. (b) Fossil AOM in SEM showing depressions in alveoli.

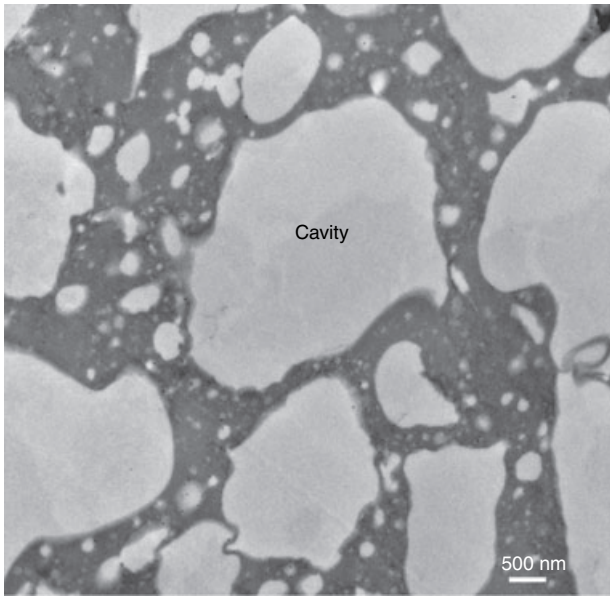

Fig. 5 Fossil amorphous organic matter (AOM): ultrathin section in transmission electron microscopy showing the alveolar network.

tified through organic geochemistry (lipids), and bacterial remains such as sheaths have been observed in SEM (Kazmierczak *et al.*, 1996). Previous studies at Orbagnous using TEM (Mongenot *et al.*, 1997, 1999) indicate that AOM is entirely composed of gel-like, nanoscopically amorphous, sulphur-rich, organic particles. The latter authors' observations refer to the lower part of the Orbagnoux section (Fig. 2), where dark-coloured parallel laminae are the most characteristic and abundant. Although the precise location of their studied sample(s) is not available, the two samples studied here in this lower interval (Fig. 2) present the same lithological characteristics (Mongenot *et al.*, 2000) and similar Rock-Eval parameters (TOC greater than 6%, HI greater than 800). In order to confirm the observations made in this lower interval, another sample with similar lithological facies and Rock-Eval parameters was also studied in the upper interval of the section where dark-coloured parallel laminae are less frequent (Fig. 2). The investigation of these three samples through a combination of TEM and SEM methods reveals that in fact AOM has a complex structure at the nanoscale. The subsequent comparison between fossil AOM and the recent biofilm leads to the following observations:

1 Both AOM and biofilm exhibit a similar alveolar structure in the same size range (compare Figs 4b and 11, Figs 5 and 14). The biofilm structure is constituted by EPS, and consequently, that observed in the fossil AOM can be interpreted as the relics of a biofilm framework. This correlation precludes that this morphology may be related to diagenesis. Moreover, the ubiquitous presence of alveoli in the recent biofilm (which contains less than 5% of calcite) makes it highly improbable that most of the alveoli observed in the fossil AOM might be attributed to solution cavities resulting from the loss of mineral phases.
2 Thylakoids observed in the acid-etched biofilm (Fig. 13) closely resemble the ultralaminae present in fossil AOM (Fig. 9). Therefore, part of the ultralaminae in Orbagnoux AOM can be interpreted as

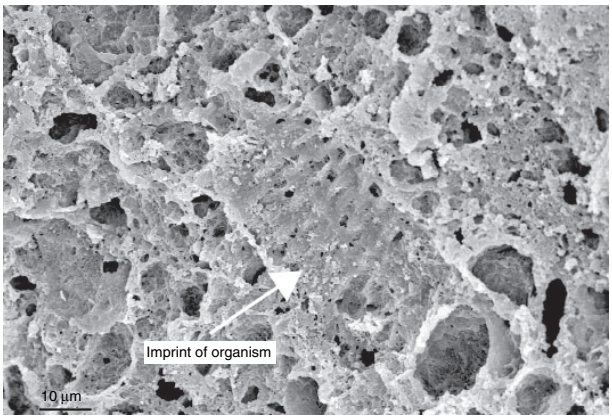

Fig. 6 Fossil amorphous organic matter (AOM) in scanning electron microscopy showing an imprint of organism.

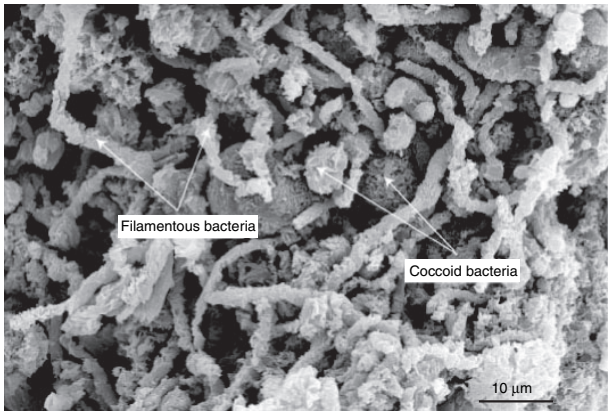

Fig. 7 Fossil amorphous organic matter (AOM) in scanning electron microscopy: communities of filamentous and coccoid bacteria.

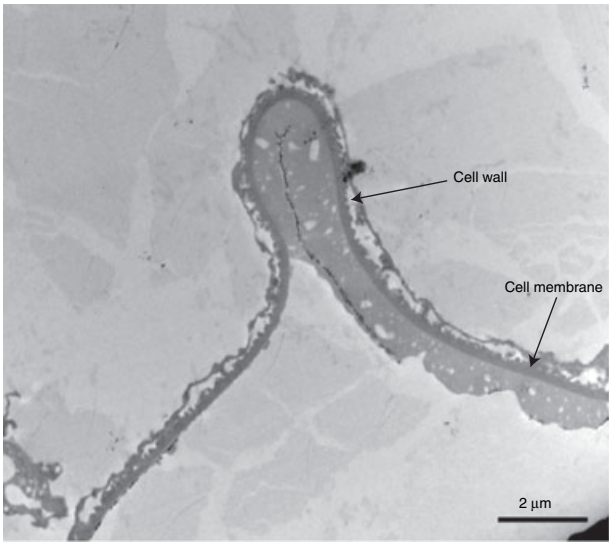

Fig. 8 Fossil amorphous organic matter (AOM): ultrathin section in transmission electron microscopy illustrating a filamentous bacterium characterized by typical cell wall and cell membrane.

originating from cyanobacteria rather than only from microalgae as previously thought (Derenne *et al.*, 1991).

3 The presence in fossil AOM of ultralaminae and other structured elements such as filamentous and coccoid bacteria (Figs 7–9) sheds new light on OM preservation mechanisms. So far, AOM at Orbagnoux has been described as gel-like and amorphous at the nanoscale by Mongenot *et al.* (1997, 1999). This amorphization is commonly attributed to the vulcanization preservation pathway, which is well identified through organic geochemistry (Sinninghe Damsté *et al.*, 1988, 1989; Mongenot *et al.*, 2000). The better resolution of the data presented here permits the distinction of structures that can be attributed to cyanobacterial biofilms. The presence of these structures indicates that the selective preservation pathway (Derenne *et al.*, 1991) in Orbagnoux dark laminae has been strongly underestimated and/or that the vulcanization pathway in the Orbagnoux palaeoenvironment does not result in OM amorphization.

Conclusions

The combined use of SEM and TEM leads to a better characterization of AOM at the nanoscale. These techniques show clearly that this AOM is nanostructured, whereas it has been previously described as gel like and amorphous. The morphological interpretation of these structures is supported by similar observations on one type of recent cyanobacterial biofilm and can be summarized as follows:

1 alveolar structures are reminiscent of bacterial exopolymeric substances (EPS) within the cyanobacterial biofilm;
2 fossil AOM at Orbagnoux is dominated by the presence of bacterial structures (filamentous and coccoid bacteria, EPS);
3 ultralaminae can be directly related to cyanobacterial thylakoids, whereas it has so far been ascribed only to microalgal cell walls.

These results demonstrate that AOM at Orbagnoux is structured at the nanoscale, by contrast to what has

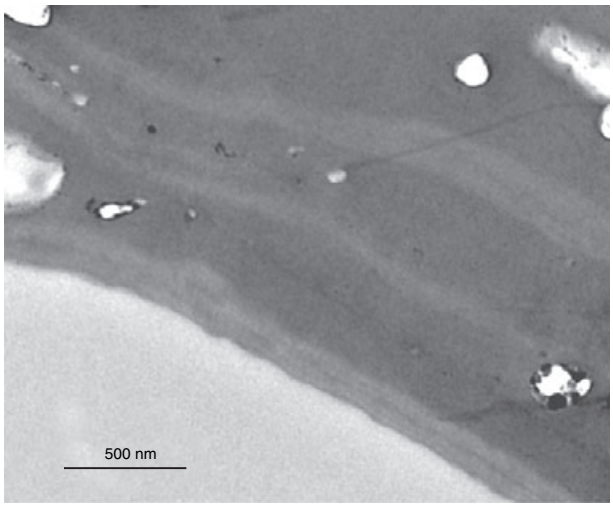

Fig. 9 Fossil amorphous organic matter (AOM) in transmission electron microscopy showing ultralaminae.

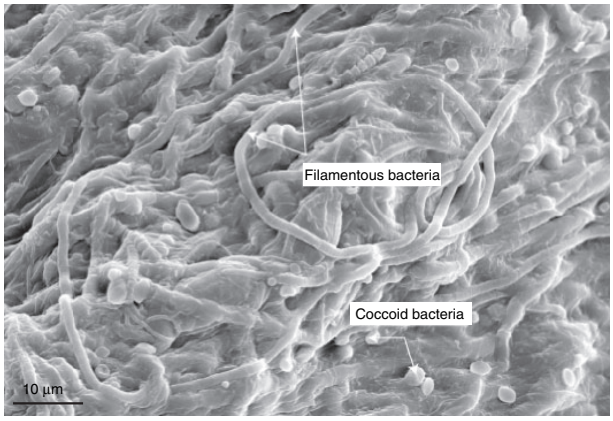

Fig. 10 Recent biofilm (from Lagoa Vermehla) prior to acid etching: scanning electron microscopic picture illustrating communities of coccoid and filamentous bacteria.

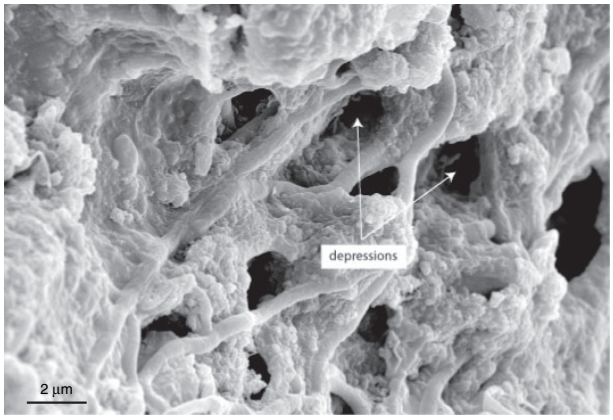

Fig. 11 Recent biofilm prior to acid etching: scanning electron microscopic picture showing exopolymeric substances (EPS) between filamentous bacteria creating depressions similar to an alveolar network.

always been observed before (Mongenot *et al.*, 2000). This amorphous aspect has been interpreted as the result of the vulcanization process which is well documented through geochemical analyses. The identification of a strong microscopic cyanobacterial imprint highlights the so-far underestimated contribution of the selective preservation pathway and/or that the vulcanization pathway does not automatically lead to OM amorphization.

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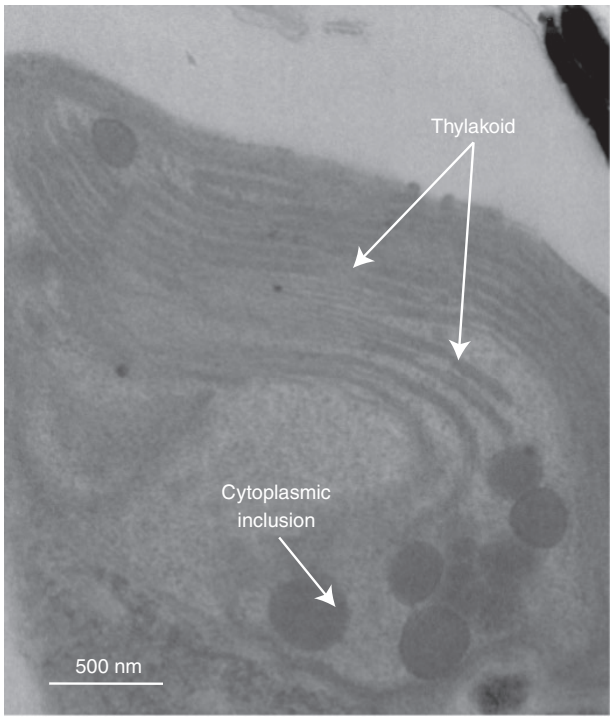

Fig. 12 Recent biofilm prior to acid etching: ultrathin section in transmission electron microscopy illustrating a cyanobacterium containing thylakoids and cytoplasmic inclusions.

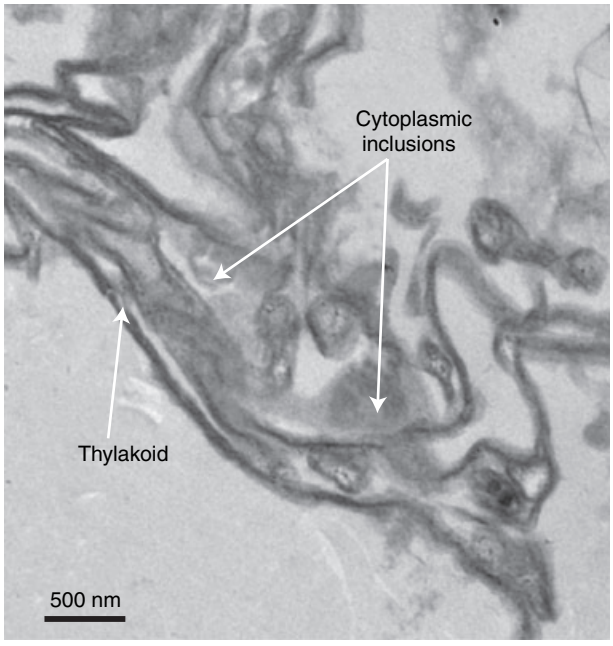

Fig. 13 Recent biofilm after acid etching: ultrathin section in transmission electron microscopy showing thylakoids and cytoplasmic inclusions.

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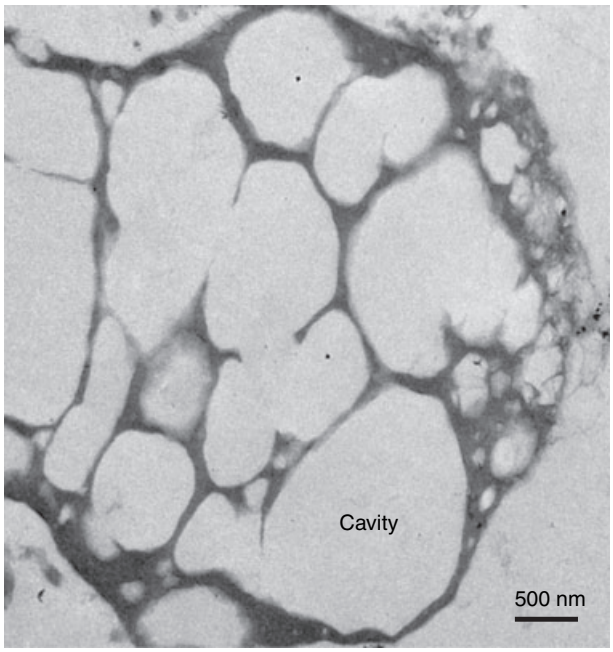

Fig. 14 Recent biofilm after acid etching: ultrathin section in transmission electron microscopy showing the exopolymeric substances (EPS) alveolar network.

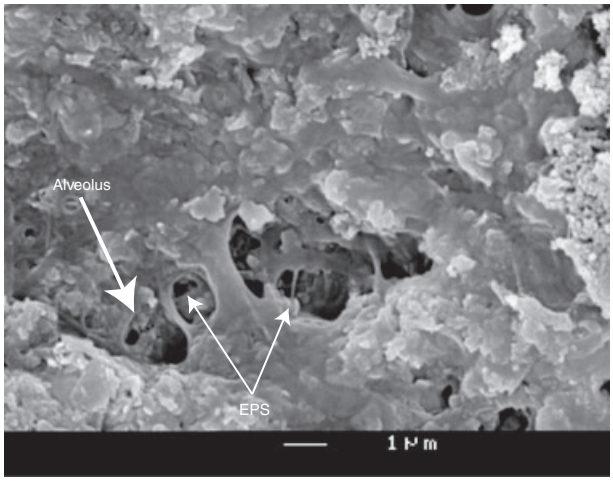

Fig. 15 Recent biofilm after acid etching: scanning electron microscopic picture illustrating alveoli and filaments within exopolymeric substances (EPS).

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