

Experimental Colonization and Alteration of Orthopyroxene by the Pleomorphic Bacteria *Ramlibacter tataouinensis*

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The colonization of orthopyroxene crystals by a pleomorphic bacterium and the mineralogical products resulting from a prolonged interaction have been studied. We used *Ramlibacter tataouinensis* (strain TTB310), which is an aerobic β -Proteobacterium moving over surfaces by gliding motility and whose life cycle includes rods and spherical cysts. Analysis of cultures grown on solid media with micrometer-sized pyroxene and quartz crystals scattered over the surface revealed a taxis of the bacteria toward the crystals. Given the mineralogical non-specificity of the interaction, a mechanism of elasticotaxis is inferred. After the rods had adhered to the pyroxene surface, they differentiated into cysts leading to the formation of microcolonies that were centered on a crystal grain. This suggests an original coupling between the life cycle of *R. tataouinensis* and the interaction with the crystals. The alteration of orthopyroxene was studied by high-resolution transmission electron microscopy at the interface between cysts and pyroxene crystals. The pyroxene surface showed an amorphous layer that was more developed than that of abiotic control samples processed under the same conditions. Moreover, chemical analyses showed that the dissolution of pyroxene was reduced in the presence of *R. tataouinensis*. The origin and the significance of the amorphous layers is discussed.

Keywords bacterial colonization, bio-alteration, elasticotaxis, gliding, pyroxene, *Ramlibacter tataouinensis*, TEM

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INTRODUCTION

Most of the microorganisms inhabiting the surface of the Earth are attached to solid surfaces. Many studies have questioned whether the mineralogical substrate is only an anchorage site for the bacteria or a source of nutrients (for example, Bennett et al. 2001). It has been demonstrated that bacteria can modify the mineral chemistry at their attachment sites (for a review see Banfield and Hamers 1997), and that the physiology of bacteria can vary depending on whether they are attached to surfaces or not (Bonin, Rontani, and Bordenave 2001). One interesting system exhibiting both aspects is the interaction between Fe(II)-silicates and bacteria. While iron may represent a significant electron donor (Jakosky and Shock 1998) as well as a key component of numerous enzymes for bacteria, the dissolution rates of Fe(II)-rich silicate minerals may also be influenced by the metabolic activity of the bacteria (Welch and Banfield 2002). Although these minerals are very common in the surface rocks of the Earth and are major components of the geochemical cycle of iron, only few studies have been devoted to the study of the alteration of such phases in the presence of aerobic microorganisms (e.g., Welch and Banfield 2002).

Ramlibacter tataouinensis TTB310, a bacterium isolated from an arid sand near Tataouine (South Tunisia), belongs to a recently described species (Heulin et al. 2003). Previous observations (Gillet et al. 2000) suggested that this β -Proteobacterium could be involved in the colonization and alteration of orthopyroxene crystals (Fe²⁺, Mg²⁺ containing silicates) collected in the Tataouine sand. *R. tataouinensis* is a slow-growing, aerobic, and chemo-organotrophic bacterium (Heulin et al. 2003), whose life cycle includes both rods and spherical cysts. The rods can move across solid surfaces, whereas the nonmotile cysts can divide (Heulin et al. 2003). To better understand the former observations by Gillet et al. (2000) on natural samples

and the processes involved in the interactions between bacteria and Fe(II)-silicates, it is of interest to understand how *R. tataouinensis* colonizes orthopyroxenes, to determine what links exist between the colonization and the life cycle of the bacteria, and what are the resulting modifications of the orthopyroxene chemistry and crystal structure. In this study, the results of two sets of experiments carried out on the *R. tataouinensis*/orthopyroxene system are presented and discussed: (1) colonization of orthopyroxene crystals by *R. tataouinensis* in solid culture media; (2) study of chemistry and microstructures of orthopyroxene alteration by *R. tataouinensis* in liquid culture media.

EXPERIMENTAL METHODS

Preparation of Powdered Pyroxene and Quartz

Experiments were carried out using bronzitic (orthopyroxene) cumulates from Zimbabwe. Light microscope observations and electron microprobe analyses showed high chemical homogeneity of the pyroxene; its composition corresponded to $(\text{Mg}_{0.82}\text{Fe}_{0.12}\text{Ca}_{0.03})\text{SiO}_3$. Bronzite crystals were ground in an agate mortar, in acetone. The powders were cleaned ultrasonically in distilled water. The $<5 \mu\text{m}$ fraction was obtained by sedimentation in distilled water, then dried at room temperature. Powders were checked by X-ray diffraction and prepared for TEM observations with ultramicrotomy (blank crystals). Quartz (SiO_2) powder was obtained from highly pure white sand (Fontainebleau, France) processed using the same procedure to obtain a $<5 \mu\text{m}$ powder. Both dry pyroxene and quartz powders were sterilized by autoclaving 30 minutes at 121°C .

Cultivation

Experiments were conducted using both solid and liquid cultures. Solid culture media are appropriate for light microscopy studies of the spatial dynamics of colonization of orthopyroxene by *R. tataouinensis*, whereas liquid cultures are more adequate for following chemical modifications of the medium due to pyroxene alteration as well as for preparing pyroxene grains for high resolution transmission electron microscopy.

The study of pyroxene colonization by *R. tataouinensis* was performed on a solid medium. Strain TTB310 was first cultured in 10 ml of 10-fold diluted tryptic soy broth (TSB/10, Difco Laboratories) in 50 ml polycarbonate flasks with shaking at 200 rpm. After incubation at 30°C for 5 days with shaking, the cells were harvested by centrifugation for 5 minutes at $15,000 \times g$ and subsequently washed in sterile distilled water. The bacterial preparation was then serially diluted in TSB/10 to 10^{-5} ($300\text{--}500 \text{ CFU ml}^{-1}$) and approximately 1 mg of orthopyroxene or quartz was added to 1 ml of the higher dilution (10^{-5}). Microscopic observations conducted immediately after the mixing showed that many crystals were not colonized by bacteria. One hundred microliters ($30\text{--}50 \text{ CFU}$) of this mix were spread

on 10-fold diluted tryptic soy broth agar medium (TSA/10, agar concentration: 15 g l^{-1}) and incubated at 30°C . After 1 week, plates were analyzed by light microscopy and scanning electron microscopy.

Analysis of the study of alteration chemistry and microstructures was performed by using liquid cultures (batch reactors) of *R. tataouinensis* TTB310 grown in presence of fine grained orthopyroxene powder. One hundred milligrams of sterile orthopyroxene powder (abbreviated as Px in the sample names) was added to 100 ml of sterile TSB/10. This solution was used to prepare 10 ml solutions of TSB/10 with the same pyroxene load. The solutions were either inoculated with TTB310 (#TSB/10+Px+TTB) or left uninoculated (#TSB/10+Px). Inoculated and sterile TSB/10 solutions (#TSB/10+TTB and #TSB/10) were prepared to be used as controls for the inductively coupled plasma-atomic emission spectroscopy (ICP-AES) analyses. All the solutions were incubated at 30°C for 1 month.

Analytical Methods

Light Microscopy. Direct observations of agar plates were performed using a differential interferential contrast (DIC) microscope (BX50, Olympus) equipped with a $100\times$ objective lens (UPlanApo, numerical aperture = 1.35, oil immersion) and a $60\times$ objective lens (PlanApo, numerical aperture = 1.40, oil immersion). The images obtained with the microscope were captured with a CCD camera (F-View, Olympus). Digital acquisition, processing and archiving were performed using analySIS software (Soft Imaging System). For each observation, a small piece of agar supporting one colony was cut with a sterile blade and placed on a glass slide. A cover slide mounted on a square rubber joint was placed above the sample. The height of the rubber joint was adjusted so that the cover slide lay just above the surface of the colony. With this setting, the sample remained wet for several hours.

Chemical Analyses. Chemical analyses were performed by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES, Perkin Elmer Optima 3000, LISA). All solutions were filtered with a $0.2\text{-}\mu\text{m}$ acrodisc syringe filter to remove the bacteria and mineral particles, diluted 10-fold, and then acidified with two drops of 14 N HNO_3 . TSB/10 was buffered with phosphate to $\text{pH} = 7.1$. No pH change was detected over one month in the cultures. Since batch dissolution experiments are often irreproducible, especially in the early stages of the experiments due to rapid dissolution of the very reactive material created by sample preparation, the measurements were made after 30 days to suppress possible irreproducibilities coming from the initial stages. Moreover, the same stock solution containing the culture medium and the pyroxene powder was used to prepare both abiotic and biotic dissolution experiments, which guarantees identical conditions between the different samples. Finally, two replicates for each condition were analyzed and yielded consistent results.

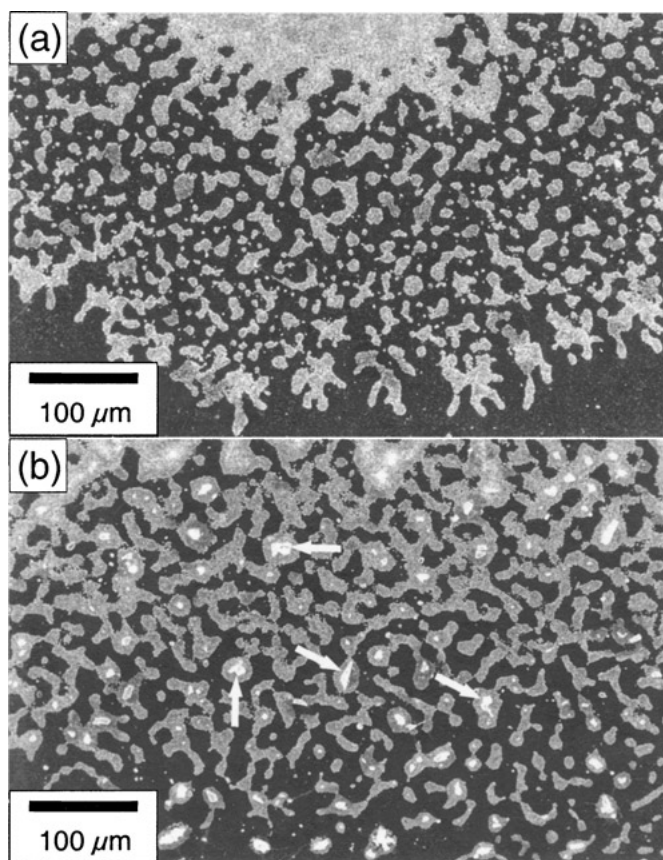


Figure 1. Scanning electron micrographs (secondary electrons) showing the peripheral zone of *R. tataouinensis* TTB310 colonies cultured on a solid medium (TSA/10) with or without pyroxene grains. (a) TSA/10 without pyroxene. The flat core of the colony (grey) is partly visible on the top of the photograph. The peripheral zone consists of spotted microcolonies (arrows). Observation at a higher magnification of these microcolonies shows that they are comprised of aggregates of spherical cysts (see Figures 2 and 3). (b) Close up of the peripheral zone of a colony grown on TSA/10 with pyroxene. Pyroxene crystals are bright. Each pyroxene crystal is surrounded by a microcolony.

Transmission Electron Microscopy. For each preparation, one drop of 100 μl of the liquid cultures reacted for 1 month was directly deposited on a copper grid coated with a carbon film. Cultures were resuspended by slight agitation just before pipetting the drop in order to sample the bacterial flocs trapping pyroxene grains, which settled at the bottom of the flasks. TEM examinations were performed on a Jeol 2010F microscope operating at 200 kV, equipped with a field emission gun, a high-resolution UHR pole piece, and a Gatan energy filter GIF 100. X-ray Energy Dispersive Spectroscopy (XEDS) analyses were performed using a Kevex detector with an ultrathin window allowing detection of low atomic mass elements. XEDS analyses were systematically performed for 100 seconds with a probe size of 1 nm.

RESULTS

Colonization of Orthopyroxene Grains by R. tataouinensis

Following the procedure described above, pyroxene and quartz (as a control) grains were heterogeneously scattered on the agar surface. A few colonies grown at some distance from any crystal were used as control colonies. These control colonies displayed either a dome-shaped or a flat core and always had a heterogeneous concentric peripheral zone (Figure 1). Observation of the peripheral zone showed (i) spots corresponding to cyst-containing microcolonies, (ii) swarms of thousands of rods, and (iii) isolated rods at the border of the colony. The microcolonies derived from cyst-differentiating rods. The overall morphology of colonies growing near quartz or pyroxene grains was similar to that of colonies growing far away from any crystal. There were, however, several noticeable features visible at a smaller scale (Figure 1a and 1b). First, all the crystal grains (either quartz or pyroxene) lying in the peripheral zone were colonized by cyst-containing microcolonies (Figure 1b and Figure 2. See also caption Figure 3). Secondly, every time a crystal was close to a colony front, it was colonized by an emerging swarm (Figure 3).

To better appreciate the progression and the timescale of the process, several grains lying near the front of the colonies were observed over 20 hours. The magnification was high enough to resolve the 200-nm-wide rods. Capturing images every 3 seconds allowed the estimation of the speed of the border of the colony, i.e., a few microns per hour. Figure 3 illustrates the progression of a swarm near pyroxene grains (see also Web

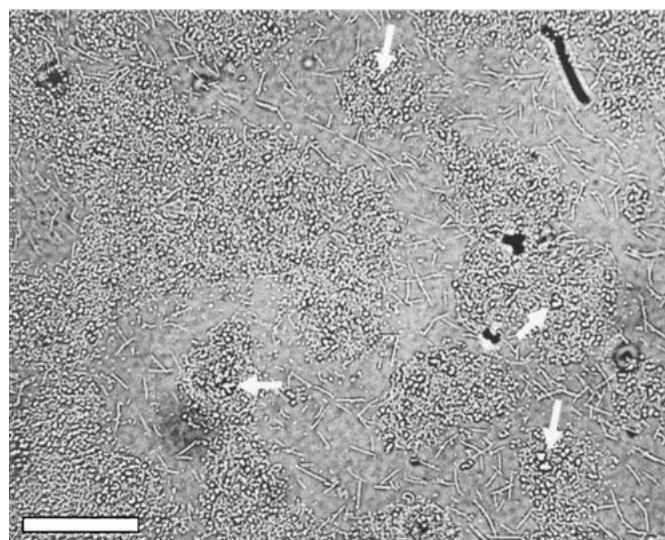


Figure 2. Optical phase-contrast micrograph of cyst-microcolonies formed at the border of colonies grown on TSA/10 with quartz. Very refringent micron-sized spots are quartz crystals (arrows). Similar to pyroxene crystals, quartz crystals are systematically colonized by cyst-microcolonies which appear as light grey areas. Rods swarm between the cyst-microcolonies. Scale bar is 30 μm .

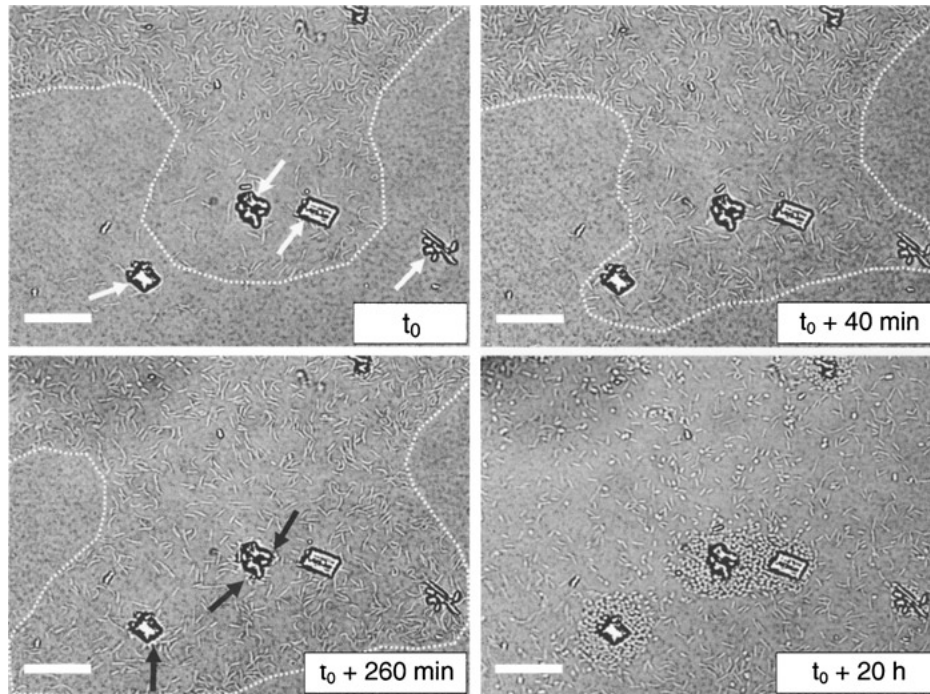


Figure 3. Optical time-lapse micrographs of the periphery of a colony grown on TSA/10 with pyroxene, scale bars are 12 μm . (a) $T = 0$ min. Only rods are observed in this micrograph. They propagate in the direction of refringent pyroxene grains (arrows) forming a swarm emerging from the colony. (b) $T = 40$ min. Densification of the different swarms directed towards the four main pyroxene grains. (c) $T = 260$ min. All pyroxene grains on the micrograph are colonized by the rods. The first appearance of spherical cysts in contact with pyroxene crystals is outlined by arrows. (d) $T = 1,200$ min. Cyst-containing microcolonies centered on the pyroxene grains. Except for microcolonies associated with a pyroxene grain no other microcolony was visible in this area, suggesting that contact with a crystal accelerated the differentiation of rods into cysts. Animated film showing bacterial colonization of pyroxene crystal is available at the following URL: (<http://www-dsv.cea.fr/geomicrobiology>).

site (<http://www.dsv.cea.fr/geomicrobiology>)). The rods were attracted by the crystals at short distances (nearly 10 to 20 μm ; Figures 3a, 3b, and 3c). Rods then adhered to the pyroxene grains and after few hours (3 to 4 hours), rods differentiated into spherical cysts that formed concentric microcolonies around the grains (Figure 3d).

Alteration of the Orthopyroxene Crystals

After 30 days of incubation, filtered cultures and abiotic controls were analyzed by ICP-AES (Table 1). Filtration was performed with 0.2- μm filters in order to remove the pyroxene crystals from the solutions. Under both abiotic and biotic conditions, Mg/Si ratios were similar in solution and in orthopyroxene starting material (Table 1). This was not the case for iron (Table 1). Of particular interest is the comparison between the abiotic and biotic dissolution (Table 1). First, the total quantity of dissolved elements (Fe, Mg, and Si) is reduced in the presence of *R. tataouinensis*. Regarding the standard deviations, the differences between abiotic and biotic experiments were significant for each repetition and for each analyzed element. Second, compared to silicon, less iron is released in the liquid medium in the

presence of *R. tataouinensis*. TEM observations were performed in order to better understand the lowered dissolution in the presence of *R. tataouinensis*. Control crystals (reacted in uninoculated TSB/10) and some pyroxene crystals cut by ultramicrotomy just after grinding (blank crystals) were observed. The lattice fringes of the orthopyroxene control grains not exposed to bacteria persisted from a fraction of a nanometer to less than 2 nm below the surface of each grain (Figure 4). Many orthopyroxene grains reacted in liquid cultures with *R. tataouinensis* were associated with adhering cysts (Figure 5). Cysts displayed an extracellular slime gluing the pyroxene grains. The bacteria/crystal interface was investigated. In contrast to the control and to the blank grains, the first nanometers (up to 10 nm in some cases) of orthopyroxenes in contact with cysts did not display any lattice fringe (Figure 5). Such developed amorphous or very poorly organized zones were only observed in contact with bacteria. We did observe, on the same grain, areas displaying an amorphous layer and corresponding to the site of adhesion of a cyst and areas where the orthopyroxene structure was intact (Figure 5). Focusing of the electron beam on amorphous zones for a few minutes induced crystallization of unidentified nanometer-sized domains displaying 1.9 \AA fringes (data not shown). XEDS analyses were

Table 1
ICP-AES chemical analyses of liquid culture media

Sample name ^a	[Si] ^c (ppb)	[Fe] ^d (ppb)	[Mg] ^e (ppb)	Fe/Si ^b (molar ratios)	Mg/Si ^b (molar ratios)
TSB/10					
r. 1	368 (7)	79 (2)	1018 (20)	—	—
TSB/10+TTB					
r. 1	381 (7)	76 (2)	958 (20)	—	—
r. 2	413 (8)	87 (2)	1305 (26)	—	—
TSB/10+Px					
r. 1	10766 (108)	959 (19)	9057 (90)	0.042	0.895
r. 2	10030 (100)	984 (20)	8640 (85)	0.047	0.913
TSB/10+Px+TTB					
r. 1	8914 (90)	232 (4)	7148 (75)	0.009	0.828
r. 2	9017 (92)	170 (3)	7702 (78)	0.005	0.893
Px	—	—	—	0.12	0.83

^aAbbreviations: TSB/10: culture medium; TTB: *R. tataouinensis* strain; Px: Zimbabwe orthopyroxene whose Fe/Si and Mg/Si ratios determined by electron microprobe are given in the last line of the table; r. #: replicate label.

^bMolar ratios of Fe/Si and Mg/Si released by the dissolution of pyroxene. These ratios were obtained by subtracting the average composition of TSB/10 to the measured compositions in the pyroxene dissolution experiments.

^{c,d,e}Number in parentheses correspond to uncertainties derived from the relative deviations of 5 multiple analyses on each individual sample.

performed on both well-crystallized and amorphous areas. No significant chemical difference could be detected between the amorphous and the well-crystallized zones. However, due to thickness variations, low count ratios, and the predominant mag-

nesium signal in the orthopyroxene, small variations could not be detected.

DISCUSSION

Mechanism of Colonization

As previously mentioned by Heulin et al. (2003), the rods of *R. tataouinensis* are able to glide, i.e., they are nonflagellated and able to move on a surface in the direction of their long axis. *Ramlibacter* is the third β -Proteobacteria genus, along with *Simonsiella* and *Vitreoscilla* for which gliding has been described (Reisenbach 1999). Such motility has been thoroughly studied in *Myxobacteria* (e.g., Spormann 1999) and *Flavobacterium johnsoniae* (e.g., Agarwal, Hunnicutt, and McBride et al. 1997). Isolated cells of *Myxobacteria* can move (adventurous motility) but they often aggregate to form cooperatively spreading swarms (social motility). The subsequent swarms coalesce with other streams or turn on themselves, forming aggregation centers, which finally evolve into fruiting bodies within which cells differentiate into spores. The colonization of agar surfaces by *R. tataouinensis* displayed very similar patterns; rods differentiate into cysts leading to the formation of microcolonies. Formation of microcolonies by *R. tataouinensis* can take place in the absence of crystals. However, this study shows that every crystal near a colony is colonized suggesting a particular interaction between crystals and colonizing bacteria. For better scattering of both, the bacteria and the crystals were mixed in a water solution before spreading on agar. The live observation (see Figure 3)

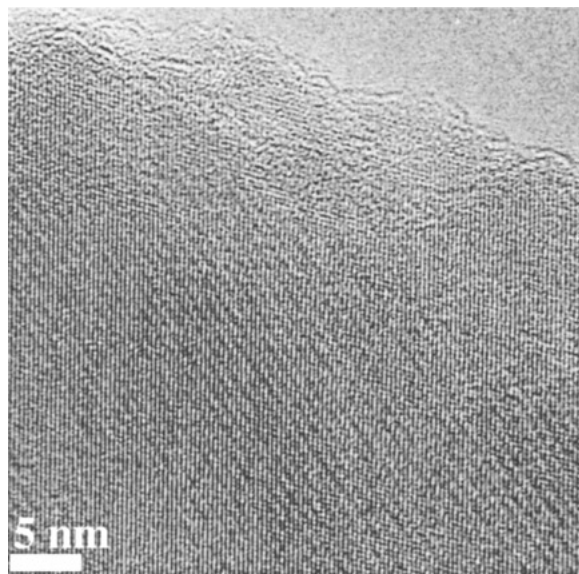


Figure 4. High resolution transmission electron microscopy micrograph of the surface of a control pyroxene crystal (reacted with uninoculated TSB/10). The lattice fringes of the orthopyroxene persist to a fraction of a nanometer below the surface of the grain (top).

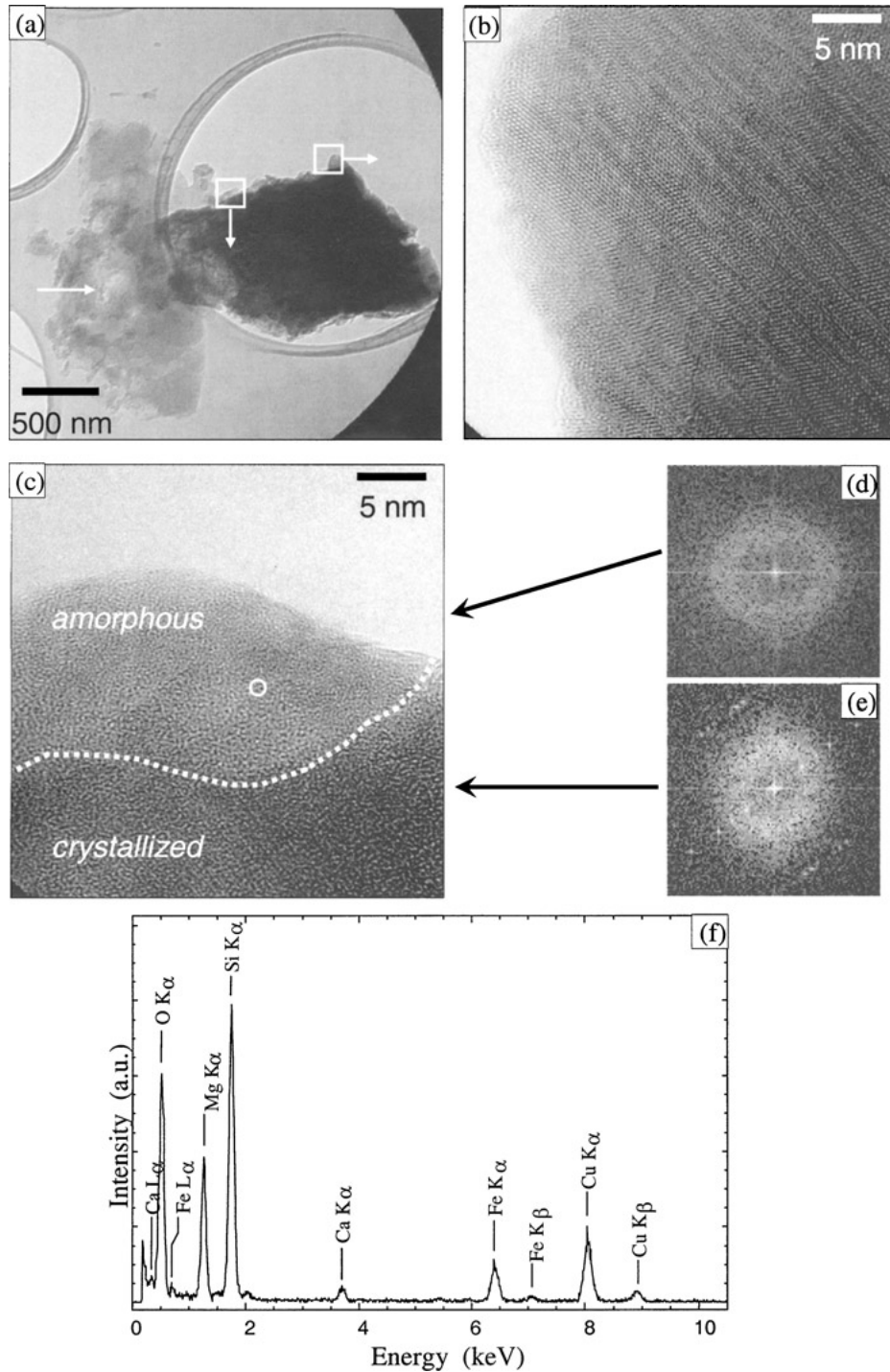


Figure 5. Transmission electron micrographs of the interface between *R. tataouinensis* TTB310 and a pyroxene crystal obtained from direct deposition of a drop of a liquid culture on a carbon film-coated copper grid. (a) Adhesion of a *R. tataouinensis* cell (arrow) to a pyroxene crystal. (b) High resolution image of the surface of the pyroxene away from the cell (enclosed in the right rectangle in 5a). The lattice fringes persist to a fraction of a nanometer below the surface (left hand side), and no significant amorphous layer is observed. (c) High resolution image of the surface of the pyroxene in contact with a bacterial cell (enclosed in the left rectangle in 5a). Observation of the lattice fringes shows a crystallized inner part (crystallized) and a 10-nm thick amorphous layer (amorphous). (d) Fourier Transform of the area located above the dashed line in c) showing its amorphous state. (e) Fourier Transform of the area located below the dashed line in c). The SW-NE bright spots indicate that this area is crystallized. (f) XEDS analysis performed with a 1-nm wide probe on the amorphous layer (see circle in c). No significant difference was detected between analysis of the crystallized part and the amorphous layer.

shows that the formation of microcolonies around the crystals was not the result of an early attachment in the solution followed by cell divisions (rods do not divide), but involved gliding of the rods on agar and a long-range attraction of *R. tataouinensis* rods by orthopyroxene and quartz crystals. Three hypotheses can be proposed to explain this:

1. The attraction is a chemotactic response of the rods. Except for a few trace elements for which a role of chemoattractants seems unlikely, silicon is the only element present in both orthopyroxene and quartz. However, the silicon concentration in TSA/10 far exceeds that which is potentially released by the dissolution of micrometer-sized quartz and orthopyroxene grains. This first hypothesis is thus unlikely.
2. The attraction of the swarms to the grains may result from cell-to-cell communication (for example Budrene and Berg 1991). Isolated rods may precede the swarms and emit attractant signals when adhering to a crystal. However, in several cases we did not observe any isolated rods near attracting grains; this hypothesis is thus unlikely.
3. The preferred hypothesis is that *R. tataouinensis* rods are able to sense the mechanical deformation of the agar surface induced by weight of the crystals. This phenomenon has already been described only for *Myxobacteria* and has been named "elastictaxis" (e.g., Stanier 1942; Dworkin 1983).

However, only a few mechanisms have so far been proposed for elastictaxis (Fontes and Kaiser 1999). Compression could align the polysaccharides bundles in the agar, and cells could sense this orientation and align their long axes with the polysaccharides chains (Wolgemuth et al. 2002). This is not actually incompatible with the model by Jelsbak and Sogaard-Andersen (2002), who proposed that a cell surface-associated C-signal is involved in the formation of swarms in *M. xanthus*. The cooperative movements of the cells in a swarm are thus a self-organizing process and result from changes in motility behavior by end-to-end contacts between the cells. These C-signal-induced cooperative movements of the cells could be coupled with a global elastictactic attraction of the swarm. A mechanical stimulus is likely more efficient than a chemical one, because a highly diffusible signal might disperse before *M. xanthus* cells could orient in the gradient due to the slow gliding velocities (Jelsbak and Sogaard-Andersen 2002). The role of elastictaxis in nature has also been questioned and has been interpreted as a consequence of the feeding habits of myxobacteria (Fontes and Kaiser 1999). Myxobacteria may in this way sense the presence of colonies of prey bacteria lying on elastic surfaces such as EPS polymers. The determination of the feeding habits of *R. tataouinensis* could reveal valuable information for testing the applicability of this hypothesis.

The observed stability of the crystal-based aggregates may be linked either with an irreversible adhesion of the rods to

the grains or with their inability to escape the depression lying around the crystals. As for the attraction, this phenomenon is independent of the mineralogical nature of the grains. Jelsbak and Sogaard-Andersen (2002) proposed that the sporulation of *M. xanthus* is induced by the high level of C-signaling in the aggregates. This mechanism known as quorum sensing could be involved in the differentiation of *R. tataouinensis* rods to cysts, and crystals would simply play the role of gathering a high density of rods. Molecular studies have to be conducted to investigate this hypothesis and the annotation of the *R. tataouinensis* genome, which is in progress, should provide valuable information regarding this issue. Regardless of the mechanism, the differentiation of rods into cysts induces a modification of the surface chemistry of the cells with, for example, the secretion of a thick capsular material (Heulin et al. 2003), which potentially affects the mineral surface reactivity. The modifications of the pyroxene chemistry and structure were thus investigated in more detail.

Amorphous Layers on Pyroxene

The incongruent dissolution of iron-bearing pyroxenes under oxic conditions has been observed in previous studies (e.g., Schott and Berner 1983). Many geomicrobiological studies have stressed the enhancing effect of microorganisms on the dissolution of silicates (e.g., Welch and Ullman 1999; Liermann et al. 2000). However, some studies have observed an inhibition of the dissolution due to the bacterial metabolism (Santelli et al. 2001) or to the production of alginate exopolysaccharides (Ullman et al. 1996). One hypothesis is that *R. tataouinensis* consumes complexing organic ligands contained in the culture medium which results in the lowering of the dissolution of the pyroxene crystals. It also has to be assumed that the ligands produced by *R. tataouinensis* are less complexing than those it consumes. This hypothesis is difficult to test in a culture medium as complex as TSA/10. The future design and use of a minimum medium with well-characterized organic molecules would be of great interest. Iron (II) oxidation or pH modifications promoted by the bacteria could also reduce the releasing of elements to the solution. No metabolic utilization of iron as an electron donor was evidenced in *R. tataouinensis* (Heulin et al. 2003). *R. tataouinensis* could however indirectly catalyze the oxidation of iron as suggested for *E. coli* and *B. subtilis* by Chatelier et al. (2001) but such a mechanism is still unclear. No pH change was detected at the macroscopic scale; however such changes at the nanometer scale in contact with the cells (local pH) cannot be excluded. Alternatively, Ullman et al. (1996) proposed that the adsorption of EPS to the silicate surfaces could limit the exchange between the mineral surface and the surrounding fluid, thus reducing the rate of dissolution. This idea is consistent with the observations of this study showing concomitant reduction in dissolution rates and presence of a well-developed amorphous layer observed by TEM at the surface of the pyroxene grains.

In these experiments, one might invoke three mechanisms for forming an amorphous layer at the surface of the pyroxene grains:

1. The focusing of an intense electron beam could have disrupted the atomic structure of the thin edges of pyroxene (see for example Gloter et al. 2000). However this reaction has not been observed in this study. Instead, the opposite phenomenon was observed since focusing the electron beam induced the crystallization of the amorphous areas.
2. Peck, Farnan and Stebbins (1988) have shown that dry grinding of diopside crystals ($\text{CaMgSi}_2\text{O}_6$) results in the formation of a thin amorphous layer. Despite the narrow XRD peaks obtained on the pyroxene powders (data not shown), a thin amorphous layer resulting from grinding might be present. However, such thin amorphous layers are highly reactive (Peck et al. 1988) and their observation on crystals incubated for one month in shaken liquid cultures is unexpected. Moreover, no such amorphous layers were evidenced on grains observed just after the grinding process. A poorly crystallized layer of less than 2 nm was observed on control grains reacted in sterile TSB/10.
3. Banfield et al. (1995) observed a 3 to 7 nm-thick amorphous leached layer resulting from the chemical alteration of MnSiO_3 crystals. According to the XEDS analyses, this layer was silica rich and was interpreted to have formed as a result of the exchange of Mn for protons and a subsequent collapse of the protonated silicate chains. The only other reported occurrence of an amorphous weathering product of a pyroxene was observed by Nahon and Colin (1982) on natural samples. The product was amorphous and displayed a chemical composition very similar to the parent mineral. Our XEDS analyses are not inconsistent with the existence of small chemical differences between the amorphous layers and the pyroxene cores.

The amorphous areas observed in our study are thus likely the result of chemical alteration of the orthopyroxene grains. The main observation is that the amorphous layers are thicker when in contact with bacteria. A possible explanation is that bacteria enhanced the dissolution of the orthopyroxene grains, but that the exopolymers produced by *R. tataouinensis* limited the diffusion of the chemical elements to the solution and locally induced the precipitation of an amorphous layer. Alternatively, the amorphous layer might be produced in the liquid medium at the same rate on all the crystals, but the bacteria could hinder its dissolution by reducing the fluid/solid effective contact area. Both explanations would be consistent with the observed solution chemistry. Moreover, the dissolution of orthopyroxenes would release various elements (Fe, Mg, Mn), which can be essential for enzyme function and are potentially toxic. Schott and Berner (1983) have studied the kinetics of dissolution of bronzites and mentioned the formation of a hydrous ferric oxide layer under oxic conditions at near neutral pH. Such a layer was not present in our study, and despite thorough examination of

the samples, no iron oxides were detected. ICP-AES analyses have shown that iron was efficiently trapped by *R. tataouinensis*. This could be related to the presence of organic chelating molecules, such as the exopolymers surrounding *R. tataouinensis* cysts. High-resolution chemical imaging of iron at the interface between crystals and cells would be an important advance for understanding this aspect of pyroxene alteration.

CONCLUSIONS

R. tataouinensis colonizes preferentially crystals scattered at the surface of solid culture media. The colonization process involves the gliding of the rod form and a long range attraction which is presumably elasticotaxis, the sensing by the bacteria of the deformation of agar under the weight of the crystals. The rods then differentiate into cysts after contact with the crystals. This process may be due to a bacterial density-dependent signal, with the crystals only acting as gathering points. The molecular study to investigate this assumption has to be conducted. Release of elements by the dissolution of pyroxene is hindered by the presence of *R. tataouinensis*. Although many hypotheses can be stated, the observation of an amorphous layer on the pyroxene grains only in contact with the cells is consistent with the Ullman et al. (1996) study, suggesting that the cells have the effect of limiting the exchange between the mineral surface and the surrounding fluid, thus reducing the rate of dissolution.

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