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Bacterial colonization of artificial substrate in the vicinity of deep-sea hydrothermal vents

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Abstract

Artificial substrata of different material composition were deployed at deep-sea hydrothermal areas on the Mid-Atlantic Ridge for exposure times ranging from 1 to 12 days. After 4 days of exposure, a very thick but loosely-bound biofilm formed on all surfaces. Two bacterial morphotypes dominated the attached microbial community: rod-shaped bacteria sometimes several cell layers thick and large filamentous forms attached to the substratum at one end of the filament. Quantitative extraction of biofilm lipids associated with the substratum surface indicated the accumulation of a large amount of bacterial biomass after 4 days of exposure for all substrata. Microbial biomass accumulated at different rates on the different substrata. The greatest biomass was associated with 316L stainless steel and titanium substrata. Polar lipid fatty acid (PLFA) analysis of lipid extracts contained signatures of sulfate reducing bacteria and fatty acids (FA) previously reported in filamentous sulfur-oxidizing bacteria. The results demonstrate rapid in situ colonization of artificial substrata by hydrothermal vent microbial populations irrespective of the nature of the substratum.

Keywords: Hydrothermal vent; Biofilm; Artificial substrate

1. Introduction

Deep-sea hydrothermal vent ecosystems depend on microbial systems for the conversion of reduced inorganic forms of energy to organic forms of energy and carbon. Attached microbial populations have been described in these environments and likely represent an important contribution to the primary production [1,2]. Little is known on the diversity of these attached microbial populations, microbial colonization rates and biomass associated with surfaces in hydrothermal vent fields [3].

Analysis of cellular lipids provides a satisfactory way to gain insight into microbial community structure and biomass [4]. Microbial biomarkers are chemical components of microorganisms which can be extracted directly from the environment, provide qualitative information on the types of microorganisms present as well as quantitative information on in situ microbial biomass [5]. Membrane lipids and

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their associated fatty acids are particularly useful biomarkers as they are essential components of every living cell and have great diversity coupled with high biological specificity [6]. Phospholipid ester-linked fatty acids (PLFA) have proved to be of great value in describing bacterial community structure in sediments, the establishment of a biochemical basis to bacterial phylogeny and taxonomy, and in the detection of specific physiological groups of bacteria associated with microbiologically influenced corrosion studies [7–10]. The use of PLFA from the polar lipid fraction eliminates interference from many contaminants as well as endogenous storage lipids present in the neutral or the glycolipid fractions of the extractable lipids. Phospholipids are not found in storage lipids and have relatively rapid turnover in sediments [11].

A study was carried out to investigate the extent to which the nature of the substratum influenced biofilm development, population structure and biomass in areas under the influence of venting hydrothermal fluids at a site (Snake Pit) along the Mid-Atlantic Ridge during the French 'Microsmoke' cruise. A spectrum of artificial substrata was used in this study to determine the importance of substratum properties on microbial colonization rates, population structure and succession in the vent environment.

2. Materials and methods

2.1. Substratum deployment and recovery

Artificial substrata were deployed at three locations on the ocean bottom along the Mid-Atlantic Ridge at the Snake Pit site (23°22N, 45°57W) using the manned submersible 'Nautile' during the French oceanographic cruise in November 1995. Two locations (sites 1 and 2) were under the influence of venting hydrothermal fluid. The temperatures at sites 1 and 2 fluctuated between 5–20°C. A third location (site A) was outside the influence of the hydrothermal fluids where the temperature maintained a constant 2°C. Water samples were collected from the 3 locations using a Niskin bottle operated by the mechanical arm of the submersible.

The following materials were used as artificial substrata: 316L stainless steel (316L SS), titanium (Ti), aluminum 5052 (Al), copper 90/10 (Cu), copper-nickel alloy (Cu-Ni), Teflon (PTFE), polyamide (PA), polyacrylate (PAc) and polycarbonate (PC). SS and Ti were selected on the basis of their resistance to marine corrosion; Al was selected for its extensive oxide film in seawater; Cu and Cu-Ni were selected for their ability to inhibit microbial colonization. The non-metallic surfaces were selected to offer a range of surface energies in natural seawater (PTFE, 18 $ml \cdot m^{-2}$; PA, 38 $ml \cdot m^{-2}$; PC, 35.5 $ml \cdot m^{-2}$; and PAc, 40 $ml \cdot m^{-2}$) different from those provided by the metallic substrata.

One-cm diameter coupons of each material were glued on one surface to a sample holder, allowing the other surface exposure to the surrounding environment (Fig. 1). A set of sample holders (one for each site and sampling time), each containing an array of coupons of different composition, was transported from the sea surface to the sea floor in the externally-located, seawater-filled box mounted at the front of the Nautile. A mechanical arm transferred the sample holders from the box to their respective location on the sea floor. After 1, 4, 8 and 12 days exposure, a sample holder from each location was retrieved by the mechanical arm of the submersible, and transported to the surface in the seawater-filled, externally-located box on the submersible.

As soon as the submersible was brought on board the support ship, Nadir, the sample holders (l/location) were immediately retrieved from the box and 3 coupons of each type of material removed from each holder and treated as follows: one coupon was lyophilized and stored for lipid analysis; the two remaining coupons were preserved in artificial seawater containing 2.5% glutaraldehyde for subsequent microscopic observations.

2.2. Scanning electron microscopy

Coupons were sputter-coated with a thin film of gold to minimize charging and examined with a Philips XL 30 Lab 6 scanning electron microscope.

2.3. Lipid analysis

Lipids were extracted using a modified Bligh/Dyer

method [11,12]. Samples were placed for 1 h in an ultrasonic bath with adequate volumes of methanol, dichloromethane and water (2:1:0.8, v/v). Identical volumes of water and dichloromethane were then added to the solution and the two resulting phases allowed to separate in a funnel for 18 h. The total lipids were separated into three general lipid classes by silicic column chromatography using a series of mobile phases of increasing polarity (dichloromethane, acetone and methanol). Fatty acid methyl esters were prepared from the esterified lipids in the polar (methanol) lipid fraction by mild alkaline methanolic trans-esterification and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

An aliquot of the polar lipid fraction was analyzed for its ether glycerol content as a biomarker of archaebacteria. A fraction of the polar phase was subjected to a strong acid hydrolysis and the resulting core ether lipids digested with 55% HI solution for 18 h at 100°C. The resulting alkyliodides were extracted with hexane and successively washed with $Na_2S_2O_3$ and Na_2CO_3 solutions [13,14]. After extraction with hexane, the iodide derivatives were analyzed using GC equipped with an electron capture detector using 1,2 di-*O*-hexadecyl-rac-glycerol as internal standard. Authentic glycerol diethers and diglycerol tetraethers were purchased from Sigma or isolated from cells of *Sulfolobus* sp.

2.4. Gas chromatography and gas chromatography-mass spectrometry analyses

GC analyses were performed on a Carlo Erba (Rodano, Italy) HRGG 5360 gas chromatograph equipped with a fused silica column coated with a non-polar phase (60 m × 0.2 mm i.d.; film thickness 0.25 μm). Hydrogen was used as carrier gas (33 cm s^{-1}).

GC-MS analyses were performed on a Carlo Erba (Rodano, Italy) model 5160 HRGC chromatograph coupled to a quadrupole Nermag (Delsi) r10-10H mass spectrometer. The fatty acid separation was achieved on a CP Sil5CB capillary column (60 m × 0.20 mm i.d.; film thickness 0.25 μm; J&W Folson, California) with helium as carrier gas (1.5 bar). Standard fatty acids were purchased from Sigma along with fatty acids of pure culture of *Vibrio natriegens*, *Desulfovibrio desulfuricans* and members of the *Alteromonas* group isolated from an hydrothermal environment [15,16].

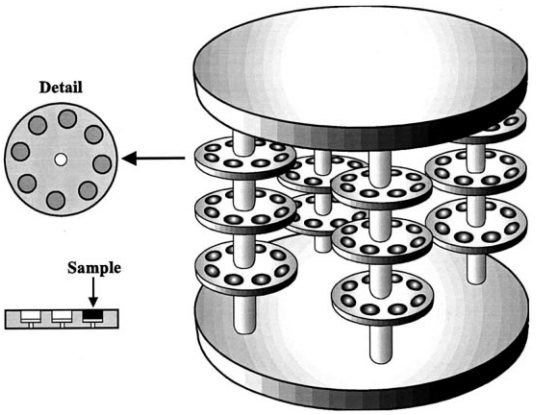

Fig. 1. Sample holder.

2.5. Fatty acid nomenclature

A shorthand nomenclature is used which is in the form of numbers separated by a colon. The number before the colon indicates the carbon chain length and the figure after the colon corresponds to the number of double bonds. The position of the double bond is defined by the symbol 'ω' followed by the number of carbons from the methyl end. The prefixes 'i' and 'a' refer to iso and anteiso, respectively. The geometry of the double bonds is indicated by *cis* and *trans*.

3. Results

Coupons of different material composition, deployed at 2 vent-influenced sites (sites 1 and 2) accumulated a thick, loosely-bound biofilm on their surface, whereas coupons deployed at control site A, not under vent influence, accumulated a sparser biofilm. The chemistry of water samples from the 3 locations is presented in Table 1. With the exception of the silica and ammonia concentrations, the chemistry of the fluids collected at sites 1 and 2 was similar to that of control site A.

Fig. 2a–j shows the most commonly observed morphological types of microorganisms on the sur-

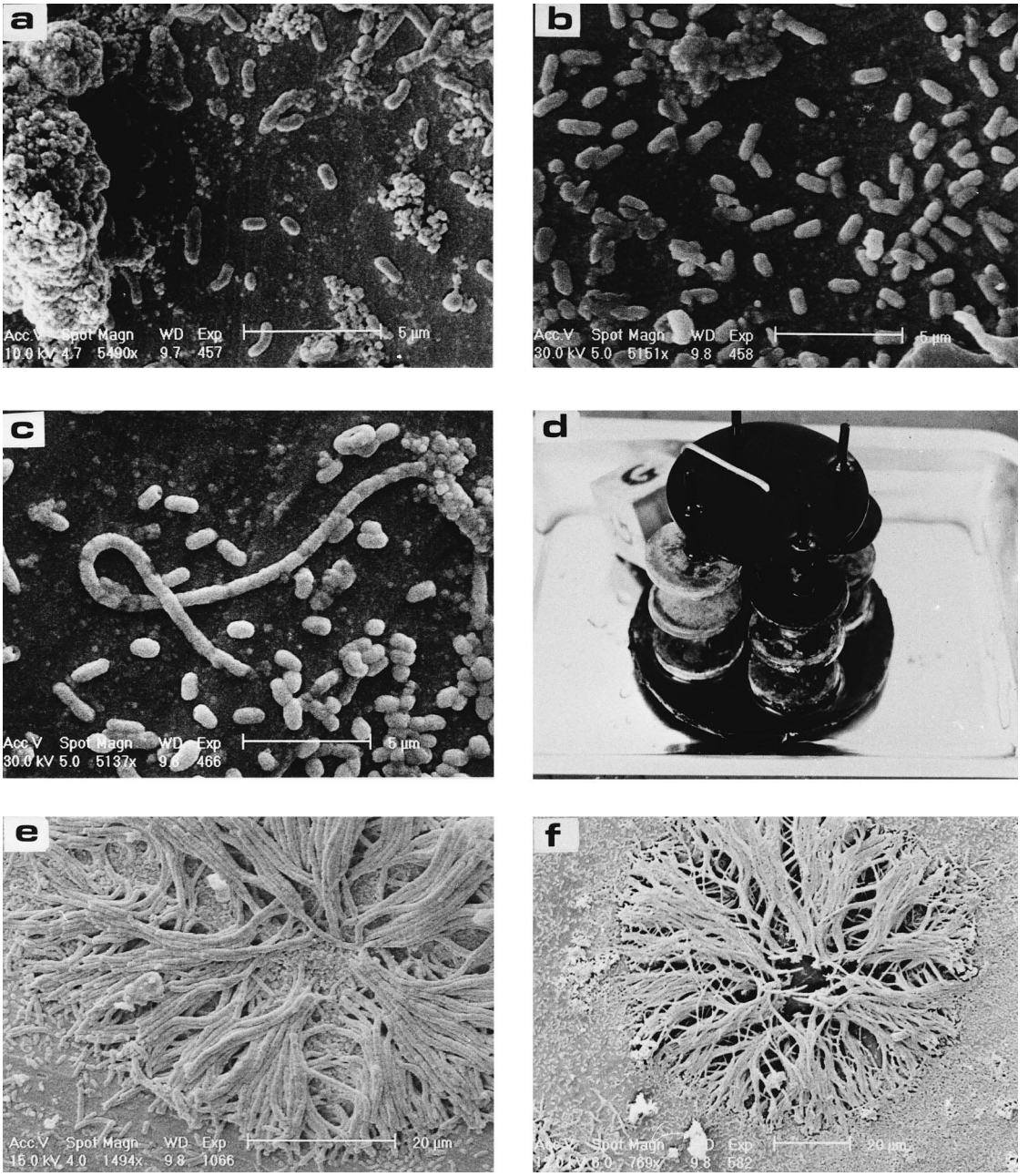

Fig. 2. a–j: Selected SEM photographs from colonized surfaces. a: 316L SS (control, 9 days at site A). b,c: One day colonization (Ti, 316L SS). d: Sample holder after 4 days in the vicinity of the vents; e,f,g: Two main bacterial morphotypes commonly found on all surfaces (4 days). Filamentous bacteria forming rosettes on the surfaces and rod-shaped bacteria (Ti, 316L SS, PTFE); h: Filaments perpendicular to the surfaces (PTFE); i,j: Multilayers of bacteria on 316L SS and titanium (8 days).

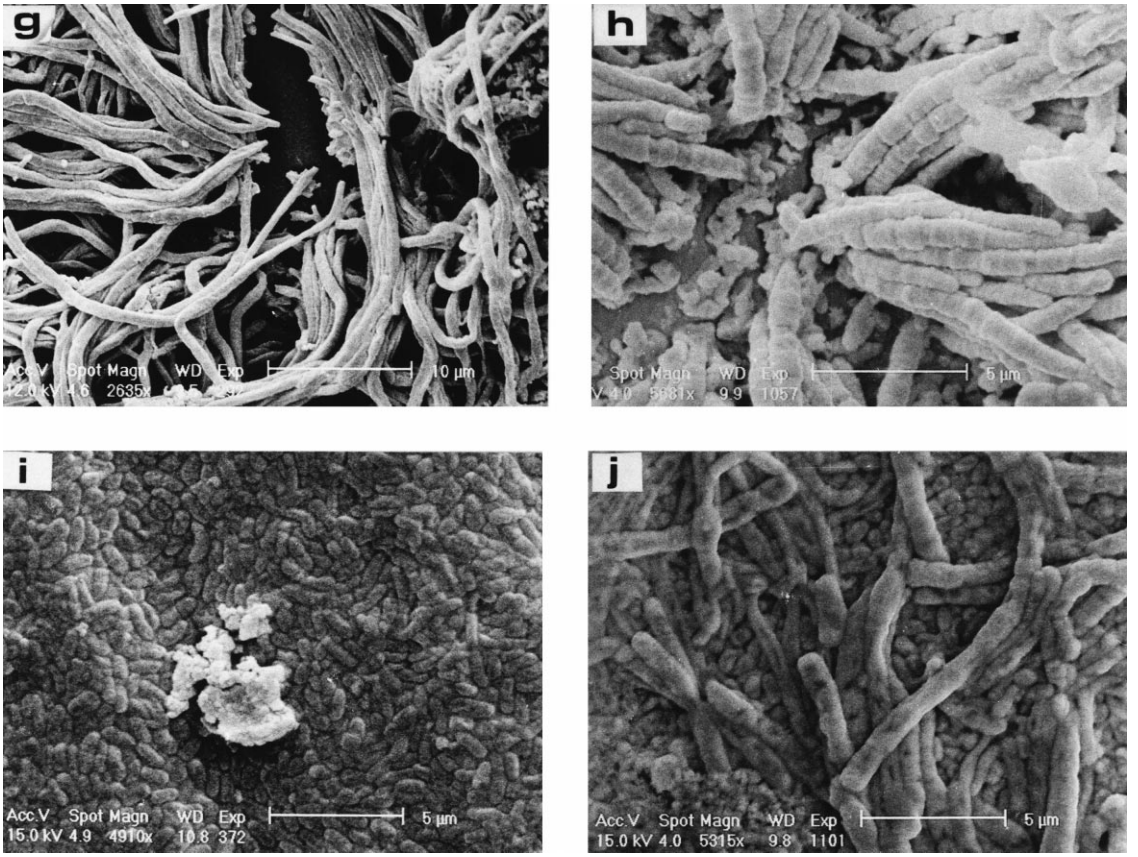

Fig. 2. (*continued*)

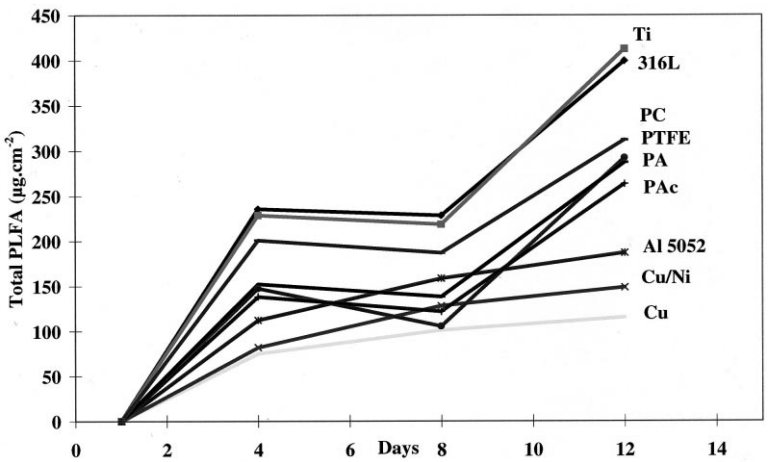

Fig. 3. Biomass expressed as total PLFA µg cm^{-2} vs. time.

face of coupons from the three sites. Contamination during the different steps of the deployment/recovery procedures was estimated by examination of coupons kept in the shuttle box on the submersible for a typical one-day dive. Both SEM and lipid analyses indicated that contamination of surfaces during coupon deployment and retrieval was negligible.

Most coupons retrieved from sites 1 and 2 after one day of exposure to the vent environment were colonized by rod-shaped bacterial colonies heterogeneously distributed over their surface (Fig. 2b,c). The Cu, Cu-Ni and Al coupons were sparsely colonized after one-day exposure.

After four days, all coupons and adjacent areas of the sample holders at sites 1 and 2 were covered with a very thick white biofilm filamentous microorganisms (Fig. 2d). Rod-shaped and rosette-forming filamentous microorganisms were the two dominant morphotypes on the surfaces of all coupons, including the Cu and Cu-Ni coupons exposed at these sites (Fig. 2e,f). The filaments were up to 50 μm length and appeared to be anchored to the surface at a single pole, extending perpendicularly to the substratum (Fig. 2g,h).

After 8 days exposure to the vent environment the surface of all coupons deployed at sites 1 and 2 were colonized by a filamentous biofilm. Biofilm morphology varied with substratum type. Cu and Cu-Ni coupon surfaces exhibited the most heterogeneous distribution of microorganisms. The biofilm on Al coupons was contained within the aluminium oxide film on the surface. Windows of filaments were frequently observed on the surface of the SS coupons. Ti coupon surfaces accumulated a multi-layer of similarly-sized, tightly-packed, rod-shaped bacteria (Fig. 2i,j). Biofilms dominated by rod-shaped bacteria were also observed on the surfaces of the non-metallic coupons.

After 12 days exposure to the vent environment, the surface of all coupons deployed at sites 1 and 2 had accumulated a multi-layered biofilm containing both rod-shaped bacteria and filamentous forms. Metallic and non-metallic surfaces were similarly fouled by these microorganisms based on SEM observations.

Surfaces of coupons exposed to ambient seawater for 9 days at control site A contained fewer rod-shaped bacteria than those exposed to hydrothermal fluids (Fig. 2a). No filamentous forms were observed. No bacteria were detected on the copper and aluminium surfaces.

3.1. Lipid analysis

Twenty-six PLFA were recovered from the surface of the different coupons exposed to the hydrothermal vent environment. The total PLFA-based biofilm biomass associated with the surface of the different coupons was determined between 1 and 12 days exposure to the hydrothermal vent environment (Fig. 3). The most rapid increase in biomass occurred between one and four days exposure on all coupons regardless of composition. Biomass remained relatively unchanged between days 4 and 8 on coupons composed of Ti, 316L SS, and the 4 synthetic polymers PC, PTFE, PA and PAc, while the biomass on Al, Cu-Ni and Cu increased slowly between days 4–12. Between days 8–12, biomass on the Ti, SS and the 4 synthetic polymers increased at rates comparable to those observed between days 1–4. After 12 days exposure, Ti and 316L SS coupons had accumulated the greatest biomass, Cu, Cu-Ni and Al accumulated the least biomass, and 4 synthetic polymers accumulated intermediate amounts of biomass.

In contrast to the coupons exposed to the venting fluids, control coupons exposed to ambient seawater at Side A accumulated a low PLFA-based

Table 1
Seawater composition for exposure sites (sites 1 and 2) and site A

Sampling sites	pH	T (°C)	Ca[a]	Mg[a]	SO_4^{-}[a]	S[−a]	NO_3^{-}[b]	NH_4^{+}[b]	PO_4^{3-}[b]	Si[b]
Site 1	7.8	10–15	9.2	47.9	26.2	<1	11.2	5	0.26	655
Site 1(2)	7.9	10–15	9.8	48.1	26.2	<1	11.1	5.1	0.26	648
Site 2	7.8	10–15	9.4	48.7	26.6	<1	13.4	8.8	0.47	460
Site A	8.1	2	9.8	52.8	28.2	ND	15.1	0.5	1.28	152

[a]mmol l^{-1}; [b]μmol l^{-1}.

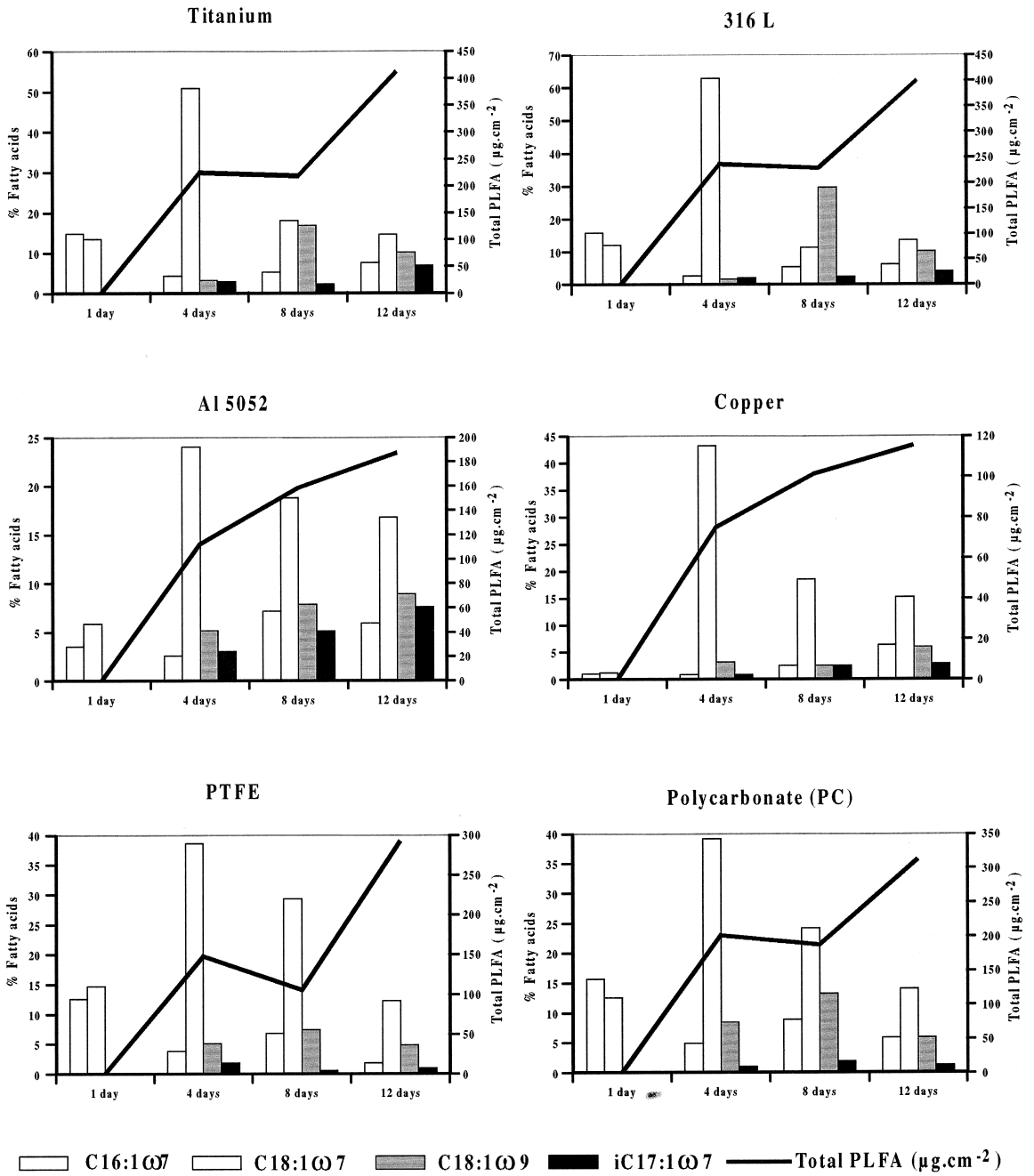

Fig. 4. C16:1, C18:1 isomers and iC17:1ω7c vs. exposure time.

microbial biomass after 9 days of exposure (data not shown).

For all coupons the common microbial fatty acids C16:1ω7, C18:1ω7 and C18:1ω9 were the major monounsaturates, i and a C15:0 and C17:0 were the major branched chain acids, and C14:0, C16:0 and C18:0 constituted the main saturates. With the exception of C18:2ω 6,9 no polyunsaturates were recovered from the biofilms. Cycle C19:0 was in low abundance (up to 1.98% of the total acids) on some surfaces after 4 days. iC17:1ω7c fatty acid was present in most biofilms after 4 days and its proportion increased with exposure time (Fig. 4). 10Me C16:0 fatty acid was present in low proportions after 12 days on non-metallic surfaces, aluminium and copper alloys as well. The diversity of PLFA increased with exposure time, with C16:1 and C18:1 ω9, ω7 and ω5 isomers appearing only after 12 days. The absence of ether glycerol in lipid extracts of the various coupon surfaces indicated that archaebacteria were either absent or below the level of detection.

Coupons exposed to site A for 9 days yielded PLFA profiles that were similar to those recovered from surfaces exposed for 1 day at sites 1 and 2: equivalent proportions of C16:1ω7 and C18:1ω7. No C18:1ω9 fatty acid was present in the biofilms at site A.

4. Discussion

Bacterial mats have been observed on the surface at many hydrothermal sites [17,18]. Whitish bacterial mats, similar in morphology to those described here, reportedly cover substrata in the vicinity of vents at the Guaymas basin [19]. Natural and artificial materials exposed to hydrothermal features at the Galapagos Rift Ocean Spreading Zone at a depth of 2550 m for periods ranging from several days to one year accumulated massive biofilms, 5 μm to 10 μm in thickness, containing populations of coccoid microorganisms interspersed with filamentous microorganisms [1]. However, no biomass estimates were obtained from these substrata. That microbial morphotypes similar to those observed at the Galapagos Rift Ocean Spreading Zone occurred in biofilms accumulating on the various artificial substrata deployed at the Snake Pit site along the Mid-Atlantic Ridge supports previous evidence that filamentous microorganisms are widely dispersed in the world's oceans. The dominance of filamentous forms on substrata under hydrothermal influence but not in surrounding areas supports the idea that the venting hydrothermal fluids provide the limiting nutrient or energy source for these organisms.

It has been difficult to assess biomass accumulation rates in the vicinity of deep-sea hydrothermal vents, primarily because of inaccessibility. The 'Microsmoke' cruise permitted frequent excursions to the vent environment, facilitating a study on in situ microbial biomass accumulation rates of different artificial substrata. Coupons of varying composition were evaluated to take into account the possible influence of substratum features on the types of microbial biofilms that colonize the different natural substrata at these sites and on rates of colonization and biomass accumulation. Both SEM observations and PLFA profiles indicated that the coupons deployed at the two sites under the influence of the hydrothermal vents were colonized by similar biofilm-forming microbial populations. After only 4 days exposure, all surfaces were covered with a biofilm composed of microbial morphotypes similar to those found in the white mats observed in many hydrothermal deep-sea vents. That similar populations of microorganisms accumulated on all the different artificial substrata deployed does not contradict the possibility that the natural surfaces may be colonized in a similar manner.

Bacterial biomass associated with coupon surfaces at the control site (site A) was similar to that reported for metallic and non-metallic surfaces exposed to natural flowing seawater [20]. That bacterial biomass was three orders of magnitude higher on surfaces exposed to the vent environment than on those exposed to the surrounding ambient deep-sea environment suggests that the conditions of the vents were more conducive for colonization and growth of these microbes. The only measured variables that appeared different at the vent and control sites were temperature, ammonia and silica concentrations. Interestingly, sulfide concentrations were not significantly different in spite of the fact that sulfur oxidizers and reducers were detected at significant levels at the vent sites but not at the control site. The sulfide concentrations reported here may not

reflect in situ concentrations as it is unstable and some may have been lost from the gas phase during sample manipulation.

Some interesting trends were observed in the colonization behavior of the microorganisms over the 12-day exposure to the vent environment. Surfaces of 316L SS and Ti coupons accumulated the highest biomass, while non-metallic surfaces, Al, Cu and Cu-Ni surfaces accumulated the least biomass over the duration of the experiment. All non-metallic surfaces were similarly colonized at the end of the experiment (Fig. 3). The delay observed in the colonization of pure Cu and Cu alloy may be related to the formation of toxic copper (Cu^{2+}) species at the surface of the coupon. The protective oxide film formed on the Al coupons in seawater may have obscured the attached microorganisms during SEM examination or, alternatively, impeded attachment of microorganisms on these surfaces.

Two distinct morphological types of microorganisms contributed to the biofilm observed on both metallic and non-metallic surfaces. Filamentous microorganisms, sometimes in rosettes, were heterogeneously distributed over the surfaces, often overlaying a layer of rod-shaped bacteria. The latter formed mono- or multi-layers by the end of the experiment. However, morphological data based on SEM offers limited insight on microbial diversity of the attached microbial populations.

PLFA profiles offer insight into biofilm bacterial community structure. The different fatty acid profiles obtained at different sampling times suggest a succession in bacterial community structure between 1–12 days in the vent environment. After one day, monounsaturates C16:1ω7 and C18:1ω7 were in similar proportions for substrata that had acquired a detectable attached microbial population. Interestingly, *trans* isomer was only detected on non-metallic surfaces. The ratio of C16:0/C16:1ω7c/C18:1ω7c was different in biofilms from the Cu, Cu-Ni and Al surfaces than that of other coupon materials after the one-day exposure period, suggesting that the microbial populations on these three material surfaces were similar and distinct from those on the other material surfaces.

The appearance of large filaments after 4 days coincided with the predominance of C18:1ω7 (up to 62% of the total surface-associated PLFA). The ω7 PLFAs are common to many Gram-negative eubacteria and have been shown to be major fatty acids of the membrane of mat-forming bacteria at hydrothermal deep-sea vents [21]. Membrane lipids of sulfur-oxidizing bacteria have been described by several authors and are usually characterized by large amounts of monounsaturated fatty acids with either C16:1ω7 or C18:1ω7 predominating [22–25]. *Thiomicrospira crunega*, an obligate chemoautotrophic sulfur-oxidizing bacterium isolated from a deep-sea hydrothermal vent, exhibited a fatty acid profile dominated by C16:1ω7 and C18:1ω7c [17]. Filamentous bacteria, identified as members of the genus *Beggiatoa* by gliding motility and internal globules of elemental sulfur have been identified in massive red or white aggregations at the deep-sea hydrothermal vents of the Guaymas basin [19,26]. Other filamentous bacteria common to the vents included the genera *Leucothrix* and *Thiothrix* [27]. Only the latter forms rosettes during growth on the surfaces.

C18:1ω9 fatty acid was only present in high proportions in biofilms formed after 8 days on 316L SS, accounting for 30% of the total acids. Interestingly, high concentrations of this acid corresponded to the presence of multiple layers of rod shaped bacteria on surfaces. Some budding bacteria have oleic acid (C18:1ω9) as the second most abundant component [28]. This acid is also common to many psychrophilic microorganisms such as *Pseudomonas* sp. and some *Colwellia*/*Vibrio* sp. (D. Nichols and J. Bowman, personal communication). Moreover, one species of the genus *Desulfobacter*, *D. latus*, also contained oleic acid as the dominant PLFA [29]. The occurrence of this acid as a major component of the total FA pool in 8-day-old biofilms is unusual, however. To our knowledge, the occurrence of C18:1ω9 as a major fatty acid in sulfur-oxidizing bacteria has never been reported. Its presence in significant proportions in mature microbial biofilms on surfaces exposed to hydrothermal venting in the deep sea deserves further investigation.

At the end of the 12-day experiment the presence of C16:1 and C18:1 ω9, ω7 and ω5 *cis* and *trans* isomers indicated a more diversified bacterial population on the surfaces.

Significant contributions of SRB biomarkers were detected after 12 days, particularly on the metallic surfaces with the highest SRB biomass associated

with the Al surface (Fig. 4). Iso and anteiso C15:0, and particularly i C17:1ω7c, are specific biomarkers for sulfate-reducing bacteria. Previous studies performed on sulfate reducers isolated from the deep-sea environment have demonstrated the presence of these markers for *Desulfovibrio* species [30]. iC17:1ω7c is the predominant and characteristic PLFA of *Desulfovibrio* spp. with the exception of *Desulfovibrio gigas* [31,32]. The presence of 10 Me C16:0 in the absence of other 10 methyl-branched PLFA is a signature for the SRB genus, *Desulfobacter* [33]. These data indicate that the sulfate-reducing bacteria, the genus *Desulfobacter* in particular, appear after the *Thiothrix*-like sulfur-oxidizing organisms during biofilm succession on surfaces exposed to the hydrothermal fluids at these slow-spreading centers on the seafloor.

5. Conclusions

Microbial biomass accumulation rates varied with substratum. 316L SS and Ti were colonized faster than Al, Cu and Cu-Ni alloy. Biomass accumulation rates varied less among the non-metallic substrata. Rod-shaped bacteria colonized all the substrata while large filamentous forms became abundant only after 4 days exposure to the vent environment. The elevated levels of C18:1ω7 fatty acid that coincided with the increasing abundance of the filamentous forms suggest they are similar to those shown to oxidize sulfur at other vent sites. The occurrence of oleic acid (C18:1ω9) indicated a change in community structure after 8 days, coinciding with the presence of multiple layers of bacteria found on artificial substrata deployed in the vicinity of the vents. Sulfate reducing bacteria appeared on surfaces exposed to the vent fluids after 12 days. Filamentous forms were not observed on substrata deployed outside the vent field. On the basis of ether lipid analysis, archaebacteria do not contribute to the bacterial biomass present on all surfaces evaluated in this study.

Acknowledgments

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References

[1] Jannach, H.W. and Wirsen, C.O. (1981) Morphological survey of microbial mats near deep-sea thermal vents. Appl. Environ. Microbiol. 41, 528–538.
[2] Jannasch, H.W. (1983) Microbial processes at deep sea hydrothermal vents. In: Hydrothermal Processes at Seafloor Spreading Centers (Rona, P.A., Bostrom, K., Laubier, L., Smith, K.L. Jr., Ed.), pp. 677–701. Plenum, New York, NY.
[3] Jannasch, H.W. and Mottl, M.J. (1985) Geomicrobiology of deep-sea hydrothermal vents. Science 229, 717–725.
[4] Vestal, J.R. and White, D.C. (1989) Lipid analysis in microbial ecology. Qantitative approaches to the study of microbial communities. Bioscience 39, 535–541.
[5] Balkwill, D.L., Leach, F.R., Wilson, J.T., McNabb, J.F. and White, D.C. (1988) Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface sediments. Microb. Ecol. 16, 73–84.
[6] White, D.C. (1988) Validation of quantitative analysis for microbial biomass, community structure, and metabolic activity. Adv. Limnol. 31, 1–18.
[7] Lechevalier, M.P. (1977) Lipids in bacterial taxonomy – A taxonomist's view. Crit. Rev. Microbiol. 7, 109–210.
[8] Guckert, J.B., Anthworth, C.P., Nichols, P.D. and White, D.C. (1985) Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiol. Ecol. 31, 147–158.
[9] Dowling, N.J.E., Guezennec, J.G. and White, D.C. (1988) Methods for insight into mechanisms of microbially influenced metal corrosion. In: Biodeterioration (Hougton, D.R., Smith, R.T. and Eggins, H.O.U., Eds.), Elsevier, London.
[10] Guezennec, J.G. (1991) Influence of cathodic protection of mild steel on the growth of sulphate-reducing bacteria at 35°C in marine sediments. Biofouling 3, 339–348.
[11] White, D.C., Nickels, J.D., King, J.D. and Bobbie, R.J. (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62.
[12] Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

[13] Langworthy, T.A., Holzer, G., Zeikus, J.G. and Tornabene, T.G. (1983) Iso- and anteiso-branched glycerol diethers of the thermophilic anaerobe *Thermodesulfotobacterium commune*. Syst. Appl. Microbiol. 4, 1–17.
[14] Pauly, G.G. and Van Vleet, E.S. (1986) Acyclic archaebacterial ether lipids in swamp sediments. Geochem. Cosmochim. Acta 50, 1117–1125.
[15] Vincent, P., Pignet, P., Talmont, F., Bozzi, L., Fournet, B., Guezennec, J., Jeanthon, C. and Prieur, D. (1994) Production and characterization of an exopolysaccharide excreted by a deep-sea hydrothermal vent bacterium isolated from the polychaete *Alvinella pompejana*. Appl. Environ. Microbiol. 60, 4134–4141.
[16] White, D.C., Bobbie, R.J., Herron, J.S., King, J.D. and Morrison, S.J. (1979) Biochemical measurements of microbial mass and activity from environmental sample. In: Native Aquatic Bacteria: Enumeration, Activity and Ecology. ASTM STP 695.
[17] Jannash, H.W. (1985) The chemosynthetic support of life and the microbial diversity at deep-sea hydrothermal vents. Proc. R. Soc. London 225, 277–297.
[18] Jannasch, H.W., Nelson, D.C. and Wirsen, C.O. (1989) Massive natural occurence of unusually large bacteria (*Beggiatoa* sp) at a hydrothermal deep-sea vent site. Nature 342, 834–836.
[19] Nelson, D.C., Wirsen, C.O. and Jannasch, H.W. (1989) Characterization of large autotrophic *Beggiatoa* spp abundant at hydrothermal vents of the Guaymas basin. Appl. Environ. Microbiol. 55, 2909–2917.
[20] Fera, P. (1985) Etude expérimentale de la colonisation par les bactéries de surfaces immergées en milieu marin. Thèse de l'Université de Bretagne Occidentale, 203 pp.
[21] Guezennec, J.G. and Fiala-Médioni, A. (1996) Bacterial abundance and diversity in Barbados Trench sediments determined by phospholipid analysis. FEMS Microbiol. Ecol. 19, 83–93.
[22] Katayama-Fujimura, Y., Tsuzaki, N. and Kuraishi, H. (1982) Ubiquinone, fatty acid and DNA base composition determination as a guide to the taxonomy of the genus *Thiobacillus*. J. Gen. Microbiol. 128, 1599–1611.
[23] Larkin, J.M. (1980) Isolation of *Thiothrix* in pure culture and observations of a filamentous epiphyte on *Thiothrix*. Cur. Microbiol. 4, 155–158.
[24] Jacq, E., Prieur, D., White, D.C., Porter, T. and Geesey, G. (1989) Microscopic examination and fatty acid characterization of filamentaeous bacteria colonizing substrata around subtidal hydrothermal vents. Arch. Microbiol. 152, 64–71.
[25] Durand, P. (1992) Taxonomie des bactéries oxydant les composés soufrés réduits en milieu hydrothermal profond: cas du Sud-ouest pacifique. Thèse de l'Université de Bretagne Occidentale, 185 pp.
[26] Jorgensen, B.B. (1982) Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments. Phil. Trans. R. Soc. Lond. B 298, 543–561.
[27] Brock, T.D. (1981) The genus *Leucothrix*. In: A Handbook on Habitats, Isolation and Identification of Bacteria (Starr, M.P., Stopl, H., Trüper, H.G., Balows, A. and Schlegel, H.G., Eds.), Vol. 1, pp. 400–408. Springer Verlag, New York, NY.
[28] Kerger, B.D., Mancuso, C.A., Nichols, P.D., White, D.C., Langworthy, T., Sittig, M., Schleser, H. and Hirsch, P. (1988) The budding bacteria, Pirellula and Planctomyces, with atypical 16S rRNA and absence of peptidoglycan, show eubacterial phospholipids and uniquely high propertions of beta-hydroxy fatty acids in the lipopolysaccharide lipid A. Arch. Microbiol. 149, 255–260.
[29] Kohring, L.L., Ringelberg, D.B., Devereux, R., Stahl, D., Mittelman, M.W. and White, D.C. (1994) Comparison of phylogenetic relationship based on phospholipid fatty acid profiles and ribosomal RNA sequence similarities among dissimilatory sulfate-reducing bacteria. FEMS Microbiol. Lett. 119, 303–308.
[30] Parkes, R.J. and Taylor, J. (1983) The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. East Coast Shelf Sci. 16, 173–189.
[31] Vainshtein, M., Hippe, H. and Kroppenstedt, R.M. (1992) Cellular fatty acid of *Desulfovibrio* species and its use in classification of sulfate-reducing bacteria. Syst. Appl. Microbiol. 15, 554–556.
[32] Elsgaard, L., Guezennec, J.G., Benbouzid-Rollet, N. and Prieur, D. (1991) Fatty acid composition of sulfate-reducing bacteria from deep-sea hydrothermal vents (13°N, East Pacific Rise). Kieler Meeresforsch. 8, 182–187.
[33] Dowling, N.J.E., Widdel, F. and White, D.C. (1986) Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate-reducing bacteria and other sulphide forming bacteria. J. Gen. Microbiol. 132, 1815–1825.