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Biomass and Organic Acids in Sandstone of a Weathering Building: Production by Bacterial and Fungal Isolates

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Abstract. Ten fungal and nine bacterial strains were isolated from a weathering sandstone building. Their growth, organic acid production, and acidification capacity were assessed in culture under nutritional conditions similar to those in situ. Biomass (10–50 nmol phospholipid-PO_4 g^{-1}) within the rock was small compared to soils. The isolated organisms were able to produce high amounts of those acids found in the sandstone, but acid production did not cause a drastic reduction in culture pH. It is suggested that the importance of acidification in microbial degradation of sandstone has been overestimated and that, under in situ pH and nutritional conditions, cation chelation by microbially produced organic acid anions may be more relevant to the weathering process.

Introduction

Studies of rock weathering through heterotrophic microbial metabolism have generally taken two directions. Several investigations have been concerned with growth of bacteria [5, 25, 26, 27] or fungi [6, 10, 27] in media containing pulverized rock or minerals as the sole inorganic ion source; it was taken for granted that little growth would occur without minerals and that ions in solution at the experiment's end resulted from biological mineral dissolution. Alternatively, and rarely in growth experiments such as those cited above, the ability of bacteria [24, 25] or fungi [8, 12, 24] to produce organic acids has been examined in laboratory pure cultures; it was assumed that these compounds would be excreted in situ and thereby cause mineral degradation through acidification and/or cation chelation. The studies involving organic acid production have been, with one exception [8], qualitative. In addition, media were employed that contained high concentrations (> 1%) of total organic carbon (TOC) and other nutrients, relative to those found in stone. Field and laboratory work have rarely been combined to show that microbes can weather minerals under the low-nutrient conditions occurring in nature. An exception is the work of Wolters et al. [29] in which a strong case was made for the role of chemoautotrophic nitrifying bacteria in stone degradation.

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The aim of the present study was to assess the role of organic acid production by heterotrophic microorganisms in the degradation of a sandstone building. Bacteria and fungi were isolated from weathered sites, and their growth, organic acid production, and capacity to acidify the culture medium were measured under low-nutrient conditions. Biomass and weathering activity of the microbial population were assessed by measuring in situ concentrations of phospholipid-PO_4 and organic acids.

Methods

Site Description

Construction of the Schleswig regional courthouse (Oberlandesgericht) was completed in 1860. The building is primarily of brick, but a molding (2.4 m above the foundation) and much of the lower walls are of red sandstone. Sampling Site 1 was an area of blistered, gray/black crust on the molding with a north exposure; this prevailing form of weathering can be seen on roughly 75% of the total molding surface. Site 3 (on the molding approximately 2 m away from Site 1) had no crust but was visibly weathered and crumbled on touch. This type of weathering could be atypical as it was not seen elsewhere. Sites 5 and 6 (west and north exposures, respectively) were on walls directly beneath the molding and showed exfoliate weathering; thin sheets of material were eroded away, leaving behind a mosaic pattern in the stone. Approximately 50% of the total wall surface displayed this damage. Unweathered stone was obtained from the inner side (8–10 cm beneath the weathered surface) of a large piece of the wall that was accidently knocked loose during restoration work. Sites 2 and 4 were areas of brick facade and are not discussed in the present article.

Geological Description

Unweathered stone: unstructured, carbonate-free sandstone; very fine to fine grain size (0.063–0.2 mm according to Wentworth scale); grains iron-coated (Fe^{III}); mainly quartz, less than 1% (vol) mica and feldspar; silicate-cemented; lower Triassic in origin.

Site 1: Very fine to find sand, sanding; mainly quartz, less than 5% (vol) mica and weathered feldspar; kaolinites possible; manganese crusts; weathered silicate cement, iron and clay binding possible.

Site 3: Fine sand, sanding; no manganese crusts, otherwise as for Site 1.

Sites 5 and 6: Very fine to fine sandstone, sanding; grains Fe^{III}-coated; mainly quartz, but mica detectable; weathered silicate cement, iron and clay binding.

Estimated weathering intensity: 1 > 3 > 5, 6.

Sample Collection

Stone samples (1–10 g) were collected aseptically using alcohol-flamed forceps and sterile plastic bags. Samples were taken in July and September 1988, for isolation of microorganisms, and in September 1988, and April 1989, for analysis of in situ phospholipid-PO_4. For analysis of organic acids, samples were taken in April 1989. Samples were composed of the easily removable (highly weathered) material from each site and generally reached a depth of 2–4 mm. Procedures described below were begun usually within 3 hours (but not more than 24 hours) after sample collection. Storage, when required, was at 8°C.

Isolation and Enumeration of Microorganisms

Stone samples (1–2 g) were ground to homogeneity (single sand grains) within a laminar flow hood using an alcohol-flamed mortar. Aliquots (20 mg) of each sample were sprinkled onto PYGV plates

(solidified with 2% agar) in triplicate. PYGV [20] contains 20 ml liter^{-1} of modified Hutner's Basal Salts Medium [4], 10 ml liter^{-1} vitamin solution [20], plus 0.025% each of glucose, Bacto peptone, and Bacto yeast extract (Difco). The analyzed TOC content is 0.044% without agar (P. Hirsch, unpublished). The plates were incubated in darkness at 25°C and examined after 48 hours, and after 1, 2, and 3 weeks. Fungal and bacterial colonies were differentiated on the basis of fruiting bodies and colony morphology; they were isolated, purified, and transferred to PYGV-agar slants. As a traditional enumeration technique, the dilution spread-plate method was used in which 200–500 mg ground stone were suspended in 2 ml liquid PYGV, vortexed twice for 5 sec, diluted in tenfold steps, and 0.2 ml were spread onto PYGV plates in triplicate.

Growth and Organic Acid Production in Culture

All cultures were maintained at 25°C in darkness. Organisms were transferred from slants to 65-ml aliquots of liquid PYGV and incubated for 2 days (bacteria) or 4 days (fungi) on a reciprocal shaker; 10- to 25-ml aliquots of these starter cultures were homogenized in a home-made Dounce homogenizer (wall clearance approximately 0.1 mm), and 0.1 ml (bacteria, yeasts) or 0.5 ml (filamentous fungi) of the homogenate were transferred to 65 ml PYGV. Stationary cultures, in triplicate, were incubated 2, 4, 6, 8, 12, and 24 days (fungi, yeasts) or 1, 2, 4, and 8 days (bacteria). At each time point, triplicate cultures were harvested for growth estimation by mycelial dry weight (80°C) or, for bacteria and yeasts, by protein concentration based on binding of Coomassie Blue [2] (reagents from BioRad). Filtered (0.2 μm) culture supernatants (10 μl) were analyzed directly for organic acids by HPLC on an Aminex HPX-87H column (BioRad, 300 × 7.8 mm) at 36°C using 0.008 N H_2SO_4 (0.6 ml min^{-1}) as eluent. Detection was by UV light (210 nm, 0.010 absorbance units full scale). Identification and quantitation of acids was based on retention time and concentration curves (peak area vs concentration) of known free acids or their salts (Sigma, Merck). Acetate and fumarate coeluted in this chromatographic system, as did succinate/glycolate and glucuronate/citrate. Phenacyl-derivatized samples [3] yielded no peaks for acetate or glycolate. Therefore, the overlapping peaks on the BioRad column were quantified on the basis of fumarate and succinate. Phenacyl-citrate was detected, but glucuronate does not derivatize; we cannot be certain that this coeluting peak on the BioRad column was entirely citrate. However, this peak was always minor, and it was quantified using data for citrate. Therefore, the results for citrate represent maximum values.

Biomass and Organic Acid Concentration in Stone Samples

Two to seven grams of stone were picked free of visible organic residue, such as insect remains, under a dissecting microscope. Phospholipid-PO_4 (PLP) was extracted from 1–5 g stone using a single-phase $CHCl_3/CH_3OH/H_2O$ system, and quantified using a molybdate-based assay [27, volumes of buffer and solvents changed according to sample weight]. Organic acids were extracted from 1–2 g mortar-ground stone with 0.15 M NaCl (1 g stone: 1 ml solvent) at 4°C for 30 min on a rotary shaker. The samples were centrifuged and 10 μl of 0.2 μm-filtered supernatants were analyzed by HPLC as above.

Results

Isolation and Enumeration of Microorganisms

Listed in Table 1 are the distribution abundance, and capacity for growth on PYGV-agar of fungal isolates, as well as the dilution-plate counts (total fungal

Table 1. Fungal strains isolated from the Schleswig Courthouse. Abundance, growth, and dilution-plate counts on PYGV-agar

		On trim				On wall			
		Site 1		Site 3		Site 5		Site 6	
Strain	Identification	Abundance[a]	Growth[b]	Abundance	Growth	Abundance	Growth	Abundance	Growth
1L3.6	*Aspergillus* sp.	+++	+++	+++	+++				
2L1.13	*Penicillium* sp.	+++	+++	+++	+++	+++	+++		
1L1.10	*Paecilomyces* sp.	+	+						
1L1.5	*Alternaria* sp.	+	++						
2L1.3	*Phoma* sp.	+++	+++	+++	+++			+	+++
1L1.9	*Cladosporium* sp.	+++	+++	+++	+++			+++	+++
2L1.12*P*	unidentified	+++	+++	++	++				
2L1.12	unident., sterile	+++	+++						
2L1.2	unidentified	+	++						
1L3.1	budding yeast	+	++	++	++				
Dilution-plate count (total fungal CFU g^{-1}, SD in parentheses, n = 3)		1.6×10^4 (0.23)		6.7×10^3 (1.89)		1.1×10^4 (0.18)		5.6×10^3 (2.08)	

[a] +++ = abundant (>5 colonies per plate), ++ = present (1–5 colonies per plate), + = infrequent (1 colony per plate), blank = not observed
[b] +++ = luxurious (rapidly spreading), ++ = good (slowly spreading), + = poor (little or no spreading)

Table 2. Bacterial strains isolated from the Schleswig Courthouse. Abundance[a] on PYGV-agar plates

Strain	Description	On trim		On wall	
		Site 1	Site 3	Site 5	Site 6
1L1.3	G+[b] rod, endospores, light brown colonies with fuzzy edge	+	+++		
1L6.3	G+ rod in chains with endospores	+++		++	+++
2L6.2	G+ rod in chains with endospores	++			+++
1L3.2	G+ rod in chains with endospores		+		
1L1.2	G+ rod, granules, pale yellow colonies	+			
1L1.4a	large G+ coccus, pink colonies	+			
1L1.4b	motile G+ rod, yellow colonies	+			
1L3.3	G+ coccus, yellow mucoid colonies	+			
2L1.16	G+ rods, white punctiform colonies	+			
Dilution-plate count (total bacterial CFU g^{-1}, SD in parentheses, n = 3)		1,200 (900)	5,700 (3,040)	400 (360)	600 (580)

[a] Abundance as in Table 1
[b] Gram-positive

CFU g^{-1}) from the sampling sites. Site 1 had the greatest morphological diversity; all 10 strains occurred here. Site 5 yielded only one strain. Five strains were found only at Site 1, and three of these strains occurred infrequently. The remaining strains were found at other sites as well, and in abundance. No strain was found at all 4 sites, but 3 strains (*Penicillium* sp., *Phoma* sp., and *Cladosporium* sp.) occurred at 3 sites. Table 2 presents the distribution and abundance of the nine bacterial isolates, and the dilution-plate counts (total bacterial CFU g^{-1}) from the sites. As was true for the fungi, the greatest diversity in bacterial morphotypes was seen at Site 1. Site 5 yielded only one bacterial morphotype, and Sites 3 and 6 yielded two morphotypes. All bacterial isolates were gram-positive.

Numbers of bacteria and fungi, as measured by direct plating, were low. The most abundant organisms produced 5–8 colonies per plate (250–400 CFU g^{-1}). Thus, total fungal counts by direct plating were at most 10^4 CFU g^{-1}, and total bacterial counts were 10^3 CFU g^{-1}. Dilution spread-plate counts (Tables 1 and 2) confirmed these results; 1.6×10^4 CFU g^{-1} (fungi) and 5.7×10^3 CFU g^{-1} (bacteria) were the highest numbers obtained. To examine input of organisms from air, PYGV plates were exposed near the building for 2 min. Less than 10 CFU/plate were recovered; the morphotypes did not correspond to those from the building (data not shown).

Relationship Between Bacterial Growth and Culture pH

Growth of bacterial isolates (Table 3) ceased sometime after 4 days; between 4 and 8 days, total protein decreased or was unchanged (i.e., standard deviations overlapped) except in strains 1L1.4a and 1L1.4b. When a decrease in pH (initially 7.2) was observed, it took place early during incubation (pH minimum

Table 3. Bacterial growth (μg protein ml^{-1}) corrected for PYGV (3.26 μg ml^{-1}), and pH of culture medium during 8 days incubation

	Day 1		Day 2		Day 4		Day 8	
Strain	Protein	pH	Protein	pH	Protein	pH	Protein	pH
1L6.3	4.9[a]	5.23	16.5	5.10	29.2	6.33	11.8	7.30
	(0.4)	(0.09)	(0.9)	(0.01)	(0.2)	(0.01)	(1.5)	(0.03)
2L1.16	5.4	6.43	11.3	5.90	17.1	5.90	14.1	5.83
	(0.6)	(0.09)	(0.8)	(0.01)	(0.2)	(0.01)	(1.5)	(0.03)
1L3.2	0.1	6.90	1.2	7.13	11.8	7.83	15.1	8.05
	(0.1)	(0.01)	(1.2)	(0.05)	(2.3)	(0.06)	(4.2)	(0.05)
1L1.2	24.7	5.40	27.6	5.32	30.7	5.28	31.9	5.27
	(0.4)	(0.07)	(3.1)	(0.01)	(0.4)	(0.01)	(1.0)	(0.04)
1L1.3	6.4	5.62	23.3	5.91	29.3	6.82	23.2	7.86
	(0.1)	(0.02)	(4.4)	(0.01)	(0.8)	(0.12)	(0.7)	(0.19)
1L1.4a	6.0	6.80	18.1	6.96	17.3	6.95	24.6	6.83
	(0.1)	(0.01)	(0.6)	(0.03)	(2.7)	(0.05)	(1.0)	(0.09)
1L1.4b	3.6	6.96	5.4	7.11	13.3	7.25	21.0	7.34
	(0.2)	(0.01)	(1.0)	(0.01)	(0.4)	(0.04)	(1.3)	(0.01)
1L3.3	3.2	6.85	11.2	6.93	16.7	7.15	18.0	7.49
	(0.3)	(0.01)	(0.7)	(0.01)	(2.0)	(0.01)	(1.3)	(0.01)
2L6.2	4.4	5.65	15.2	5.14	27.2	5.62	10.9	6.45
	(1.0)	(0.07)	(0.8)	(0.03)	(1.1)	(0.05)	(0.1)	(0.01)

[a] Standard deviation (n = 3) in parentheses

reached within 48 hours). Thereafter, pH usually rose to near neutrality. Exceptions were strains 2L1.16 and 1L1.2, in which pH reached a minimum after 8 days. Strain 1L1.2 was the strongest acidifier and reached a pH minimum of approximately 5.3.

Relationship Between Fungal Growth and Culture pH

Growth of fungal isolates in culture (Table 4) ceased between days 8 and 12. By day 24, dry weight was less than on day 8 or 12 in all strains except 1L1.9 in which dry weight did not increase significantly. In general, pH of the culture medium dropped slightly or did not change over the first 4 days of incubation. Strain 1L1.10 was the strongest acidifier and reached a pH of 6.07 after 4 days. In all cases, pH rose during the final 16 days to $\geq$8.0.

Organic Acid Production by Bacteria

Bacterial strain 1L3.3 produced no organic acid and strain 1L1.4b produced very small amounts of fumarate (data not shown). The remaining strains excreted combinations of lactate and formate (Table 5). In addition, two unidentified acidic compounds which also occurred in PYGV (retention times of

Table 4. Fungal growth (mg dry wt/culture) and pH of culture medium during 28 days incubation

	Day 2		Day 4		Day 6		Day 8		Day 12		Day 28	
Strain	Dry wt	pH	Dry wt	pH	Dry wt	pH	Dry wt	pH	Dry wt	pH	Dry wt	pH
2L1.2	13.3[a]	6.43	n.d.	n.d.	33.2	7.59	n.d.	n.d.	26.5	7.95	17.9	8.32
	(2.5)	(0.05)			(1.3)	(0.04)			(1.9)	(0.04)	(0.3)	(0.06)
1L3.6	7.6	6.40	n.d.	n.d.	18.3	7.68	n.d.	n.d.	14.5	8.27	8.6	8.63
	(6.5)	(0.36)			(2.0)	(0.07)			(1.2)	(0.11)	(0.6)	(0.21)
2L1.13	4.7	6.83	6.2	6.97	n.d.	n.d.	n.d.	n.d.	13.6	7.60	11.1	8.40
	(1.4)	(0.02)	(0.6)	(0.02)					(0.9)	(0.06)	(1.2)	(0.01)
2L1.12	5.9	6.74	10.6	7.00	n.d.	n.d.	n.d.	n.d.	16.8	7.60	16.3	8.46
	(0.9)	(0.01)	(1.9)	(0.01)					(0.7)	(0.32)	(3.8)	(0.01)
1L1.10	4.6	6.91	14.1	6.07	n.d.	n.d.	20.8	8.33	n.d.	n.d.	7.5	8.05
	(1.9)	(0.06)	(6.3)	(0.35)			(3.1)	(0.02)			(0.4)	(0.02)
1L1.5	10.1	6.83	58.2	6.73	n.d.	n.d.	20.0	8.20	n.d.	n.d.	8.8	8.26
	(2.3)	(0.07)	(30.9)	(0.06)			(1.2)	(0.16)			(0.5)	(0.03)
2L1.3	4.8	6.97	26.9	6.48	n.d.	n.d.	21.1	7.71	n.d.	n.d.	14.8	7.96
	(0.5)	(0.03)	(5.1)	(0.02)			(1.0)	(0.07)			(0.2)	(0.09)
1L1.9	3.1	6.71	6.9	6.79	n.d.	n.d.	10.5	8.03	n.d.	n.d.	13.5	8.32
	(1.2)	(0.35)	(0.7)	(0.06)			(2.0)	(0.10)			(1.8)	(0.01)
2L1.12*P*	0.8	6.40	6.7	6.68	n.d.	n.d.	19.5	7.46	n.d.	n.d.	10.4	8.14
	(0.6)	(0.03)	(4.0)	(0.05)			(13.1)	(0.09)			(8.5)	(0.02)

[a] Standard deviation (n = 3) in parentheses; n.d. = not determined

Table 5. Organic acid concentrations in bacterial culture supernatants during an 8 day incubation

Strain	Time (days)	Organic acid concentration (μM)[a]						
		cit	pyr	mal	suc	lac	for	fum
1L1.4a	1	nc	2	nc	<	nc	<	nc
	2	nc	nc	nc	nc	nc	=	nc
	4	nc	3	nc	nc	nc	nc	nc
	8	nc	16	nc	nc	nc	11	nc
1L1.3	1	7	8	nc	<	920	263	2
	2	16	10	nc	nc	670	215	nc
	4	nc	nc	nc	nc	31	138	<
	8	nc	2	nc	nc	166	229	nc
1L1.2	1	nc	9	nc	<	488	770	1
	2	1	nc	nc	nc	439	680	2
	4	5	nc	nc	nc	511	767	3
	8	3	5	nc	nc	518	692	4
1L3.2	1	nc	2	nc	<	81	nc	nc
	2	nc	5	nc	nc	177	nc	nc
	4	nc	2	nc	nc	nc	nc	nc
	8	nc	=	nc	nc	nc	nc	nc
2L1.16	1	nc	9	nc	<	nc	nc	nc
	2	nc	18	nc	nc	nc	nc	1
	4	nc	11	nc	nc	nc	nc	2
	8	nc	19	nc	nc	nc	nc	3
1L6.3	1	nc	53	154	<	1,085	488	7
	2	nc	3	nc	nc	=	419	6
	8	nc	12	65	nc	305	756	26
2L6.2	1	nc	2	nc	<	126	nc	1
	2	nc	nc	nc	nc	387	nc	2
	4	nc	=	nc	nc	107	nc	1
	8	nc	<	nc	nc	=	nc	nc

[a] <, less than concentration in PYGV; =, equal to concentration in PYGV; nc, no change from previous time point, or from t_0 (PYGV), respectively. Concentration of acids in PYGV (μm): cit, 0.55; pyr, 1.4; lac, 20; fum, 22
Abbreviations used: cit = citrate, pyr = pyruvate, mal = malate, suc = succinate, lac = lactate, for = formate, fum = fumarate

20.3 and 22.4 min) were excreted (data not shown). Pyruvate and fumarate were frequently detected, but at relatively low levels. Citrate and malate were excreted only rarely. Succinate was always removed from the medium. Usually, the highest acid concentrations were seen early during incubation and often decreased after day 4. This result implies that acid excretion is dependent on cell growth and is unrelated to lysis. In two of the three formate-producing strains, stable concentrations during the incubation indicated that this acid was excreted only during the first 24 hours. In contrast, concentrations of lactate varied; acid excreted early was taken up later. Under our chromatographic conditions, oxalic acid was difficult to quantitate in cultures because it occurred as a shoulder on the void volume peak; however, oxalate had a high extinction coefficient at 210 nm and thus was easily detected. Only one strain (1L6.3) produced oxalic acid; concentrations were less than 10^{-6} M (data not shown).

Table 6. Organic acid concentrations in fungal culture supernatants during a 24 day incubation

Strain	Time (days)	Organic acid concentration (μM)[a]								
		cit	glu	pyr	mal	suc	lac	for	fum	oxa
1L3.1	2	nc	nc	2	nc	51	nc	nc	12	nc
	4	nc	nc	nc	nc	19	101	<	nc	nc
	8	nc	nc	4	1	117	40	nc	nc	nc
	24	nc	nc	1	7	230	36	nc	2	nc
2L1.13	2	nc	nc	2	nc	11	nc	<	nc	nc
	4	nc	nc	nc	nc	70	nc	nc	nc	nc
	8	nc	nc	1	nc	66	nc	nc	nc	nc
	24	nc	nc	<	nc	<	nc	nc	<	nc
2L1.12*P*	2	nc	nc	2	5	130	nc	nc	nc	nc
	4	nc	nc	1	<	60	nc	nc	nc	14
	8	nc	nc	<	nc	41	nc	nc	<	120
	24	nc	nc	1	nc	<	nc	nc	nc	98
2L1.2	2	17	nc	5	81	1,143	139	406	<	nc
	6	13	nc	<	44	145	nc	634	nc	144
	12	<	nc	nc	nc	102	124	nc	nc	132
	24	nc	nc	nc	48	<	245	299	2	180
2L1.12	2	nc	58	nc	nc	nc	nc	nc	nc	nc
	4	nc	104	nc	nc	nc	nc	nc	nc	nc
	12	nc	85	nc	nc	nc	nc	nc	nc	nc
	24	nc	nc	nc	nc	nc	nc	nc	nc	nc
2L1.3	2	16	nc	20	nc	718	168	338	<	nc
	4	<	nc	23	nc	559	158	634	2	nc
	8	nc	nc	34	38	95	174	230	nc	nc
1L1.5	2	1	nc	7	nc	706	189	323	<	nc
	4	nc	nc	10	60	677	290	344	4	nc
	8	=	nc	<	29	<	347	252	2	nc
	24	nc	nc	nc	<	nc	<	<	<	nc
1L1.9	2	nc	91	4	187	1,136	nc	406	<	nc
	4	nc	nc	nc	109	1,788	924	496	3	nc
	8	nc	32	<	113	148	247	457	<	nc
	24	nc	<	nc	<	<	<	<	nc	nc
1L1.10	2	1	3	1	6	494	243	nc	<	nc
	4	<	<	<	<	439	1,088	nc	nc	nc
	8	6	nc	3	nc	<	161	216	6	nc
	24	<	nc	<	nc	nc	<	<	<	nc

[a] Symbols and abbreviations as in Table 5. oxa = oxalate; concentration in PYGV, $\leq$74 μm

Organic Acid Production by Fungi

As was true for bacterial isolates, most fungal strains excreted several acids (Table 6). Strain 1L3.6 produced small (<75 μM) amounts of pyruvate and succinate, and strains 1L1.5, 1L1.9, and 1L1.10 produced the same unidentified acidic compounds seen in bacterial cultures (data not shown). Other fungal strains produced succinate, malate, and/or gluconate in addition to lactate and/or formate. Small amounts of citrate, pyruvate, and fumarate were also detected. Acid concentrations in fungal cultures were similar to those in bacterial cultures, and acid excretion was again dependent on growth; the highest concentrations were observed on or before day 8 (or day 12) of incubation. Strains 2L1.12*P*

Table 7. Organic acid concentrations and biomass (phospholipidphosphate [PLP]) in stone samples

Site	Organic acid concentration (nmol g^{-1})[a]								PLP (nmol g^{-1}, Sept 1988, Apr 1989)
	cit	pyr	suc	lac	for	fum	oxa	Total	
1	X	19	X	X	1,353	22	X	1,394	49, 41
3	4	4	19	108	607	12	19	773	17, n.d.[b]
5	1	28	X	69	6,088	7	X	6,193	22, 39
6	X	21	X	X	283	X	X	304	11, 17

[a] Abbreviations as in Tables 5 and 6; X = not found
[b] Not determined

and 2L1.2 produced oxalate in sufficient quantities to allow an estimate of the minimum concentration. In all other strains, oxalate was detected at concentrations below 10^{-6} M.

In situ Biomass

The concentration of phospholipid-PO_4 (PLP) was highest at Site 1, lowest at Site 6, and intermediate at sites 3 and 5 (Table 7).

In situ Organic Acid Concentrations

No organic acids were detected in the control sample (unweathered stone). Formate was detected at all sites and in relatively high amounts at sites 1 and 5 (Table 7). Fumarate was not detected at Site 6, but was found in low amounts at the other sites. Pyruvate occurred at all sites, but at a concentration at least ten times lower than that of formate. Site 3 had the greatest diversity in acids and was the only site where oxalate was detected. Site 5 had the highest total acid concentration. The absolute amounts of acids reported in the present paper could be subject to seasonal fluctuation, and, because sample material was limited, statistical analysis was not possible.

Discussion

Counts of microorganisms in the present study (whether by direct plating or by dilution spread-plating) were similar to the lowest dilution-plate counts reported by Webley et al. [27]. They were much lower than those reported by Wagner and Schwartz [24] or, in a study of a sandstone monument, by Eckhardt [8]. In the latter two studies, dilution-plate counts of bacteria were in the same range as those generally reported for soil (10^6 CFU g^{-1}), and dilution-plate counts of fungal propagules (10^5–10^6 CFU g^{-1}) were 10–100 times greater than those commonly obtained for soil. The usefulness of plate-count methods for enumeration of organisms, especially for those of terrestrial environments, is

widely disputed; the influence of medium selectivity and of sampling procedures are well documented [8, 11]. Also, CFU-based methods count spores and cell clumps as colonies and thus are inherently inaccurate for estimating living, viable biomass. For these and many other reasons, the use of plate counts as indicators of active microbial biomass has fallen out of favor. Quantitation of phospholipid-PO_4 (PLP) is simple and provides a better estimation of viable biomass than plate-count methods [23]. PLP concentration in the courthouse sandstone (10–50 nmoles g^{-1}) was several orders of magnitude lower than that in upper soil horizons (100–10,000 nmol g^{-1}) [9], but similar to that reported for subtropical estuary sediment (25–33 nmol g^{-1}) [28] and Antarctic sandstone (approx. 29 nmol g^{-1}) [22]. However, the courthouse sandstone had a PLP concentration several orders of magnitude greater than that of groundwater sediments (0.050–0.200 nmol g^{-1}) [16, 20]. All biomass values should be normalized to total (colonizable) surface area because of differences in bulk density of the various substrata. However, rough comparisons based on dry weight, as well as the low TOC content of sandstones, imply that the numbers of viable microorganisms in sandstones must be much lower than those in upper soil horizons.

Medium composition, naturally, controls culture pH. Drastic changes in culture pH can be observed by varying buffer capacity and concentrations and/or sources of carbon and nitrogen. In studies of rock weathering, nutritional conditions under which microorganisms are grown should be qualitatively and quantitatively similar to those in situ. The sandstone in the present study had a TOC content of 1.5–3.5 mg g^{-1} (combustion determination, data not shown), and amino acids [18] as well as proteins (unpublished data) were present, sometimes in large quantities. Therefore, a medium having a low TOC concentration, a relatively low C:N value, and biologically available N predominantly in oligopeptide form (e.g., PYGV) was indicated for isolation and physiological studies of the relevant heterotrophic microflora. The present results contrast with the reported acidification of media by fungi isolated from weathered stone [6, 7]. However, it should be reiterated that acidification is highly dependent on medium composition (Fig. 4 in reference 8; cf. Tables 1 and 2 in reference 12). Extremely low pH values in microbial cultures, such as those reported by Kuroczkin [12] and Eckhardt [6], were not observed in the present study even though organic acids were excreted by nearly all organisms and the production of these acids was shown to be correlated with growth. As growth slowed, presumably due to carbon limitation, organic acid concentrations generally decreased; acids were probably reabsorbed and catabolized as carbon sources. Eckhardt [8] reported lactate concentrations of 100–300 mg liter^{-1} at pH 2.5–3.0 for bacteria and fungi cultured in MF-1, a medium that has a greater buffering capacity than PYGV (titration with 50 mM HCl, data not shown). The highest concentration of any organic acid reported in the present study was approximately 1 mM at pH 5–7, i.e., 90 mg lactate liter^{-1}. This comparison suggests that concentrations of organic acids similar to those seen in highly acidified cultures can indeed occur without an extreme drop in pH, and it seems that extremely low pH values cannot result solely from organic acid production even in a poorly buffered medium such as PYGV. Perhaps an additional acidification mechanism, unrelated to organic acid excretion, is activated by a high

concentration of organic carbon and/or a high C:N value. A further implication of the present results is that rapid screening methods for microbial acid production based on pH changes (microtiter plate methods, etc.) could overlook organisms capable of producing large amounts of organic acids.

Results of Eckhardt [8] suggest that numbers of bacteria at various sites in a sandstone monument did not vary greatly with season (at most by a factor of ten). In the present work, this type of analysis was not performed. Although comparison of data from samples obtained on different dates is therefore questionable, some generalized conclusions can be drawn, particularly when it is kept in mind that biomass as measured by PLP concentration (i.e., viable organisms, not propagules or cell clumps as in CFU determinations) did not vary greatly between September 1988 and April 1989.

The spectrum of organic acids produced in culture was generally the same as that reported by other investigators [12, 25], and, in the present study, the acids produced in culture were shown to occur in the stone. Site 1 displayed the highest morphological diversity of microorganisms and also the highest PLP concentration, but did not have the highest diversity or concentration of acids. This result suggests that perhaps the complete spectrum of organisms isolated from this site was not active there, and that perhaps the assemblage of organisms produces in situ a different suite of organic acids than do the individual members in culture. In addition, the nutritional conditions in the microenvironment are most likely different than those in our cultures, just as the physical conditions in the stone (e.g., matric water potential) are different than those in culture. Despite such interpretational difficulties, it is true that the site having the highest diversity in acids (Site 3) was relatively high in organismal diversity, and the culture studies indicate that the suite of microorganisms isolated from this site could have produced those acids occurring in situ. The highest total acid concentration (mostly formate) was found at Site 5: a site with an intermediate biomass concentration. Abundance data show this site to be inhabited primarily by two organisms, one of which produced high amounts of formate in culture. In general, a comparison of the laboratory culture studies with the in situ PLP/organic acid analyses suggests that the organisms isolated are active and excrete organic acids even under the conditions present in the stone. A more detailed study of in situ biomass and organic acid production vs time was not possible using this building because it is under protection as a cultural landmark; however, an experiment examining activity of isolated strains under field conditions is in progress.

It is well known that quartz is rather insoluble at pH 3–9, and acidification alone seems to play a small role in weathering of the Oberlandesgericht sandstone; pH values of the stone samples were between 4.4 and 6.5 (suspended 100% wt/vol in 0.1 M KCl; data not shown). These data agree well with pH values measured in laboratory cultures of the relevant microorganisms. The question then arises as to the significance of these microorganisms in weathering. In open, natural systems, it is the weathering rate (as influenced by chelating agents, etc.), that is important, not the solubility of quartz. In addition, sandstone consists of other substances besides quartz. Organic acids are known to chelate cations [13–15], and a correlation between the presence of organic acids and dissolution of minerals, including clay minerals found as binders in

sandstone, has been shown [1, 7, 13, 15, 17]. It should be remembered that two processes are occurring in such studies, acidification and chelation. While it has been suggested that, in the presence of organic acids, acidification alone is responsible for mineral degradation [19], the results of the present study suggest that organic acid production need not result in acidification. Research on the chelating properties of organic acids [13–15] was performed using salts, not free acids, and therefore distinguished between acidification and chelation. In one report [14], chelation was optimal at alkaline pH values and much reduced in acid solution, and recent studies on the weathering of quartz [1] and feldspar [17] by pH-controlled organic-acid solutions have demonstrated that a high rate of dissolution can occur at near neutral pH. For the Oberlandesgericht (and, in general, for sandstone with moderate pH values), the role of microbially produced organic acids in the weathering process is probably greater through chelation than through acidification because, under in situ conditions, relatively high amounts of organic acids can be produced while little acidification occurs.

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