Interaction between organic compounds, minerals and microbes: Habitability studies from Mars analogue samples

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Future Life Detection Missions to Mars

- Future space missions will investigate the possibility of past and/or present life existing on Mars.

- Preliminary analyses of Mars analogue soils are necessary to determine the best locations to find whether signatures of life may still exist in the Martian regolith.

- A good understanding of the interactions between the organic, mineralogical and microbial components of desert soils is crucial to determine target locations for future life detection missions to Mars.

- Amino acids are among the biosignatures possibly present on Mars (Parnell et al. 2007).

Field Test Mars Desert Research Station (MDRS)

- We have measured the amino acid, mineralogical and microbiology content of soils collected near the Mars Desert Research Station (MDRS) in the Utah desert, during the EuroGeoMars 2009 campaign.

Ehrenfreund et al. (2011) *IJA* 10, 239.

Field Test Mars Desert Research Station (MDRS)

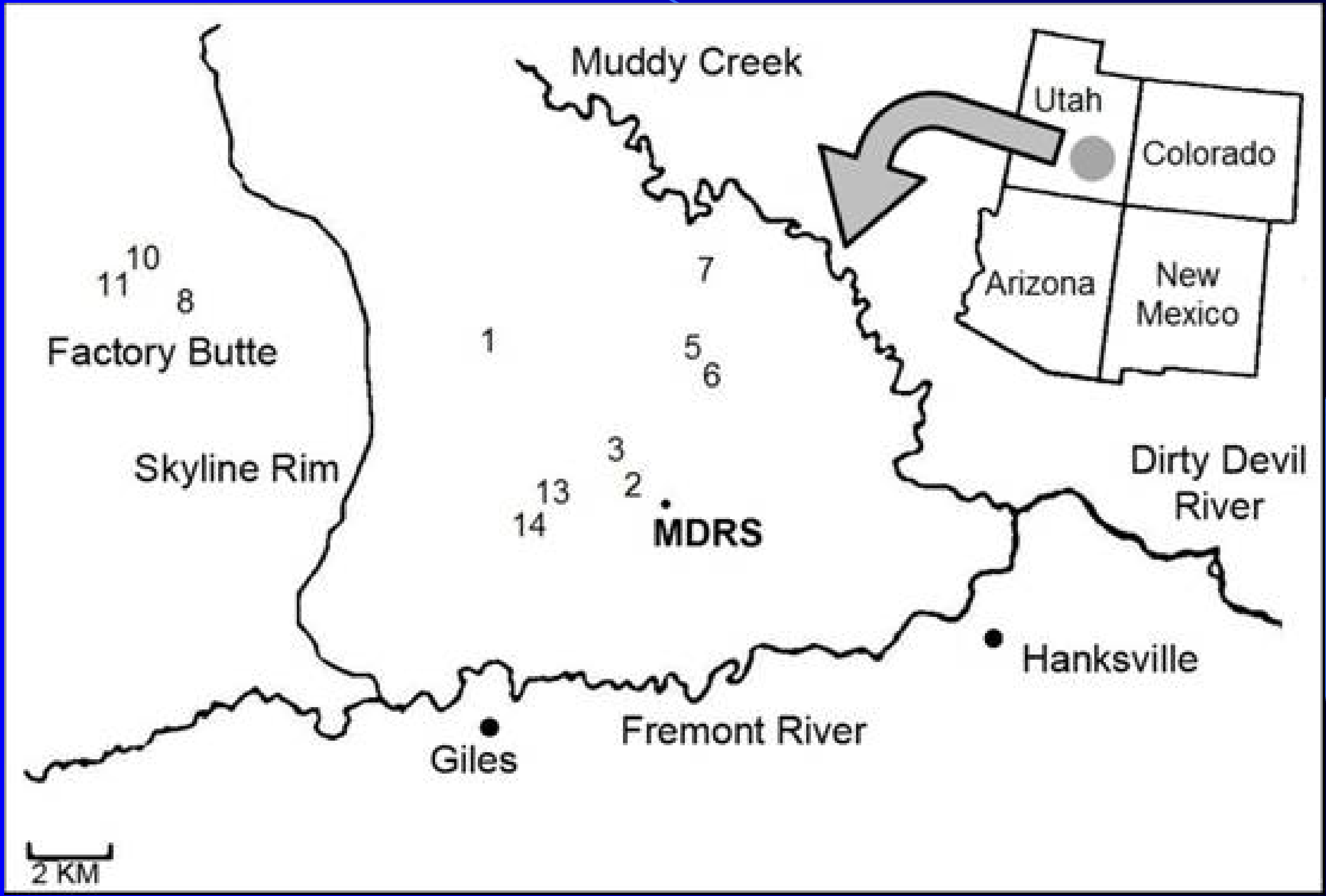


Figure 1 - Map showing the locations where the MDRS desert soil samples were collected (Martins et al. (2011) *IJA* 10, 231; Direito et al. (2011) *IJA* 10, 191)

Field Test Mars Desert Research Station (MDRS)

Table 1 - The MDRS desert soil samples and the corresponding coordinates, altitude and depth from which they were collected (Martins et al. (2011) *IJA* 10, 231)

Sample Name	Coordinates	Altitude (m)	Depth
P-1	N38. 43621° W110. 81943°	1350	surface
P-2	N38. 40746° W110. 79280°	1382	surface
P-3	N38. 40737° W110. 79261°	1375	surface
P-5	N38. 42638° W110. 78342°	1400	cliff
P-6	N38. 42638° W110. 78342°	1400	cliff
P-7	N38. 45424° W110. 79092°	1357	surface
P-8	N38. 43755° W110. 88725°	1482	surface
P-10	N38. 43896° W110. 89001°	1500	surface
P-13	N38. 40630° W110. 79547°	1405	surface
P-14	N38. 40630° W110. 79547°	1405	15cm

Experimental Procedure

- **Soil samples collected close to the MDRS**
- **Distributed to various laboratories for subsequent analysis**
- **Amino acids analysis**

  - H_2O, 100 ºC, 24h

  - Hydrolysis (6N HCl, 150 ºC, 3hrs)

  - Desalting

  - Gas Chromatograph-Mass Spectrometer (GC-MS)

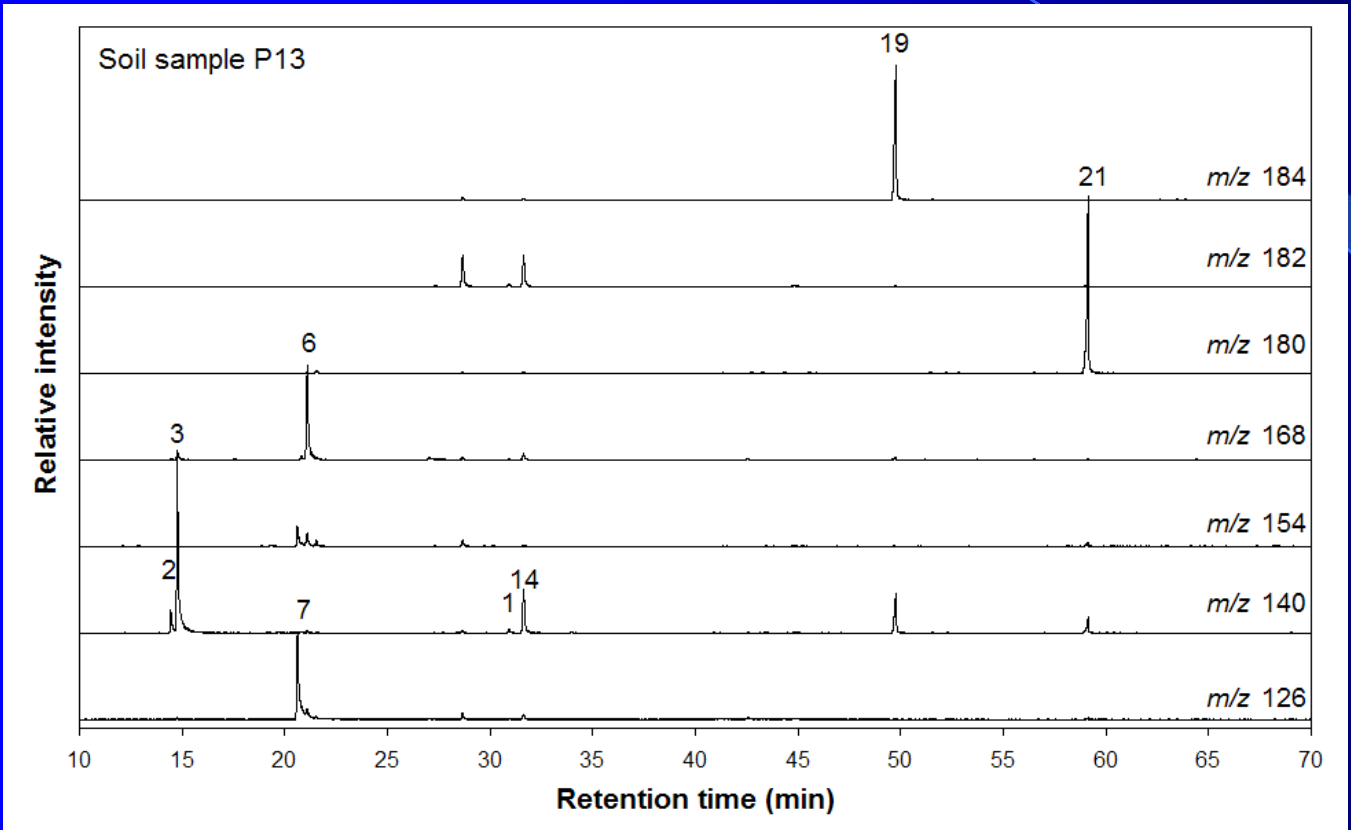
- **Mineralogy analysis**

  - IR spectroscopy and X-ray diffraction (XRD)

- **Microbiology analysis**

  - Polymerase Chain Reaction (PCR) using primers specific for ribosomal RNA

Amino Acid Results



Peak #	Amino acid	Single ion (*m/z*)
1	α-AIB	154
2	D-Alanine	140
3	L-Alanine	140
4	D,L-α-ABA[1]	154
5	D-Valine	168
6	L-Valine	168
7	Glycine	126
8	D,L-β-AIB[1,2]	182/153
9	D-Norvaline	168
10	L-Norvaline	168
11	β-Alanine	168/185
12	D,L-β-ABA[1,2]	140/182/153
13	D-Leucine	140/182
14	L-Leucine	140/182
15	D-Norleucine	182/114
16	L-Norleucine	182/114
17	γ-ABA	182/154
18	D-Aspartic acid	184/212
19	L-Aspartic acid	184/212
20	D-Glutamic acid	180/198
21	L-Glutamic acid	180/198

The 10 to 70 min region of the single ion GC-MS traces (*m/z* 126, 140, 154, 168, 180, 182 and 184) of the derivatized (*N*-TFA, *O*-isopropyl) HCl-hydrolysed hot-water extracts of each MDRS desert soil sample (Martins et al. (2011) *IJA* 10, 231).

Amino Acid Results

Table 2 - Summary of the average total amino acid abundances (in ppb) in ten Mars soils analogues collected close to the MDRS and measured by GC-MS

Amino acid	P1	P2	P3	P5	P6	P7	P8	P10	P13	P14
D-Alanine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4314 ± 216	n.d.
L-Alanine	n.d.	n.d.	76 ± 3	n.d.	n.d.	n.d.	1114 ± 98	803 ± 76	32934 ± 901	5944 ± 501
D-Valine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L-Valine	n.d.	n.d.	164 ± 4	n.d.	n.d.	n.d.	783 ± 27	322 ± 16	12793 ± 484	2622 ± 307
Glycine	n.d.	n.d.	92 ± 13	n.d.	n.d.	n.d.	1324 ± 106	327 ± 24	17390 ± 531	2535 ± 548
β-Alanine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D-Leucine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	514 ± 68	<1
L-Leucine	n.d.	n.d.	47 ± 9	n.d.	n.d.	n.d.	451 ± 48	24 ± 13	12897 ± 404	2695 ± 335
γ-ABA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D-Aspartic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L-Aspartic acid	83 ± 3	n.d.	102 ± 6	n.d.	n.d.	n.d.	220 ± 12	191 ± 22	7775 ± 155	1131 ± 48
D-Glutamic acid	53 ± 3	n.d.	57 ± 5	n.d.	n.d.	n.d.	128 ± 5	177 ± 47	n.d.	n.d.
L-Glutamic acid	139 ± 9	n.d.	194 ± 17	n.d.	n.d.	n.d.	600 ± 13	472 ± 10	10892 ± 176	1747 ± 69
Total	**280**	**n.d.**	**730**	**n.d.**	**n.d.**	**n.d.**	**4,600**	**2,300**	**100,000**	**17,000**

n.d. – Not detected

Amino Acid Results

- The most abundant amino acids were L-glutamic acid, D-glutamic acid, L-aspartic acid, L-valine, L-alanine, L-leucine and glycine.
- These amino acids were also the most abundant in other hot desert soils, such as the Oman (Martins et al. 2007), Arequipa and Atacama desert soils samples (Peeters et al. 2009).
- Total amino acid abundances were very heterogeneous, with values ranging from no amino acids detected to 100,000 ppb.
- The presence of both D- and L-amino acids (except in sample P-14) suggests that racemization has occurred over time and amino acids may be fossil remains.

Mineralogy Results

Table 3 – Bulk XRD results of the MDRS samples (Kotler et al. (2011) *IJA* 10, 221)

Mineral	P-1	P-2	P-3	P-5	P-6	P-7	P-8	P-10	P-13	P-14
Gypsum ($CaSO_4 \cdot 2H_2O$)	7		6		14		59	18		73
Quartz (SiO_2)	60	78	46	73	46	61	19	37	68	14
Albite ($NaAlSi_3O_8$)	3	3	24		2	6	<1	2	3	
K-feldspar ($KAlSi_3O_8$)	<1	1	<1	<1	<1	3			2	
Calcite ($CaCO_3$)	7		2	1		19	6	16	15	1
Dolomite ($CaMg(CO_3)_2$)	4				<1		2	4	<1	10
Total clay	15	16	18	23	33	6	11	20	9	1
Celestine ($SrSO_4$)			1							
Ankerite ($Ca(Fe, Mg, Mn)(CO_3)_2$)		<1	<1	<1		1				
Hematite (Fe_2O_3)				<1		1				
Pyrite (FeS_2)					<1		<1			
Siderite ($FeCO_3$)						1	<1	<1		<1

Mineralogy Results

Table 4 – MDRS clay fraction XRD peak positions. The 001* EG smectite crystallographic face represents diffraction analysis after ethylene glycolation (Kotler et al. (2011) *IJA* 10, 221)

Sample	001* EG smectite		001/002 illite		002/003 illite	
	d (Å)	° 2 θ	*d* (Å)	° 2 θ	*d* (Å)	° 2 θ
P-1	17.30	5.11	10.13	8.80	5.03	17.68
P-2	16.59	5.32	8.86	10.90	5.57	15.90
P-3	16.70	5.29	8.76	10.75	5.62	15.85
P-5	16.73	5.28	10.13	8.80	5.57	15.91
P-6	16.90	5.20	10.13	8.80	5.59	15.82
P-7	16.73	5.28	10.13	8.80	5.06	17.56
P-8	17.07	5.17	10.13	8.82	5.01	17.68
P-10	16.80	5.23	10.13	8.80	5.00	17.62
P-13	16.88	5.07	10.13	8.82	5.07	17.47

- Ethlyene glycol hydration shows a peak shift from the illite crystallographic face, indicating that the samples are a mixed layer illite-smectite.
- Water held in the expandable layer of smectites is released as the mineral is transformed to illite (non-expandable).

Amino acids, Mineralogy and Microbiology Results

Table 5 – Results from the amino acids, mineralogy and microbiology analyses ten Mars soils analogues collected close to the MDRS (Ehrenfreund et al. (2011) *IJA* 10, 239; Direito et al. (2011) *IJA* 10, 191; Kotler et al. (2011) *IJA* 10, 221; Martins et al. (2011) *IJA* 10, 231; Orzechowska et al. (2011) *IJA* 10, 209)

Sample	Mineralogy[a]			Organic Matter %	Amino Acids ppb[c]	Post Bacteria PS /FDNA		Post Eukarya PS /FDNA		Post Archaea PS /FDNA	
	Sulfates %	Carbonates %	Clays %								
P-1	7	11	15	2	280	+	+	+	+	+	+
P-2		<1	16	1	n.d.	+	-	-	-	-	-
P-3	7	3	18	1	730	+	+	+	+	-	-
P-5		2	23	2	n.d.	+	-	-	-	-	-
P-6	14	<1	33	2	n.d.	-	-	-	-	-	-
P-7		22	6	2	n.d.	-	-	-	-	-	-
P-8	59	8	11	4	4,600	-	+	-	+	-	+
P-10	18	20	20	5	2,300	+	+	+	+	+	+
P-13		16	9	2	100,000	+	+	+	+	+	+
P-14	73	11	1	3	17,000	-	-	-	-	-	-

PS – Power Soil   FDNA – Fast DNA   n.d. – Not detected

Amino acids, Mineralogy and Microbiology Results

- **The MDRS soils with high percentage of gypsum (calcium sulfate dihydrate) had a high/medium amino acid content, while the ones with high percentage of total clay had lower abundances of detectable amino acids.**

- **In addition, the exact clay mineral content (e.g. smectite/illite ratio) influenced the extraction of compounds such as amino acids and DNA from the mineral matrices.**

- **Clays strongly bind organics preventing efficient extraction.**

- **All three domains of life (Archaea, Bacteria and Eukarya) were observed but not in all samples.**

- **No microorganisms were detected in soil sample P-14. However, this soil sample had a high level of present life amino acid (i.e. presence of L-amino acids only).**

Amino acids, Mineralogy and Microbiology Results

- **Spiking experiments revealed that this is due to adsorption or degradation of DNA on the mineral surface.**

- **No significant correlation between amino acid content and DNA yield or detection of microorganisms.**

- **This may be explained either by different adsorption characteristics of amino acids and DNA, or the fact that different amino acids can last longer than DNA once an organism dies.**

Conclusions

- The variations in the organic matter abundances appear to reflect the ability of soils to host living organisms, to preserve their organic signatures and allow their extraction.

- It is necessary to further optimize extraction procedures that release biological compounds from host matrices to enable the effective detection of biomarkers.

- These multidisciplinary findings help in the preparation phase for future Mars missions, and are crucial to successfully target locations that may host organic matter, as well as extract and detect biosignatures on Mars.

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