

Research Article

Life Detection Using Glucose and Tetrasaccharide Enantiomer Pairs

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Abstract

A life-detection system based on the expectation that any viable organism will utilize stereoisomers of a given compound asymmetrically is examined. Aqueous extracts of common soil, Mars regolith simulant JSC Mars-1, and suspensions of *E. coli* and *S. cerevisiae* were incubated with stereoisomer pairs. The enantiomeric pairs were either D- and L-glucose or a pair of chiral tetrasaccharides. Following an incubation period of 10 days, stereoisomeric selectivity is detectable with the glucose pair by mass spectrometry in extracts made from soil at 0.5 g/ml, in extracts made from JSC Mars-1 at 2.5 g/ml, and in cell suspensions down to 1.0×10^7 cells/ml. For the tetrasaccharide pair, stereoisomeric selectivity was detected in extracts made from 0.5 g/ml or more of common soil but not in JSC Mars-1 simulant. The effective sensitivity in extracts was 2.5×10^7 cells/ml or better for the glucose pair and 5.0×10^8 cells/ml or better for the tetrasaccharide pair. The sensitivity of the glucose pair was such that it could detect life in samples that would be found to be devoid of organic matter by the GCMS system carried by the Viking landers. The results demonstrate the utility of the approach in the search for biological activity on Mars. However, sensitivity is a function of the enantiomer pair used, and this might also be different for hypothetical martian organisms. Therefore, it will be necessary to characterize additional stereoisomeric pairs and, ultimately, to include several in a single test environment. Key Words: Mars—Life detection—Enantiomers—Homochirality. *Astrobiology* 9, 297–303.

1. Introduction

1.1. Background

THE SEARCH FOR EXTANT LIFE on Mars and other planets and moons depends on the ability of landed, *in situ* instruments to detect biomarkers or other signatures of living forms and distinguish these from signals that can result from non-biological agents. One way to meet this challenge is to expose planetary samples to pairs of mirror-image stereoisomeric compounds, or enantiomers. Nonbiological, but chemically active, agents should produce the same reaction in each case, while putative life-forms would be expected to exhibit homochirality in that they would react preferentially to one compound vs. its enantiomer. The homochirality hypothesis dates back to Pasteur, who posited that biosynthetic reactions involved chiral forces. It was strengthened in the early 20th century by Fischer, who found that biology uses carbohy-

drates only in the D-form and amino acids only in the L-form (Mason, 1991). Subsequently, it has been widely reasoned that biology throughout the Cosmos should be chiral selective.

Recently, it was observed that enantiomers of ribose and glucose exhibit measurable differences in proton exchange between ¹⁷O-labeled water and one sugar vs. its enantiomer with time domain 1H nuclear magnetic resonance (Scorei *et al.*, 2007). This finding may have important implications with respect to the origin of homochirality in prebiotic or emerging biotic systems in the Cosmos. With regard to life detection on extant planetary bodies, however, this new result does not challenge the core notion that homochirality implies biology, because the effect was observed when sugars were exposed to large magnetic fields, *e.g.*, 6,000 Gauss. While magnetic fields of such magnitude or greater are present on, and in, the vicinity of certain stars (Lattimer and Prakash, 2004), they are not present on Mars or on other

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planetary bodies within the Solar System. Magnetic fields on planetary bodies are, at most, on the order of a few Gauss (Khurana *et al.*, 1998). In summary, despite this recent observation (Scorei *et al.*, 2007), there remain no known nonbiological forces native to a planet or moon that could confound the results of a life assay that is based on the concept of biological chiral selectivity.

The potential use of homochirality as the basis of a life-detection test was recognized in the mid-20th century, during the development of Project Viking. In an early form, the labeled release (LR) experiment, one of three life-detection assays that ultimately were included in the "biology package" carried to the surface of Mars by each of the two Viking landing craft (VL1 and VL2), was proposed with biological chiral selectivity in mind (Levin, 1987). In the LR experiment, samples of martian regolith were exposed to a dilute, aqueous solution that contained one-, two-, and three-carbon organic compounds labeled uniformly with ¹⁴C. The space above the sample container was then monitored for changes in radioactivity levels, such that an increase in radioactivity in the overhead space would indicate the chemical conversion of one or more of the labeled compounds into CO, CO₂, or CH₄ gas.

To control for the possibility of chemical conversion of the compounds by nonbiological catalysts in the regolith, some samples were heated in an oven for three hours at 160°C prior to testing to destroy putative microbes. While this control condition would be employed ultimately in the LR as flown on Viking, the LR principal investigator also proposed separating the test compounds into two solutions. One would have contained L-alanine and D-lactate, while the other would have contained D-alanine and L-lactate (Gilbert Levin, personal communication). Due largely to imposed limitations on mass and size, however, the LR experiment launched aboard Vikings 1 and 2 in 1975 employed only one nutrient solution. That solution contained labeled formate, glycine, and glycolate, which are not chiral, along with L-alanine and D-alanine, and D-lactate and L-lactate in racemic mixtures (Levin and Straat, 1976, 1977). Consequently, the LR experiment that VL1 and VL2 carried to Mars in 1976 did not take advantage of the phenomenon of homochirality.

Two decades later, a new version of the LR experiment was loaded into the ill-fated Russian Mars lander of 1996. This LR experiment would have employed one substrate pair, ¹⁴C-labeled L-cysteine and ¹⁴C-labeled D-cysteine, (Levin, 1987; Levin *et al.*, 2000, 2002), but would yield positive results only in the presence of biological reactions liberating the single labeled carbon of L- or D-cysteine. Due to technological advances in recent years, however, the homochirality concept can now be applied to numerous assays, which could be run by flight-capable devices that are a few centimeters in diameter and weigh less than 200 g.

While the origins of biological homochirality remain uncertain, hypotheses introduced subsequent to the Viking mission and the development of the chiral LR all share the notion that the emergence of the ability to react chemically with one enantiomer and not the other results from a process that involves Darwinian selection (Jorissen and Cerf, 2002; Goodman and Gershwin, 2006; Cartwright *et al.*, 2007; Root-Bernstein, 2007). With this in mind, we herein further explore the use of enantiomer selectivity as the basis for a life-detection assay. In particular, in the proposed approach,

rather than labeling D- and L-substrates with ¹⁴C, detection is assayed by mass spectrometry. This takes advantage of the fact that lightweight, flight-capable mass spectrometry devices of various types are increasingly available for space missions (Cotter *et al.*, 1999; Ecelberger *et al.*, 2002; English *et al.*, 2003; Ermer, 2007). Such instruments are attractive because they are capable of running assays with the use of several enantiomer pairs and are available for use in other unrelated experiments.

1.2. Experimental design

In this study, enantiomers of glucose as well as a pair of beta-linked gluco-tetrasaccharides were examined for usefulness in a life-detection assay. Because chemical changes to either, or both, compounds of a pair can be detected by mass spectrometry (MS), isotopic labeling is unnecessary when the assays are done in parallel.

The workings of the relevant MS techniques in general and in a biological context are described elsewhere (Matsuo *et al.*, 1994; Dass, 2001). Generally, for the analysis of molecules in the molecular weight range of the gluco-tetrasaccharides and glucose enantiomers used here (180 and ~700 daltons, respectively), electrospray ionization (ESI) MS is appropriate (Matsuo *et al.*, 1994). Matrix-assisted laser desorption ionization is applicable for the analysis of molecules in the 700 dalton range as well as for larger biomolecules such as peptides and oligonucleotides. Here, we present findings obtained via the ESI technique.

2. Materials and Methods

D-glucose and L-glucose were purchased from Omicron Biochemicals, Inc. The two tetra-saccharides, methyl- $[\beta$ -D-glucopyranosyl-(1-4)]3-5- β -D-glucopyranoside and methyl- $[\beta$ -L-glucopyranosyl-(1-4)]3-5- β -L-glucopyranoside (Fig. 1) were custom synthesized by, and purchased from, Omicron. In anticipation of the possibility that some D- and L-enantiomer pairs may have been mixed together in the same test solution yet could still be distinguished from one another when MS was used to determine whether chemical modification had taken place, the L-glucose carried a ¹³C atom, which gave it a molecular weight of 181, compared to 180 for the D-compound. On the D-tetrasaccharide, each glucose building block was labeled with one ¹³C atom, which gave the tetrasaccharide a

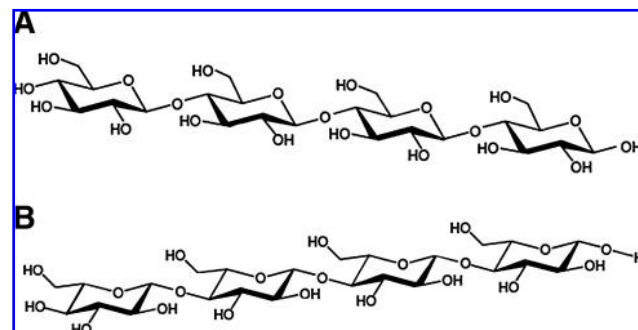


FIG. 1. Tetrasaccharide pair: (A) methyl- $[\beta$ -D-glucopyranosyl-(1-4)]3-5- β -D-glucopyranoside and (B) methyl- $[\beta$ -L-glucopyranosyl-(1-4)]3-5- β -L-glucopyranoside.

molecular weight 4 daltons higher than the molecular weight of the L-compound.

Terrestrial soil was taken from the grounds of the University of Houston, in Houston, Texas. Dr. Carl Allen of NASA's Johnson Space Center, Houston, Texas, kindly provided JSC Mars-1 regolith simulant, which was derived from Hawaiian volcanic ash (Allen *et al.*, 1997). The simulant has been determined to contain a biomass on the order of a tenth that of most common soils (Allen *et al.*, 2000).

For studies involving the glucose pair and the tetrasaccharide pair, the following sets of samples were used in triplicate: 0.25 g/ml, 0.50 g/ml, 1.0 g/ml, 1.5 g/ml of soil in water (solvent used with tetrasaccharide pair) or phosphate-buffered saline (PBS) (solvent used with the glucose pair); 0.5 g/ml, 1.5 g/ml, and 2.5 g/ml of Mars-1 regolith simulant in water or PBS; water-only control; PBS-only control. Beyond 2.5 g/ml, solvent became saturated with JSC Mars-1.

Samples were agitated in water for a minute, stirred slowly for 2 hours, and then centrifuged at low speed (272 g) to separate liquid from soil or JSC Mars-1. The extracts and controls were mixed in 1:1 ratio with aqueous solutions of each compound and then incubated for 14 days at varying temperatures, beginning at 10°C and rising to 32°C by the end of the incubation period.

In the case of the glucose pair, the technique was also tested with *E. coli* K-12 (multiple concentrations, ranging from 10⁹ cells/ml down to 10⁵ cells/ml) and *S. cerevisiae* (10⁷ cells/ml) suspensions in place of the environmental extracts. Post-incubation, samples were centrifuged to remove macroscopic particles and biological cultures, if present. Supernatants were analyzed by ESI MS.

All ESI MS experiments were performed on a LCQ Deca XP Plus ion trap mass spectrometer (Thermo Electron Corporation, San Jose, CA) with an ESI ion source. The samples

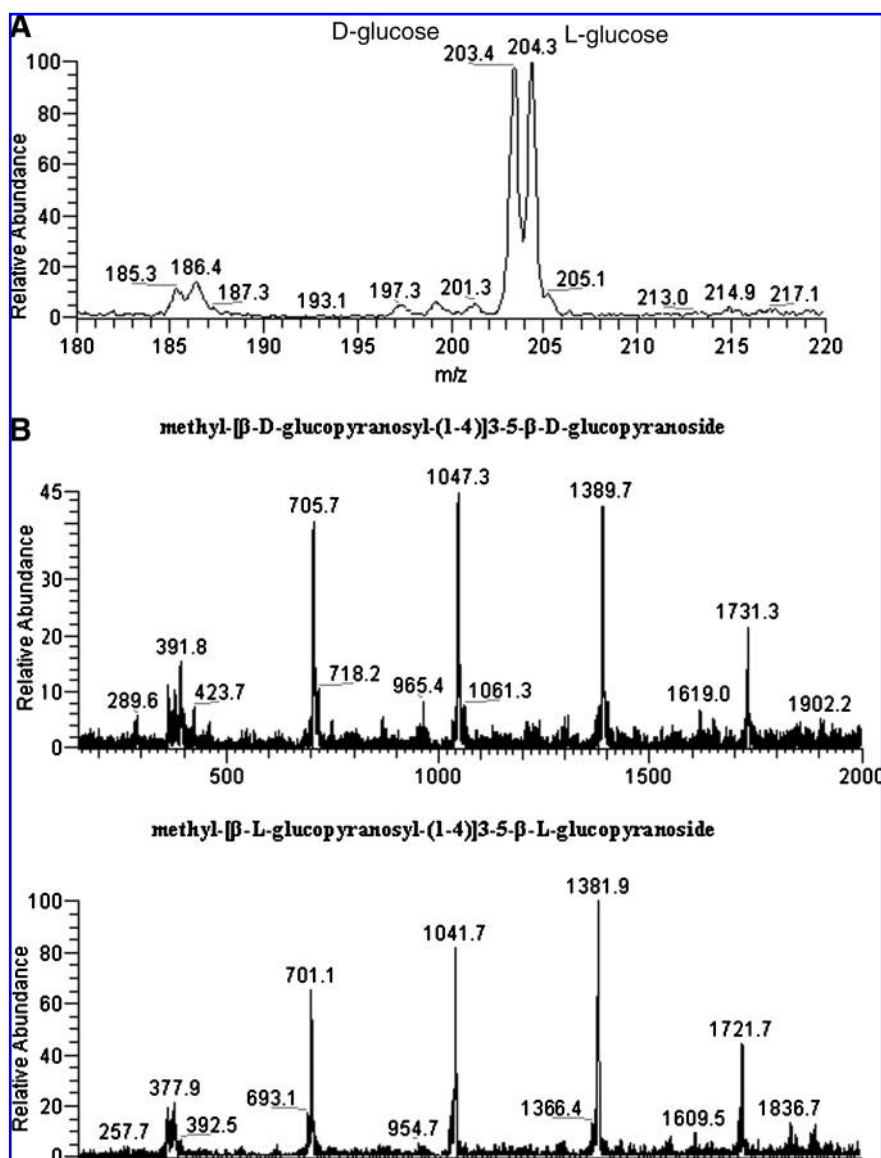


FIG. 2. (A) Peaks for D- and L-glucose in PBS. (B) Peaks of methyl- β -D-glucopyranosyl-(1-4)]3-5- β -D-glucopyranoside and methyl- β -L-glucopyranosyl-(1-4)]3-5- β -L-glucopyranoside dissolved in water.

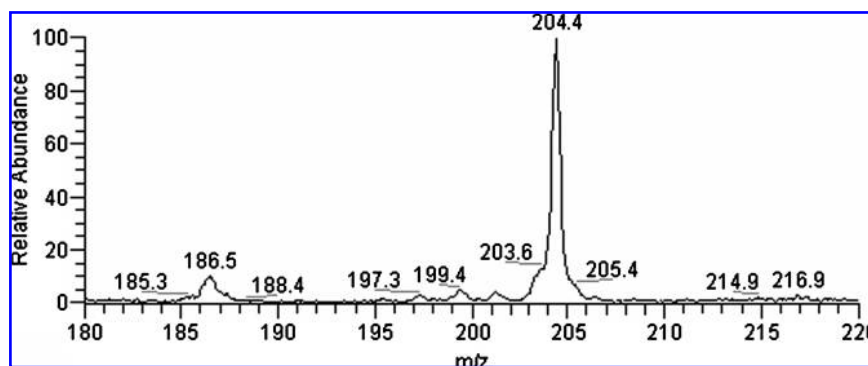


FIG. 3. Peaks of D-glucose (203) and L-glucose (204) in PBS following incubation with soil extract made from the lowest concentration of soil (5 ml soil in 30 ml PBS). D-glucose peak was absent or nearly absent among triplicate samples using extracts made from the entire range of soil concentrations.

TABLE 1. SUMMARIZED RESULTS OF ESI MS TESTING OF SAMPLES FOLLOWING INCUBATION WITH ENANTIOMER PAIRS

Substrate	Condition	Concentration	Peak present
D-glucose	<i>E. coli</i>	$\leq 10^6$ cells/ml	X
	<i>E. coli</i>	$\geq 10^7$ cells/ml	
	<i>S. cerevisiae</i>	10^7 cells/ml	
	Soil	5 ml soil/30 ml PBS	
	Soil	10 ml soil/30 ml PBS	
	Soil	20 ml soil/30 ml PBS	
	Soil	30 ml soil/30 ml PBS	
	JSC Mars-1 regolith simulant	10 regolith/30 ml PBS	X
	JSC Mars-1 regolith simulant	30 regolith/30 ml PBS	X
	JSC Mars-1 regolith simulant	50 regolith/30 ml PBS	
Control (PBS only)		X	
L-glucose	<i>E. coli</i>	All concentrations	X
	<i>S. cerevisiae</i>	10^7 cells/ml	X
	Soil	5 ml soil/30 ml PBS	X
	Soil	10 ml soil/30 ml PBS	X
	Soil	20 ml soil/30 ml PBS	X
	Soil	30 ml soil/30 ml PBS	X
	JSC Mars-1 regolith simulant	10 regolith/30 ml PBS	X
	JSC Mars-1 regolith simulant	30 regolith/30 ml PBS	X
	JSC Mars-1 regolith simulant	50 regolith/30 ml PBS	X
	Control (PBS only)		X
methyl- $[\beta$ -D-glucopyranosyl-(1-4)]3-5- β -D-glucopyranoside	Soil	5 ml soil/30 ml PBS	
	Soil	10 ml soil/30 ml PBS	
	Soil	20 ml soil/30 ml PBS	
	Soil	30 ml soil/30 ml PBS	
	JSC Mars-1 regolith simulant	10 regolith/30 ml PBS	X
	JSC Mars-1 regolith simulant	30 regolith/30 ml PBS	X
	JSC Mars-1 regolith simulant	50 regolith/30 ml PBS	X
	Control (PBS only)		X
methyl- $[\beta$ -L-glucopyranosyl-(1-4)]3-5- β -L-glucopyranoside	Soil	5 ml soil/30 ml PBS	X
	Soil	10 ml soil/30 ml PBS	X
	Soil	20 ml soil/30 ml PBS	X
	Soil	30 ml soil/30 ml PBS	X
	JSC Mars-1 regolith simulant	10 regolith/30 ml PBS	X
	JSC Mars-1 regolith simulant	30 regolith/30 ml PBS	X
	JSC Mars-1 regolith simulant	50 regolith/30 ml PBS	X
	Control (PBS only)		X

were injected through direct injection valve and then carried to the ion source with the eluate of water and methanol at the ratio 4:1 (volume per volume) and 200 $\mu\text{l}/\text{min}$. The source conditions were spray voltage, 4.5 kV; capillary temperature, 220°C; capillary voltage, 42 V; and tube lens offset voltage, 20 V.

3. Results

3.1. Control samples

Electrospray ionization MS peaks were consistent among members of the triplicate pair of control samples for each compound. Figure 2 shows the ESI MS peaks of one sample of D- and L-glucose in PBS and peaks of one sample of methyl- $[\beta\text{-D-glucopyranosyl-(1-4)}]3\text{-5-}\beta\text{-D-glucopyranoside}$ and methyl- $[\beta\text{-L-glucopyranosyl-(1-4)}]3\text{-5-}\beta\text{-L-glucopyranoside}$ dissolved in water. Because the D- and L-glucoses are monomers and differ in molecular weight due to the ^{13}C label on the L-compound, it was possible to dissolve them together and see each as a distinct peak on ESI MS as their molecular weights combined with the molecular weight of the sodium ion. Although the D-tetrasaccharide was synthesized with a ^{13}C on each monomer so as to be 4 daltons heavier than its L-counterpart, the two tetrasaccharides yielded mass-to-charge ratios (m/z) intermediate of the two compounds when mixed in the same sample. Thus, as seen in Fig. 2, the tetrasaccharide ex-

periments were carried out with each D- and L-enantiomer separated. In the case of the tetrasaccharides, in addition to the peaks corresponding to the compounds' molecular weights, other prominent peaks resulted from breakdown of the tetramers, regrouping of building blocks, and multiple charges during the ESI MS process.

3.2. Incubated samples

3.2.1. Glucose monomers. Incubation of D- and L-glucose with *S. cerevisiae* suspensions, with *E. coli* suspensions of 1×10^7 and higher cells/ml (but not with suspensions of 1×10^6 and lower cells/ml), with all concentrations of aqueous extracts of common soil, and with aqueous extracts of JSC Mars-1 regolith simulant in the highest concentration, yielded peaks markedly different for the D-compound compared to control samples. In contrast, the L-compound, in each case, was unaffected following incubation with soil extracts. In this study, we defined a positive datum as the disappearance of an enantiomer peak or the lowering of a peak to one half or less of the amplitude of the control samples, following incubation with test extracts or biological suspensions. Figure 3 shows a typical positive datum for glucose. In this case, the D- and L-compounds were mixed together and incubated with the test sample. The glucose results are summarized in Table 1.

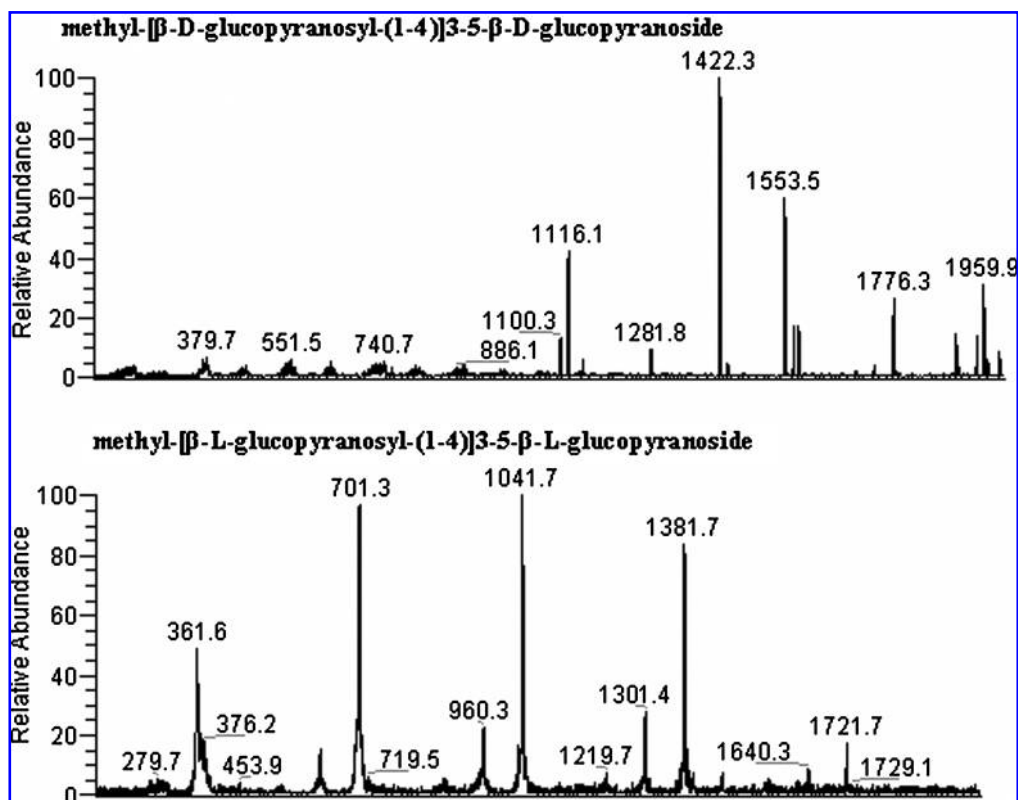


FIG. 4. Peaks of methyl- $[\beta\text{-D-glucopyranosyl-(1-4)}]3\text{-5-}\beta\text{-D-glucopyranoside}$ and methyl- $[\beta\text{-L-glucopyranosyl-(1-4)}]3\text{-5-}\beta\text{-L-glucopyranoside}$ following incubation with extract of common soil. The spectrum of the D-compound has been changed noticeably compared to control samples (see Fig. 2), with peaks at 705 and 1047 disappearing. The spectrum of the L-compound was unaffected. This was the lowest soil concentration tested; all other soil concentrations yielded results with these two peaks missing for the D-compound (see Table 1).

3.2.2. Tetrasaccharide compounds. Tetrasaccharides incubated with extracts of common soil, in all concentrations used, yielded peaks markedly different for the D-compound compared to control samples. The L-compound was unaffected. Figure 4 shows a typical positive datum for the tetrasaccharide pair. The D- and L-compounds were separately incubated with the extracts. Tetrasaccharides incubated with extracts of JSC Mars-1 regolith simulant yielded peaks that were similar to those yielded by control samples and exhibited no differences between results of the D- and L-compound. The results for all samples are summarized in Table 1.

4. Discussion

Sensitivity of the glucose pair to incubation with extracts made from JSC Mars-1 regolith simulant in PBS at 2.5 g/ml (but not to extracts made from lesser concentrations, *i.e.*, 1.5 and 0.5 g/ml) and reaction with extracts made from one tenth that concentration of common soil (0.25 g/ml) indicate that the technique is, as expected, sensitive to biomass levels. JSC Mars-1 simulant contains a biomass equivalent to 10^6 to 10^7 cells/gram, which is approximately 1 to 2 orders of magnitude below that of common soil (Allen *et al.*, 2000). Consequently, in the case of soil and regolith simulant, the effective sensitivity of the assay, with use of the glucose pair and the techniques employed for this study, lies between 1.5×10^6 cells/ml at best and 2.5×10^7 cells/ml at worst. The lower limit of detection for *E. coli* suspensions was determined to be between 1.0×10^6 and 1.0×10^7 cells/ml. This supports the finding and, furthermore, suggests that simple aqueous extraction is adequate for exposing cells from both soil and JSC Mars-1 material to the assay agents. Additionally, the detection of *S. cerevisiae* at 1.0×10^7 cells/ml lies within the effective sensitivity range found for the glucose pair.

Since the assay using the D- and L-tetrasaccharides detected life in common soil and not in JSC Mars-1, and since biomass in common soil can lie between 10^8 and 10^9 cells/gram (Prescott *et al.*, 1993), the effective sensitivity of the tetrasaccharide pair assay in this study lies between 10^7 (the upper limit of the JSC Mars-1 biomass equivalent, stated above) and 10^9 cells/gram.

These results are particularly relevant to the search for life on Mars. An earlier study showed that, to produce a positive result in the Viking GCMS, the amount of organic material in the regolith tested would have to be the equivalent of at least 3.0×10^7 *E. coli*-sized cells per gram of regolith (Glavin *et al.*, 2001). Since the Viking GCMS found no organic material in the martian regolith (Biemann *et al.*, 1977), the actual levels had to be less than that. If the actual Mars regolith suspends in aqueous solvents, similarly to the simulant, then the upper limit of organic carbon would be approximately 2.5 g/ml. This means that a regolith containing the organic matter equivalent of up to 7.5×10^7 *E. coli*-sized cells would not have provoked a positive result on the Viking GCMS. With a sensitivity of 2.5×10^7 cells/ml, the glucose pair assay, via the techniques employed in this study, could detect life at concentrations lower than the effective detection limit for organic matter of the Viking GCMS. Moreover, given that the biomass in our JSC Mars-1 simulant may in fact be as low as $\times 10^6$ cells/gram, biomass as low as 1/75 the detection limit of

the Viking GCMS could be detected by our assay with no conflict with the negative Viking GCMS results.

5. Conclusions

Ultimately, the goal is to develop a life-detection instrument that employs several nutrient pairs, such that asymmetric response of one or more of the pairs would be an indication of biological activity. When configured for use on Mars, the instrument would likely be associated with a deep-drilling lander. This would allow samples to be obtained from the subsurface, where possible life-forms would avoid the hostile surface environment. Multiple enantiomeric pairs are essential because sensitivity will be different between pairs. One cannot assume that substances that are readily metabolized by Earth life will necessarily be the preferred substrates of martian life as well. Thus, while the findings presented here suggest that the glucose pair may be a more sensitive life-detection tool than the tetrasaccharide pair, this might not be the case with actual martian organisms. Furthermore, it would be useful to develop additional enantiomer pairs such as oligonucleotides and peptides. The use of mass spectrometry in the analysis offers the additional advantage that it will be possible to incubate simultaneously each sample with multiple compounds, as the compounds' individual spectra will be distinctly different. If isotopic labeling is used as well, it will be possible in some cases to incubate single samples with both members of the same enantiomer pair.

6. Acknowledgments

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7. Abbreviations

ESI, electrospray ionization; LR, labeled release; MS, mass spectrometry; *m/z*, mass-to-charge ratio; PBS, phosphate-buffered saline; VL1, Viking 1 lander; VL2, Viking 2 lander.

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