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Bacterial survival following shock compression in the GigaPascal range

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ABSTRACT

The possibility that life can exist within previously unconsidered habitats is causing us to expand our understanding of potential planetary biospheres. Significant populations of living organisms have been identified at depths extending up to several km below the Earth's surface; whereas laboratory experiments have shown that microbial species can survive following exposure to GigaPascal (GPa) pressures. Understanding the degree to which simple organisms such as microbes survive such extreme pressurization under static compression conditions is being actively investigated. The survival of bacteria under dynamic shock compression is also of interest. Such studies are being partly driven to test the hypothesis of potential transport of biological organisms between planetary systems. Shock compression is also of interest for the potential modification and sterilization of foodstuffs and agricultural products. Here we report the survival of *Shewanella oneidensis* bacteria exposed to dynamic (shock) compression. The samples examined included: (a) a "wild type" (WT) strain and (b) a "pressure adapted" (PA) population obtained by culturing survivors from static compression experiments to 750 MPa. Following exposure to peak shock pressures of 1.5 and 2.5 GPa the proportion of survivors was established as the number of colony forming units (CFU) present after recovery to ambient conditions. The data were compared with previous results in which the same bacterial samples were exposed to static pressurization to the same pressures, for 15 minutes each. The results indicate that shock compression leads to survival of a significantly greater proportion of both WT and PA organisms. The significantly shorter duration of the pressure pulse during the shock experiments (2–3 μs) likely contributes to the increased survival of the microbial species. One reason for this can involve the crossover from deformable to rigid solid-like mechanical relaxational behavior that occurs for bacterial cell walls on the order of seconds in the time-dependent strain rate.

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1. Introduction

Life on Earth is traditionally considered to occupy a relatively narrow range of pressure (P-) and temperature (T-) conditions at or near the surface of our planet. However, sampling expeditions have demonstrated that life can exist under deep subsurface conditions, extending to several km below the oceanic and continental crust (Daly et al., 2016; Huber 2015; Inagaki et al., 2015; Anderson et al., 2013, Borgonie et al., 2011, Colwell and D'Hondt, 2013, Meersman et al., 2013; Picard and Daniel, 2013, Oger and Jebbar, 2010; Ono et al., 2010). It has also been suggested that the origins of life might lie at depth, associated with submarine volcanic activity (Lane and Martin, 2012). Laboratory studies have also demonstrated that microbes can survive even more extreme pressures extending to within the GigaPascal (GPa) range (Hazael et al., 2014; Kish et al., 2012; Griffin et al., 2012; Vanlint et al., 2011; Sharma et al., 2002), raising the possibility that organisms might exist within the deep interiors of colder planetary systems (Hazael et al., 2016; Vance et al., 2016). In addition to their relevance for Earth and planetary biology, studies of the survival of organisms have been conducted for the food industries, where the techniques of "Pascalization" *vs* "Pasteurization" can be applied to remove unwanted pathogens while maintaining color, texture, flavor and nutritional value (Demazeau and Rivalain, 2011).

Most investigations of microbial survival under extreme high pressure conditions have been conducted using static compression techniques, where the microbes are typically exposed to the pressure stress on timescales ranging from minutes to hours. However,

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other studies have focused on dynamic shock compression, where the pressure is applied as a pulse rising to a peak value on a much shorter timescale, on the order of tens of nanoseconds (ns), and is maintained within the sample for a few microseconds (μs), for example. Such studies are relevant to the possibility that organisms might have been transported between planetary bodies, giving rise to the potential phenomenon of "panspermia" (Melosh, 1988). That hypothesis presupposes that bacteria or other primitive life forms could survive the extreme environments of space trapped inside cometary or meteoritic bodies and then be delivered intact to the early Earth during an impact event (Howard et al., 2013; Paulino-Lima et al., 2010; Fajardo-Cavazos et al., 2009; Willis et al., 2006). Several pioneering studies have now investigated the survival of living microorganisms during the transient high-P,T conditions encountered during shock compression (Gruzielanek et al., 2010; Hazell et al., 2010; Horneck et al., 2008; Burchell et al., 2004; Burchell et al., 2001). These experiments have been conducted using light gas guns (Burchell et al., 1999) on various broths, spores and bacterial organisms to achieve peak pressures between 1–8 GPa (Price et al., 2013; Hazell et al., 2010; Hazell et al., 2009; Burchell et al., 2004; Burchell et al., 2001). Reported proportions of surviving colony-forming units (CFU) have been remarkably high (Fajardo-Cavazos et al., 2009), with survivors recorded following exposure to peak shock pressures as high as 78 GPa (Burchell et al., 2004).

Here we report results of the effects of dynamic shock compression on the survival of samples of *Shewanella oneidensis* following exposure to peak pressures of 1.5 and 2.5 GPa, using a target assembly designed to facilitate recovery of the bacterial cells, and also to maintain the temperatures developed during the shock compression as low as possible. The experiments were carried out using a light gas gun apparatus at the Shrivenham campus of Cranfield University, U.K., using bacterial strains developed at University College London (UCL). Previously we had investigated colony formation among survivor populations of this organism following static pressurization to pressures extending up to 2.5 GPa using a piston cylinder apparatus at UCL (Hazael et al., 2014). In our initial experiments in that work, colonies of bacteria were raised directly to the target pressure, retained at that value for 15 minutes, and then returned to ambient conditions for examination of the survival statistics. In further series of runs, bacteria were sequentially exposed to successively higher pressures, in pressure increments of 250 MPa. The survivors from each compression experiment were cultured and used to provide feedstock for the subsequent treatments at progressively higher pressures, resulting in increased survival rates for the "pressure adapted" (PA) or more pressure resistant members of the population. A similar protocol had been previously described in our work on *E. coli* by Vanlint et al. (2011). For the present shock compression study, we compared survival results for wild type (WT) and PA examples of *S. oneidensis,* shocked to peak pressures of 1.5 and 2.5 GPa. The PA samples had been developed from survivors that had previously been compressed to 750 MPa, following prior culturing of survivor populations at 250 and 500 MPa (Hazael et al., 2014). In this way we could directly compare the survival rates obtained in the shock compression study with the previous static compression results, for both WT and PA bacterial samples. The results provide new information about the bacterial response to dynamic *vs* static compression.

2. Materials and methods

Shewanella oneidensis MR-1 (CIP 106,686) was purchased from the Collection Institut Pasteur (Paris, France) (Venkateswaran et al., 1999) and samples were rehydrated in 200 µl of Luria-Bertani Miller (LB) medium. From this stock, 50 µl was used for a liquid culture in 10 ml of LB broth grown at 30 °C and 180 rpm, and two separate plate spreads of 50 µl provided stock solutions. For each experiment a 10 ml starter culture was inoculated either from plate or liquid stock. The bacteria were harvested in stationary phase at a concentration of 1×10^8 cells/ml. For each experiment a 1 ml aliquot of the starter culture was washed three times with phosphate buffered saline (PBS) solution adjusted to pH 7.2 to remove damaged and dead cells. The cells were then re-suspended in PBS for the experiments. These samples constituted the "wild type" (WT) specimens used in both the static and shock compression experiments.

For the static compression experiments described previously (Hazael et al., 2014), a Teflon® capsule was loaded with 6 µl of the bacterial suspension. An aliquot of this solution was plated to serve as a control sample. All microbiological preparations and sample handling were carried out under aseptic conditions. Compression experiments were carried out in a stepwise manner in a piston cylinder device, to reach final pressures of 1.5 and 2.5 GPa as reported in the previous publication (Hazael et al., 2014). Those results are quoted here to provide comparison points with the present shock compression data. In order to prepare "pressure adapted" (PA) samples for the shock compression runs, bacterial samples were exposed to static high pressures in 250 MPa steps up to 750 MPa, with survivors from each intermediate step recovered and cultured before being exposed to the next highest pressure. This generated the PA strain of *S. oneidensis* bacteria used in the shock compression runs (Hazael et al., 2014).

For shock experiments, the bacterial samples were contained within a Teflon® lined capsule placed inside a specially designed target assembly in order to carry out low velocity shock loading and recovery experiments (Leighs et al., 2012) (Fig. 1). The introduction of a Teflon® sleeve reduced pressure and temperature hotspots and aided uniform pressure wave generation within the sample. The shock studies were carried out using a 5 m length, 50 mm bore single stage gas gun to accelerate 5 mm thick Al flyer plates, with the final velocity measured just prior to impact. Measured impact velocities were 273 and 360 m/s leading to peak pressures of 1.5 and 2.5 GPa, respectively. While we were able to control the capsule system and the mass of our projectile, the fact that we relied on a release of gas to drive a piston into the projectile meant there could be some variation in impact velocity. Despite these slight variations in velocity, the overall effect on pressure was deemed negligible, according to results obtained using the hydrocode models. These peak pressures were calculated using ANSYS® Autodyn® (Autodyn 2012; Robertson et al., 1994), using the compressibility factor for pure water (45.8×10^{-11} Pa^{-1}) to model the compressional behavior of the bacterial suspensions (Table 1; Fig. 2). The validity of this assumption was tested by two impact experiments where the rear free surface of (a) water and (b) bacterial solution contained within identical capsules was monitored *via* heterodyne velocimetry (Het-V). This powerful technique uses Doppler shifted light reflected from the moving end of the target during the shock experiment to determine the particle velocity (u_P) as a function of the progress of the shock wave through the sample (Strand et al., 2006) (Fig 1). The Het-V traces for the bacterial suspension and pure H_2O were indistinguishable, indicating that our use of the water compressibility factor gives reliable results for the pressure and temperature profiles simulated using ANSYS® Autodyn® codes during the dynamic compression runs.

The designed target configuration led to a complex ramped loading path lying between the principal Hugoniot and the isentrope, yielding final state temperatures of 322 and 328 K, for samples shocked to 1.5 and 2.5 GPa respectively, determined by the simulations (Fig. 2; Table 1). We tested our simulation models against the plate impact studies of pure H_2O by Nagayama et al. (2002), using the target configurations and material parameters

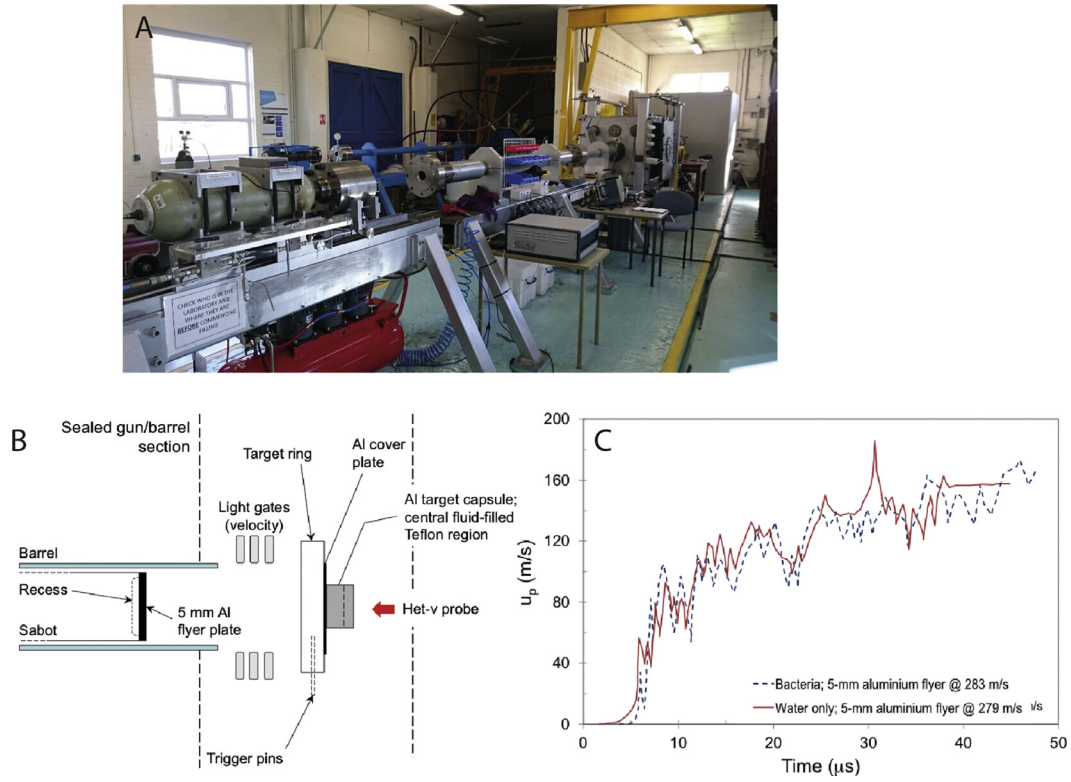

Fig. 1. Experimental details for the shock experiments. A. Photograph of the single stage gas gun and shock laboratory at Cranfield University. The sample target and recovery chamber is shown at the far end of the laboratory. The recovery chamber is packed with rags to ensure a "soft landing" for the target capsule containing the sample following the shock experiment. B. A schematic drawing of the target and flyer plate assembly used in these shock studies. Material parameters for the various components and used in ANSYS® Autodyn® simulations are provided in Table 1. C. Het-V traces comparing the evolution of the particle velocity, u_P *vs* time, for pure H_2O with that of a bacterial suspension. Both were impacted at 280 m/s to achieve a peak shock pressure of 1.5 GPa. The two systems show identical behavior with u_p asymptotically approaching a plateau near 150 m/s after approximately 25–30 μs.

Table 1
Materials and material properties used in the ANSYS® Autodyn® simulations. Impact velocities used were 273 and 360 ms^{-1} and achieved pressures of 1.5 and 2.5 GPa, respectively. Al 6061-T6 refers to an Al alloy with the highest tensile strength of the 6061 series of at least 290 MPa. [1]Taken from the Los Alamos Scientific Laboratory, Selected Hugoniots. LA-4167-MS, May 1969.

Material	Material properties					
	Density (g cm^{-3})	Strength model	Gruneisen coefficient	Thermal conductivity (J m^{-1} K^{-1} s^{-1})	Specific heat capacity (J kg^{-1} K^{-1})	Equation of State
Al 6061-T6	2.703	Steinberg-Guinan	1.97	247	885	Steinberg (1991)
Water	1.0	N/A	0.28	0.609	4.181×10^3	Nagayama et al. (2002)
Rubber	1.439	N/A	1.39	0.19	1.05×10^3	LA-4167-MS, 19,69[1]
Teflon	2.16	von Mises	0.9	0.25	1.05×10^3	Matuska (1984)

reported by these authors. Both results were in excellent agreement (with standard errors $\leq 5\%$) leading to a high level of confidence in our modelling procedures. The low temperatures developed during the shock experiments meant that thermal resistance of the bacteria was not an issue.

3. Results

Shock compression studies were carried out for WT and PA bacterial populations to peak pressures of 1.5 and 2.5 GPa. The results are compared in Fig. 3 and Table 2.

Our data clearly show that significantly larger numbers of survivors leading to colony forming units (CFU/ml) are recovered following shock compression compared with static pressurization to the same pressures for both WT and PA samples (Fig. 3). The difference in behavior is particularly striking for the 2.5 GPa experiments. At 2.5 GPa there were no recorded survivors for the WT static compression experiment (Hazael et al., 2014). This is in direct contrast to the dynamic compression study where we now observe approximately 3×10^4 CFU/ml survivors for the same WT sample. At 1.5 GPa, slightly more than 10^3 CFU/ml survivors are recorded for the static experiment, but dynamic shock compression leads to approximately 3×10^5 CFU/ml viable survivors to be recovered. For the PA population, both static and dynamic shock compression to 1.5 GPa leads to similar survival statistics with $5–7 \times 10^5$ CFU/ml recorded following both types of pressurization experiment. However, at 2.5 GPa, static compression resulted in only $\sim 10^4$ CFU/ml survival, whereas dynamic compression yielded $> 10^6$ CFU/ml among the survivor population (Fig. 3, Table 2).

4. Discussion

The survival rate found here for *S. oneidensis* subjected to shock compression at 2.5 GPa peak pressure is lower, by 1–2

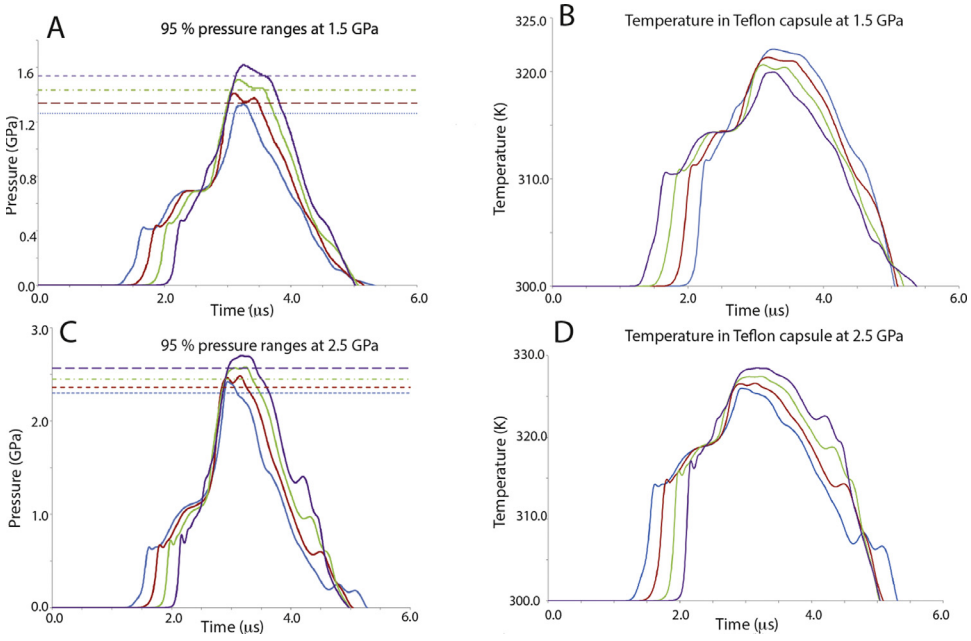

Fig. 2. Results of modelling experiments carried out to determine pressure and temperature conditions developed as a function of time during shock compression at 273 and 360 m/s using ANSYS® Autodyn® simulations. A. Calculated pressures developed within the sample as a function of time for a peak impact pressure of 1.5 GPa; B. Calculated temperatures developed within the sample at an impact pressure of 1.5 GPa; C. Calculated pressure-time trace for a shock with peak impact pressure of 2.5 GPa; D. Calculated T profile for an impact pressure of 2.5 GPa. Different coloured lines refer to different P,T profiles at different gauge points within the simulations, selected to estimate the range of P,T conditions developed at various points throughout the sample volume, and thus provide an estimate of the range of values that are expected to exist at various stages during the shock compression event.

Table 2
Results of bacterial survival expressed as the number of colony-forming units log (N) (as CFU/ml of suspended solution). All initial bacterial populations were 1×10^8 CFU/ml. [1]For the 1.5 GPa peak shock impacts a velocity matching technique was used to ensure identical peak pressures for both runs. For the 2.5 GPa shock runs, the flyer velocities varied slightly between different experiments.

	Static Compression			Shock Compression			
Sample	Pressure GPa	Survival CFU (N)	Log N (Average)	Peak Impact Pressure (GPa)	Flyer Velocity (ms^{-1})	Survival/ CFU (N)	Log (N)
WT Static	1.5	1.30, 1.32, 1.32×10^3	3.1				
WT Static	2.5	0, 0, 0	0				
PA Static	1.5	5, 6, 3×10^5	5.6				
PA Static	2.5	8,4,3 $\times 10^4$	4.1				
WT Shock				1.5 (+10.4/−9.4%)	273[1] (±1.7%)	3.14×10^5	5.4
WT vShock				2.5 (+6.1/−4.9%)	360 (±2.8%)	3.83×10^4	4.5
PA Shock Run 1				1.5 (+10.4/−9.4%)	273[1] (±1.7%)	6.6×10^5	5.8
PA Shock Run 2				1.5 (+10.4/−9.4%)	273[1] (±1.7%)	7.24×10^5	5.6
PA Shock Run 1				2.5 (+6.1/−4.9%)	354 (±1.7%)	1.93×10^6	6.2
PA Shock Run 2				2.5 (+6.1/−4.9%)	363 (±2.2%)	4.61×10^6	6.6

orders of magnitude, than that reported previously for a range of other organisms (Fajardo-Cavazos et al., 2009; Horneck et al., 2008; Burchell et al., 2004). However, several of those experiments used sporulating organisms, that can exhibit enhanced survival rates following exposure to applied mechanical stress (Fajardo-Cavazos et al., 2009; Horneck et al., 2008; Burchell et al., 2004). Burchell et al. (2004) examined an active sample of *Bacillus subtilis* as well as the non sporulating organism *Rhodococcus erythropolis,* and found greater survival rates for both samples than those found here for similar peak shock pressures. However, these authors noted that their experimental protocol might have produced uncertainties in the determined survival rates of up to 1–2 orders of magnitude, that could bring the 3 GPa data for *R. erythropolis* into general agreement with our present result for *S. oneidensis* at 2.5 GPa.

A main feature of our results reported here is that the PA population that had been cultured from survivors following previous exposure to progressively higher static pressures were more resistant than the WT species to dynamic compression, to higher peak shock pressures. That mimics the result found previously in our static pressurization experiments (Hazael et al., 2014), but the survival rates are considerably enhanced in the dynamic compression runs (Fig. 3, Table 2). In particular, bacterial survival following compression to 2.5 GPa is significantly greater in the shock experiments than found previously in static compression runs at the same pressure. We can examine some of the possible effects that could result in this markedly different behavior.

The different biochemical and microbiological factors affecting bacterial survival at high pressure are not yet understood (Meersman et al., 2013; Aertsen et al., 2004). Recent studies have suggested that the demise of microbes within the lower pressure range (up to 700–800 MPa) relevant to static compression protocols used in commercial Pascalization processes is related to formation, migration and expulsion of protein aggregates formed

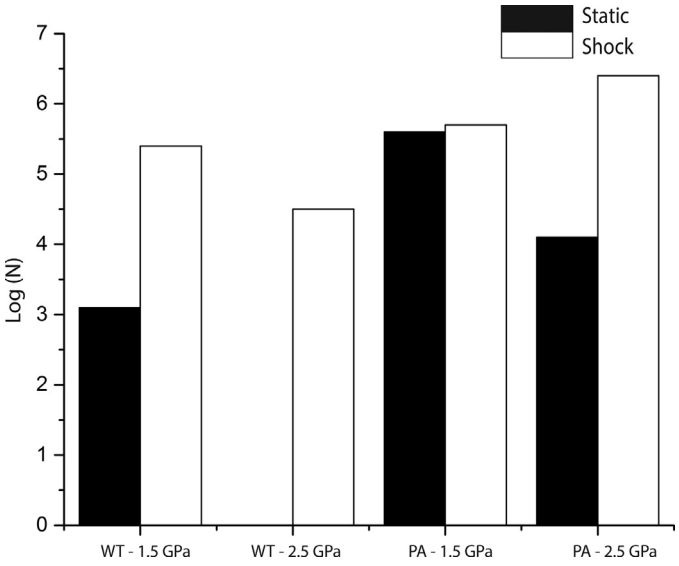

Fig. 3. Bar chart showing bacterial survival on a logarithmic scale (log (N)) with N as the number of colony forming units (CFU) per ml. These were established following recovery to ambient pressure relative to the initial concentrations (10^8 CFU/ml) for wild type (WT) and pressure adapted (PA) samples of *Shewanella oneidensis* following static (Hazael et al., 2014) *vs.* shock compression experiments (longer *vs.* shorted timescales). Note that no WT survivors could be cultured following static compression to 2.5 GPa (Hazael et al., 2014), although $\sim 3 \times 10^4$ CFU were counted following incubation of survivors following shock compression of the same WT sample to this peak pressure.

within the cells (Govers and Aertsen, 2015). However, the survival mechanisms that apply to bacteria exposed to pressures extending into the GPa range have not yet been examined in detail.

As a next step to begin to understand the differential effects of static *vs* shock pressurization on the bacterial survival, we should take account of the markedly different timescales of the static *vs* dynamic compression experiments, in relation to the mechanical and viscoelastic relaxation properties of the bacterial cell envelope. Understanding the mechanical behavior and time-dependent deformation behavior of living cells subjected to mechanical loading is becoming an important area in soft matter biophysics, with implications for medical and nanomaterials research (Bonakdar et al., 2016; Vadillo-Rodriguez and Dutcher, 2011, Fabry et al., 2001; Thwaites et al., 1991). Most living cells show a viscoelastic deformation response that follows a power law in time (Bonakdar et al., 2016; Fabry et al., 2001). Dynamic mechanical relaxation experiments and simulations carried out for bacteria indicate that the viscoelastic behaviour of the cell envelope passes from exhibiting a relaxed ("rubbery") response upon slower application of the mechanical stress to more solid-like ("glassy") behavior by increasing the speed of the applied stress, at a timescale of about ~1 s. During our dynamic compression experiments a planar shock wave was launched into the aqueous suspension medium with a peak pressure developing and persisting over a timescale of 2–3 μs (Fig. 2). That indicates that the cell walls of the *S. oneidensis* bacteria studied in our shock experiments should not deform elastically during passage of the shock wave, but instead behaved as a more rigid envelope. In that case, the biomolecular apparatus and fluids internal to the cells would not have experienced any significant effects due to compression, although protein complexes and other biomolecules located in the outer part of the membrane or external to the cell wall would be directly exposed to the shock compression conditions, and might be expected to have altered structures and functionality. On the other hand, the external cell wall could experience rupture due to the applied stress exceeding the fracture tolerance limit. Experiments have indicated that the tensile strength of bacteria is approximately 300 MPa with a Young's modulus on the order of 13 GPa (Thwaites et al., 1991). We note that the PA populations appear to have altered characteristics, including the external shape and size of the bacteria (R. Hazael, P.F McMillan et al, in prep). Those changes could indicate that the process of selection among the WT population implied by the progressive pressurization-resuscitation-culturing steps carried out as part of our static compression protocols to achieve the PA samples studied here might have an altered outer envelope structure, with enhanced pressure-resistant mechanical properties.

We must also examine the possible effects of crystallization to form ice crystals within the aqueous suspension medium or inside the bacteria themselves that might damage the cell walls and result in non-viability. In addition, the crystallization phase boundaries in the system might be altered by the presence of dissolved salts, that might also change the ionic strength as crystals of pure H_2O appear. The H_2O phase diagram shows that the high pressure crystalline phases ice VI followed by ice VII become stabilized at 1.5 and 2.5 GPa, respectively, at temperatures within the 310–330 K range achieved here. Dynamic compression experiments along the principal Hugoniot show that the P,T path lies close to the ice crystallization boundary (Nagayama et al., 2002). In our studies, the compression followed a complex dynamic loading path between the Hugoniot and isentrope, leading to lower temperatures achieved at 1.5 and 2.5 GPa peak pressures. The formation of crystalline ice phases from liquid H_2O is typically considered to be a slow process during shock events, however ramp compression studies have indicated a much faster nucleation rate as the loading conditions approach the isentrope (Dolan et al., 2007). It is possible if not likely that crystals of ice VI and/or ice VII nucleated within the aqueous suspension medium. In our static compression experiments, no WT survivors were recorded at 2.5 GPa that lies within the ice VII phase field at room temperature, whereas $\sim 1.3 \times 10^3$ survivors (a approximately 0.001% survival rate) were observed at 1.5 GPa, where ice VI would have been present during the high pressure run (Hazael et al., 2014). However, the PA specimen exhibited 10^4–10^6 CFU/ml survivors following compression to both pressures, making it unlikely that physical damage to the bacterial cell walls could have limited survival, unless the PA samples had presented a strategy to resist mechanical rupture. During a static compression study carried out to 1.4 GPa in a diamond anvil cell, the aqueous medium surrounding the microbes was observed to solidify into ice VI. However, apparently intact bacteria continued to remain visible inside fluid inclusions as well as along grain boundaries between the crystals, and metabolic activity continued to be recorded (Sharma et al., 2002). In our piston cylinder compression studies, by 2.5 GPa no viable members of the WT population exhibited colony-forming behavior, however, a substantial number of survivors from the PA populations could be recovered and cultured at ambient pressure. The shock experiments showed a significantly increased survival rate for both WT and PA bacteria at 2.5 GPa compared with the static compression results; however, static and dynamic pressurization appeared to show comparable survival rates for the PA sample exposed at 1.5 GPa. This complex series of observations leads us to suggest that H_2O crystallization can not be the main effect causing the survival or demise of bacteria following exposure to high pressures in the GPa range.

Although we have established that WT bacteria are more sensitive to shock than are the specialized survivors within PA populations, it is not known why this occurs, or what the upper limits of bacterial survival might be following a dynamic compression event. That is likely to be set by the intrinsic mechanical resistance of the cell envelope to applied stress over a short timescale. Establishing those mechanical parameters should then help determine the ultimate survival of microbes and other organisms following a shock impact event.

The impact properties of meteorites on Earth, Martian and lunar surfaces are well known. Typical speeds of impactors are expected to lie in the range of km s^{-1} with peak impact pressures estimated to be on the order of several GPa (Beck et al., 2005), dependent upon the target material and the dimensions of the impacting body. The shock wave propagation velocities inside the impactor should remain on the order of μs or faster, so that any included organisms within the bolide (or impacted body) could exhibit a similar "glassy" cellular response to the applied dynamic stress conditions. The resistance of the cell envelope to maintain its integrity would then limit microbial survival. If the temperatures developed during a bolide impact event were to remain sufficiently low (El Goresy et al., 2001), then survival of bacteria in a live as well as a dormant state could be considered as a realistic possibility.

4. Conclusions

From our data we have shown that bacterial survival following shock compression is greatly increased over that found following static compression. Specifically, shock experiments at 2.5 GPa, for which no survival can be recorded for WT samples exposed to static pressurization, exhibit some survival following shock compression. The greatest number of survivors is recorded for PA species following shock *vs* static pressurization. These results shed new light on the survival mechanisms for microbes exposed to different dynamic *vs* static pressurization conditions, as well as demonstrating the potential survival of viable species following bolide impact events and transport between planetary systems.

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