SURVIVAL OF YEAST SPORES IN HYPERVELOCITY IMPACTS EVENTS UPTO VELOCITIES OF 7.4 KM SEC^{-1}. M. J. Burchell[1], C. Solscheid[2], M. C. Price[1], L. Josse[2] , N. Adamek[2] and M. J. Cole[1]. [1]School of Physical Sciences, University of Kent, Canterbury, Kent, CT2 7NH, UK (corresponding author: m.j.burchell@kent.ac.uk). [2]Dept. of Biosciences, University of Kent, Canterbury, Kent, CT2 7NJ, UK.

Introduction: Previous studies have been conducted to verify the survivability of living cells during hypervelocity (crudely > 1 km s^{-1}) impacts to test the hypotheses of panspermia and lithospermia, and it has been demonstrated that bacteria survive impacts up to 5.4 km s^{-1} – albeit with a low survivability probability [1] whilst larger more complex objects such as seeds break up at ~1 km s^{-1} [2]. Here we report on the survivability of an organism of intermediate complexity and size, namely yeast, at impact velocities up to 7.4 km s^{-1}. Spores from a yeast strain deficient in an enzyme required for uracil production were fired into water (to simulate oceanic impacts, as described in [3]) using a light gas gun (LGG) [4]. The water was then retrieved and filtered and the resulting filtrate smeared onto growth media to check the viability of any remnant spores.

Table 1. LGG shot parameters of shot programme

Shot ID	Intended velocity (km s^{-1})	Measured velocity (km s^{-1})
G291110#2	7.50	7.40
G151110#2	5.00	4.86
G151110#1	3.00	3.10
G161110#2	1.00	1.05
G081110#2†	1.00	1.04

†Shot performed using active growth yeast cells, not spores.

Preparation of yeast strain BY 4743[1]: The yeast strain BY 4743 [5] contains a homozygous deletion of the *URA3* gene encoding an enzyme required for the synthesis of pyrimidine ribonucleotides. The diploid strain contains two copies of the URA3 gene, whereas the Haploid spores contain only one copy. Due to the homozygous deletion in the parent strain none of the four spores formed can obtain the *URA3* gene. This gene loss (and with it loss of enzymatic activity) leads to an inability to grow on media lacking uracil or uridine, which can therefore be used as a selective tool to identify the yeast after it has been shot.

Growth Media: The standard medium used was the YEPD (full medium): 2% glucose, 1% yeast extract, 1% bacto peptone and 2% granulated agar (for solid media). In addition, a selective growth medium was also used, MM-URA (minimal medium): 2% glucose, 0.67% YNB, appropriate amino acid dropout mix and 2% granulated agar (for solid media).

[1] Genotype: *MAT*a/α *his3*Δ1/*his3*Δ1 *leu2*Δ0/*leu2*Δ0 *LYS2/lys2*Δ0 *met15*Δ0/*MET15 ura3*Δ0/*ura3*Δ0

Sporulation: The diploid Saccharomyces cerevisiae strain BY 4743 was grown overnight in agitated full (YEPD) liquid medium at 30˚C. The culture was then diluted 1:10 in 25ml YEPD with 5% glucose and grown to OD_{600} ~ 1.6 (approx. 10 hours) before being harvested by centrifugation at 3220g for 10 minutes. The harvested cell pellet was re-suspended in 5ml 1% Potassium Acetate (pH 7.0) and left to sporulate at 26˚C (in agitated liquid YEPD). Once visible under the light microscope (after ~9 days) the spores were harvested by centrifugation at 855g for 5 min. The pellet was then re-suspended in 25% glycerol, 0.5% potassium acetate for long term storage at -80˚C.

Methodology: Ten full media (YEPD) plates were prepared for each shot using a fresh batch of sterile Petri dishes. Approx. 3 ml of the chilled (-20° C) BY 4743 spores were placed onto one of the Petri dishes. A sterile, hollowed out sabot was placed over the top of the spores and pressure applied to fill the sabot with a layer of spores and growth media (Fig. 1). The filled sabot was immediately transferred to the LGG for firing. Table 1 gives details of the shot programme including the intended and measured impact velocity. Harvested colonies were labeled according to their intended impact velocity.

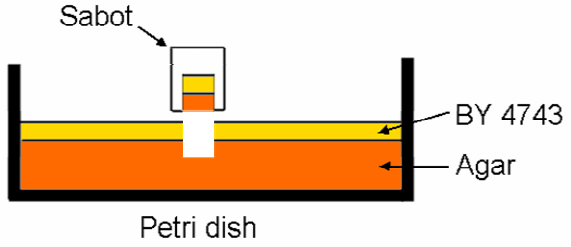

Fig. 1 Schematic showing loading of a sabot with yeast spores via a 'cookie cutter' method.

The target was a sterilised polythene bag (50 microns thick) filled with approximately 250 ml of HPLC grade water. The bag was mounted in a specially designed target holder previously autoclaved at 120 °C at 1 bar for 30 minutes prior to each shot. Sterilisation of the bag and surroundings was performed by soaking in isopropyl alcohol. Immediately after the shot, the target chamber was returned to atmospheric pressure and the target holder removed. The water remaining in the target holder was filtered through Whatman Grade 1 filter paper (also previously autoclaved). This filter paper was carefully unfolded and the filtrate dabbed onto two

Petri dishes. Two further Petri dishes were left untouched as control blanks to verify the sterlisation of the growth media. After each 'live' yeast shot, a control shot was also performed. The target (and holder) were prepared in an identical fashion to the yeast shots, but the projectile was a sterile, empty, hollowed out sabot. The recovered water was filtered and smeared onto two further Petri dishes in an identical manner to the live yeast shot.

Results: To test the viability of any collected spores from the water collected after impact, the filtrate was used to inoculate YEPD plates. These plates were then incubated at 30°C for several days until colonies became visible. The physiological characteristics of the cells were checked under a light microscope to check for the presence of Saccharomyces cerevisiae. Colonies were then transplanted onto YEPD, as well as MM – URA plates, alongside the parent strain. The plates were again incubated at 30°C for several days, and then checked for growth. As shown in Fig. 2 all strains grew well on full YEPD medium, illustrating the cells viability. Neither the parent strain, nor any of the strains collected after impact showed growth on the minimal media. These results lead to the conclusion that the strains collected were indeed produced by the spores used in the shots. The rate of growth and the appearance of the recovered yeast cells was not significantly different to that of the parent cells.

None of the ten control shot Petri dishes (two for each velocity) showed signs of yeast growth and thus we believe that spores are being delivered to the target holder via the shot sabot. All Petri dishes (except clean blanks) showed some bacterial (tentatively identified as *E-coli)* and/or (unknown) fungal contamination.

Survivability probability: No quantitative measurements have yet been performed on the survivability probability as a function of impact velocity for the yeast spores. A qualitative observation is that fewer yeast colonies were seen after the initial incubation period for the high velocity impacts (5 and 7.5 km s^{-1}), than for the lower velocity shots. This is not a very tight constraint as both the number of yeast spores delivered to the target, and the number subsequently recovered, is unknown. Work is currently in hand to quantify this and produce a survival rate.

Shock pressure experienced during impact: The approximate shock pressure, *P*, of the highest impact speed, *v* (7.4 km s^{-1}) was calculated using Eqn. (1) from [1] which allows for a finite projectile impacting a flat target:

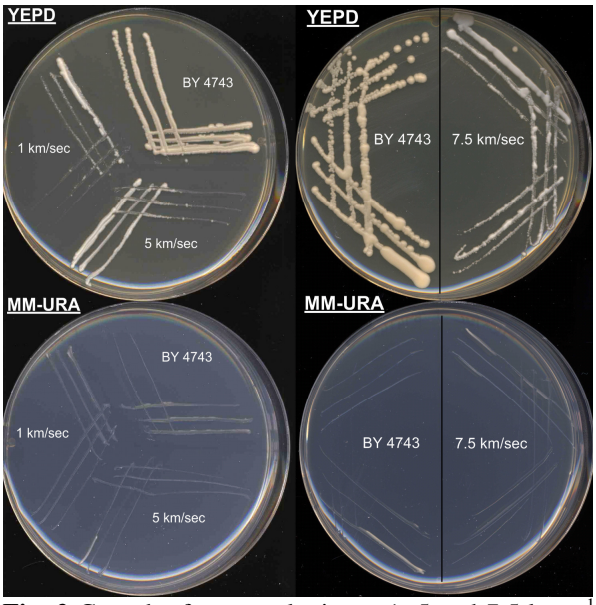

Fig. 2 Growth of yeast colonies at 1, 5 and 7.5 km s^{-1} on YEPD medium (top), and zero growth of parent or harvested cells on MM-URA growth medium (bottom).

$$P = \frac{mv}{2V_p}\left(C + \frac{sv}{2}\right), \qquad \text{Eqn. 1}$$

where V_p and m are the projectile volume (73.8 mm^3) and mass (0.08 g) respectively, C (1.48 km s^{-1}) and s (1.60) are the linear shock wave speed parameters for water [6]. This gives an approximate value for P of 30 GPa, a value similar to that calculated for shocked bacteria in [1].

Conclusions: We have extended the range of organisms that survive hypervelocity impacts at peak shock pressures of ~30 GPa to include an engineered strain of yeast. Other groups have reported elsewhere [7] that lichens can also survive shocks in similar pressure ranges. This demonstrates that not only bacteria, but more complicated (albeit microbial) life forms could survive the ejection and re-impact onto a planetary body such as Mars for example or survive ejection from Earth to the Moon [8].

References: [1] Burchell M. J. et. al. (2004) *MNRAS, 352*, 1273-1278. [2] Jerling A. et. al. (2008) *Int. J. Astrobiology, 7*, 217-222. [3] Milner D. J. et. al. (2006) *Int. J. Astrobiology, 5*, 261-267. [4] Burchell M. J. et al. (1999) *Meas. Sci & Tech.,* 10, 41 – 50. [5] Brachmann C. B. et. al. (1998) *Yeast, 14*(2); 115-132. [6] Melosh H. J., *Oxford Uni. Press,* 1989. [7] Horneck G., et al. (2008) *Astrobiology, 8*, 17 – 44. [8] Burchell M.J. et al. (2011) *Earth Moon and Planets*, in press, doi:10.1007/s11038-010-9360-5