Assessment of Gamma Radiation Resistance of Spores Isolated from the Spacecraft Assembly Facility during MSL Assembly

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Spore forming bacteria, a common inhabitant of spacecraft assembly facilities, are known to tolerate extreme environmental conditions such as radiation, desiccation, and high temperatures. Since the Viking era (early 1970's), spores have been utilized to assess the degree and level of microbiological contamination on spacecraft and their associated spacecraft assembly facilities. There is a growing concern that desiccation and extreme radiation resistant spore forming microorganisms associated with spacecraft surfaces can withstand space environmental conditions and subsequently proliferate on another solar body. Such forward contamination would certainly jeopardize future life detection or sample return technologies.

It is important to recognize that different classes of organisms are critical while calculating the probability of contamination, and methods must be devised to estimate their abundances. Microorganisms can be categorized based on radiation sensitivity as Type A, B, C, and D. Type C represents spores resistant to radiation (10% or greater survival above 0.8 Mrad gamma radiation). To address these questions we have purified 96 spore formers, isolated during planetary protection efforts of Mars Science Laboratory assembly for gamma radiation resistance. The spores purified and stored will be used to generate data that can be used further to model and predict the probability of forward contamination.

Introduction

Jupiter's moon Europa is widely regarded as the most promising extraterrestrial habitat for life in the solar system. This view is based on recent evidence suggesting the presence of a water ocean beneath Europa's fractured icy surface together with studies of microbial life in extreme environments on Earth, which suggest that living organisms emerge wherever liquid water and some form of usable energy are found. However, any spacecraft that transports scientific instruments can also carry terrestrial microbes (Space Studies Board 2000).

Since the 1970s, bacterial spores have been used as the primary indicator of the degree and level of contamination on a given spacecraft. Metabolically dormant, the spore is capable of surviving for extensive periods of time in the absence of nutrients, but is also geared to return to the vegetative state if nutrients become readily available (Driks 2002a). Several reports conclude that spores can survive for hundreds of years, and additional reports suggest that spores may survive for millions of years (Kennedy et al. 1994; Cano and Borucki 1995; Vreeland et al. 2000). To address these issues, planetary protection efforts are in place in order to analyze and reduce forward microbial contamination for spaceflight operations of the National Aeronautics and Space Administration (NASA).

In order to determine which microorganisms pose the highest threat of forward contamination, they can be categorized according to radiation sensitivity. A classification methodology has been developed to achieve this goal, resulting in 4 divisions: Types A, B, C, and D. Type A classification represents all microorganisms, regardless of any unique characteristics they may possess. Moreover, Type A is inclusive of all other classifications presented here. Type B classification represents microorganisms that are able to form spores and exhibit some degree of resistance to environmental pressures. Type C represents a population within Type B by discriminating for only microorganisms that exhibit 10% (1 log) or greater survival when exposed to 0.8 Mrad gamma (γ) radiation. Finally, Type D represents a sub-population of Type A, but is independent of Types B and C.

Type D is defined to include microorganisms that do not form spores, but have 10% or greater survival of vegetative cells after exposure to 8.0 Mrad γ-radiation (Space Studies Board 2000).

Although the mechanisms are not yet fully known, earlier studies have shown that spores exhibit a higher level of resistance to than vegetative cells to γ- radiation (Nicholson et al. 2000). This potential for spore resistance to γ-radiation generates concern regarding forward contamination (i.e. interplanetary transfer) via NASA spacecraft, thereby promoting research in identifying Type C microorganisms. Spores resistant to heat, desiccation, and radiation may also have a greater proclivity for surviving in extraterrestrial conditions. If such spores are capable of surviving cosmic exposure they could subsequently contaminate any planet that experiences a spacecraft landing. This forward contamination will severely endanger the investments and prospective success of life detection missions.

In this study, 96 isolates from planetary protection efforts on the Mars Science Laboratory (MSL) were examined for identification as Type C microorganisms. The conclusions drawn from this experiment will aid future planetary protection work and provide study subjects to better understand mechanisms for γ-radiation resistance.

Materials and Methods

Sample Source

Two polystyrene 96 well plates containing 192 MSL isolates in a glycerol stock were received from the University of Idaho. These isolates were renamed and catalogued into a system for the proposed Jupiter Europa Orbiter (JEO) project. Out of all the isolates, 108 were registered as known spore formers, and therefore Type B.

Preparation of Media

Tryptic soy broth (TSB) and tryptic soy agar (TSA) were prepared as per the standard recipes: 40 g of the respective nutrient powder per 1 L of distilled water (dH_2O). CryoVial® tubes were filled with 1.5 mL of TSA and allowed to solidify at room temperature. Additionally, a modified Schaeffer's medium (MSM) was prepared with divalent cations as described previously (Leighton & Doi,. 1971).

Cell Growth

From the 96 well plates, 100 µL of each glycerol stock isolate was inoculated into 5 mL of TSB in an individual 15 mL Falcon™ tube. Additionally, after inoculation, each pipet tip was stabbed into individual CryoVial® tubes with TSA. The 108 TSB cultures were incubated at 32°C and 120 revolutions per minute (RPM) for 24 hours, at which point turbid growth was observed. TSA stabs were incubated at room temperature for long term storage.

Sporulation

Upon achieving turbid growth, 1 mL of each TSB culture was inoculated into 5 mL of MSM in a 50 mL Falcon™ tube. Sporulation of vegetative cells was induced by limited nutrients and the presence of the divalent cations in the media. MSM cultures were incubated at 32°C and 120 RPM for 48 hours. TSB cultures were stored at 4°C.

After achieving turbid growth in 48 hour MSM cultures, spore presence was confirmed using phase contrast microscopy. Slides were prepared with 1 µL of culture sample into 5 µL of 50% molecular grade ethanol. Due to the presence and crystallization of dipicolinic acid (DPA) within the spore, spores appeared as bright bodies within vegetative cells in microscopy images.

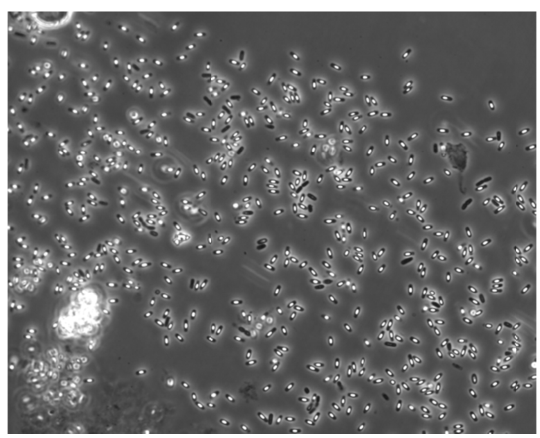

Figure 1. Vegetative cells harboring spores in the 48 hour MSM culture of sample JEO-009.

Of the 108 strains inoculated into MSM, 96 achieved 90% sporulation, and could therefore be purified.

Purification

Due to the large number of samples handled in this experiment, traditional spore purification methods would prove to be excessively time consuming and inefficient in relation to the amount of labor applied. Therefore, a fast but effective method was established for large scale purification. In order to lyse vegetative cells harboring spores, MSM cultures were heat shocked at 80°C for 15 minutes. After heat shock, and taking care to balance sample tubes, cultures were centrifuged at 5000 RPM for 5 minutes. Post centrifugation, the supernatant was decanted, leaving behind a pellet of spores. The pellet was re-suspended in 5 mL of 50% molecular grade ethanol and transferred to empty CryoVial® tubes. Lastly, 50 µL of re-suspended spores were inoculated in individual CryoBeads™ tubes and placed for long term storage at -80°C. Finally, an additional phase contrast microscopy was performed as previously, to confirm purification of spores.

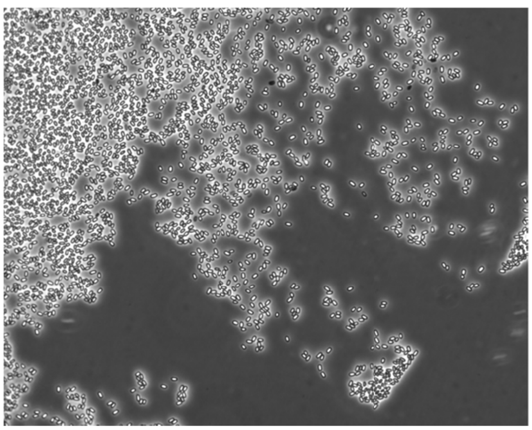

Figure 2. Purified spores of sample JEO-015.

Quantification of Spores

To effectively study survival, a known starting quantity of spores must be used, thereby necessitating the quantification of our spore suspensions. In order to do so, a large scale serial dilution was performed for each sample as follows:

In a CryoVial® tube, 10 µL of spore suspension was added to 990 µL of phosphate buffer solution (PBS), resulting in a 10^{-2} dilution. To a polystyrene 96 well plate containing 90 µL of TSB per well, add 10 µL of 10^{-2} diluted spores to the first well of each row (different sample for each row). Using a multi-channel pipet, transfer 10 µL from the first column of the plate to next, and continue for all columns. Thus, a dilution ranging from 10^{-3} to 10^{-14} is created for all 96 samples, in a total of twelve plates.

Incubate all plates at 32°C overnight. After incubation, growth can be measured by spectrophotometry at 605 nanometers. Growth continues until a dilution point is reached that contains no spores for germination. Using reverse calculation on the dilutions, an estimated log concentration of spores in the original suspension can be determined. A sample of the quantification is as follows:

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09
Sample	1	2	3	4	5	6	7
JEO-049	0.4828	0.8298	0.8076	0.8321	0.0384	0.0389	0.0387
JEO-050	0.3703	0.5719	0.4982	0.5855	0.4888	0.0391	0.0394
JEO-051	0.6995	0.6178	0.2838	0.3026	0.2958	0.3086	0.0381
JEO-052	0.6486	0.5706	0.5029	0.376	0.0383	0.0389	0.0389
JEO-053	0.3089	0.3212	0.3402	0.9219	0.822	0.5552	0.0398
JEO-054	0.9105	0.3286	0.4087	0.2871	0.6636	0.0389	0.039
JEO-055	0.4576	0.4024	0.3781	0.4498	0.0393	0.0389	0.0413
JEO-056	0.5031	0.3262	0.6896	0.3709	0.4307	0.6604	0.0378

Table 1. Spectrophotometric growth data of spores in TSB overnight after serial dilution for samples JEO-049 to JEO-056.

Sample Name	Concentration (spores/mL)
JEO-049	1.00E+06
JEO-050	1.00E+07
JEO-051	1.00E+08
JEO-052	1.00E+06
JEO-053	1.00E+08
JEO-054	1.00E+07
JEO-055	1.00E+06
JEO-056	1.00E+08

Table 2. Calculated spore concentration based on serial dilution growth levels for samples JEO-049 to JEO-056.

Preparation and distribution for γ- radiation [Future Plan]

For this experiment, spores on the order of 10^6 for each sample are to be used as the starting quantity. In a new 96 well plate, ethanol suspended spores should be distributed at various volumes (depending on concentration) to yield a total quantity of approximately 10^6 per well. Samples are to be distributed as four replicates per plate, as seen below:

JEO-001	JEO-002	JEO-003	JEO-004	JEO-005	JEO-006	JEO-007	JEO-008	JEO-009	JEO-010	JEO-011	JEO-012
JEO-001	JEO-002	JEO-003	JEO-004	JEO-005	JEO-006	JEO-007	JEO-008	JEO-009	JEO-010	JEO-011	JEO-012
JEO-001	JEO-002	JEO-003	JEO-004	JEO-005	JEO-006	JEO-007	JEO-008	JEO-009	JEO-010	JEO-011	JEO-012
JEO-001	JEO-002	JEO-003	JEO-004	JEO-005	JEO-006	JEO-007	JEO-008	JEO-009	JEO-010	JEO-011	JEO-012
JEO-013	JEO-014	JEO-015	JEO-016	JEO-017	JEO-018	JEO-019	JEO-020	JEO-021	JEO-022	JEO-023	JEO-024
JEO-013	JEO-014	JEO-015	JEO-016	JEO-017	JEO-018	JEO-019	JEO-020	JEO-021	JEO-022	JEO-023	JEO-024
JEO-013	JEO-014	JEO-015	JEO-016	JEO-017	JEO-018	JEO-019	JEO-020	JEO-021	JEO-022	JEO-023	JEO-024
JEO-013	JEO-014	JEO-015	JEO-016	JEO-017	JEO-018	JEO-019	JEO-020	JEO-021	JEO-022	JEO-023	JEO-024

Figure 3. 96 well plate arrangement of JEO-001 to JEO-024 spores in preparation for γ- radiation

In total, 4 plates are needed to accommodate all 96 spore samples. Additionally, because irradiation will take place at multiple locations and over a range of intensities, a set of plates should be prepared for each site and exposure level. Plates should be allowed to dry, evaporating the ethanol solvent and leaving behind dry spores. Upon completion, sealed plates are to be distributed to various sites for cobalt-60 γ- radiation, electron beam γ-radiation, or x-radiation. X-radiation was considered in this study due to the recent discoveries of wavelength (i.e. energy) overlap between γ and x-rays, particularly through linear accelerators (L'Annunziata et al 2003, Grupen et al 2005).

Regrowth of irradiated spores

Following return of irradiated plates, spores in each well are to be re-suspended in TSB, and incubated at 32°C. Over an extended period, wells will be checked for growth through visualization of turbidity and spectrophotometric absorption at 24 hour intervals.

Results

Due to time constraints, this project reached the spore purification and quantification stage. A catalog of all purified spores and concentration levels for each was successfully established. Further work by future students will carry out the remainder of the procedure, and results will then be analyzed for significance. The complete quantification data is presented below in Appendix A. Upon regrowth of irradiated spores, survival can be correlated and measured by the incidence of regrowth.

Conclusions

The observation of growth post-irradiation will provide evidence for γ- radiation resistance in surviving strains. This data will allow for the identification of Type C microorganisms that can be used to assess the threat level for forward contamination on NASA spacecraft, specifically for the proposed Jupiter Europe Orbiter. The information provided by this experiment will allow for the development of a common planetary protection standard for every mission to Europa. This standard will yield a probability of contaminating a Europan ocean with a viable terrestrial organism at any time in the future to be less than 10^{-4} per mission. This standard will likely employ the use of sterilization through the use of sufficient radiation, isopropyl alcohol, hydrogen peroxide, and dry heating (Space Studies Board 2000).

Additional Work

During the 15 week internship period at NASA – Jet Propulsion Laboratory (JPL), several additional projects received my attention. These projects are entitled as follows:

- Microbial Deep Diversity: Development of novel method for increasing the resolution of PCR-based microbial diversity studies
- Survival of Bacterial Spores Exposed to Germicidal Ultraviolet Radiation (254 nanometers)
- Survival of Bacterial Spores Exposed to the Cosmic Environment via the International Space Station

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Appendix A

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
Sample Name	1	2	3	4	5	6	7	8	9	10	11	12
JEO-001	0.6003	0.2385	0.6403	0.6346	0.7125	0.0408	0.0381	0.0362	0.0372	0.0368	0.0727	0.0377
JEO-002	0.4795	0.5708	0.6052	0.6369	0.6066	0.7849	0.0388	0.0388	0.0382	0.0386	0.0517	0.0387
JEO-003	0.4891	0.5286	0.5558	0.6651	0.6187	0.6718	0.0379	0.0382	0.0379	0.0383	0.0385	0.0381
JEO-004	0.5841	0.5331	0.6168	0.5707	0.5499	0.8539	0.039	0.0403	0.0384	0.0385	0.0387	0.0396
JEO-005	0.6573	0.626	0.6216	0.6175	0.6177	0.6289	0.0389	0.0948	0.0383	0.0394	1.0641	0.0385
JEO-006	0.5214	0.5403	0.5987	0.5804	0.5292	0.3489	0.4513	0.0402	0.0375	0.04	0.0439	0.0387
JEO-007	0.5186	0.5979	0.5418	0.5835	0.4345	0.6098	0.436	0.5335	0.0404	0.0295	0.0491	0.0387
JEO-008	0.5514	0.5722	0.5809	0.6051	0.5916	0.0393	0.8577	0.0307	0.041	0.0387	0.04	0.0414

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-009	0.7326	0.7765	0.696	0.6438	0.5907	0.7961	0.0393	0.0375	0.038	0.0376	0.0378	0.0382
JEO-010	0.832	0.8125	0.7492	0.5889	0.8078	0.0389	0.0388	0.0388	0.0383	0.0388	0.0388	0.039
JEO-011	0.4264	0.3701	0.3144	0.339	0.4103	0.3479	0.2862	0.0387	0.0385	0.0382	0.0403	0.0385
JEO-012	1.0343	0.763	0.6893	0.6885	0.8341	0.702	0.745	0.0403	0.0381	0.0388	0.0386	0.04
JEO-013	0.4546	0.4387	0.4571	0.5717	0.5544	0.449	0.489	0.5279	0.0389	0.0389	0.0379	0.0376
JEO-014	0.3556	0.4027	0.3031	0.3977	0.3136	0.3466	0.039	0.3304	0.0494	0.0388	0.0391	0.039
JEO-015	0.3789	0.3952	0.3634	0.574	0.3064	0.3936	0.0391	0.0381	0.0391	0.0405	0.0497	0.0372
JEO-016	0.3591	0.3597	0.4545	0.4523	0.4839	0.5713	0.0387	0.0375	0.0374	0.0387	0.0399	0.0384

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-017	0.5308	0.4496	0.4088	0.3875	0.4264	0.4255	0.6053	0.3358	0.4072	0.0378	0.0398	0.0381
JEO-018	0.4642	0.417	0.4206	0.4815	0.4737	0.3623	0.0395	0.0408	0.0393	0.0391	0.039	0.039
JEO-019	0.7047	0.81	0.6894	0.6501	0.761	0.8614	0.0392	0.0389	0.0401	0.039	0.0386	0.0391
JEO-020	0.943	0.9593	0.4055	0.7003	0.8848	0.0395	0.0396	0.0396	0.051	0.0389	0.0392	0.0386
JEO-021	0.4916	0.4843	0.5718	0.6253	0.5611	0.4031	0.4995	0.2787	0.6201	0.0415	0.0394	0.0382
JEO-022	0.7068	0.5679	0.5983	0.5566	0.5705	0.5303	0.0414	0.041	0.0393	0.0391	0.04	0.0389
JEO-023	0.4426	0.4723	0.5487	0.4465	0.4214	0.4664	0.3798	0.0386	0.039	0.0395	0.0501	0.0402
JEO-024	0.4261	0.498	0.4372	0.4132	0.5042	0.4859	0.3766	0.3824	0.0392	0.418	0.0401	0.0384

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-025	0.4557	0.5456	0.5304	0.5639	0.565	0.5099	0.0384	0.0372	0.0368	0.0361	0.0376	0.0372
JEO-026	0.6154	0.5144	0.4911	0.4699	0.6292	0.0387	0.0382	0.038	0.0379	0.0382	0.0382	0.0375
JEO-027	0.4427	0.6604	0.5514	0.4678	0.6809	0.4878	0.1507	0.0383	0.0375	0.0379	0.0387	0.0374
JEO-028	0.3265	0.3821	0.6108	0.6207	0.6148	0.3292	0.4933	0.0381	0.0381	0.038	0.0381	0.0377
JEO-029	0.9892	1.1321	1.2775	1.2989	1.3059	1.2282	0.0385	0.0389	0.0385	0.0379	0.0386	0.0378
JEO-030	0.5066	0.5083	0.4339	0.4412	0.4269	0.7818	0.0397	0.0388	0.0389	0.0388	0.0386	0.0381
JEO-031	0.3946	0.3897	0.5271	0.6418	0.5642	0.3098	0.6685	0.6297	0.2611	0.0389	0.0502	0.0377
JEO-032	0.5145	0.4624	0.5309	0.4998	0.5489	0.6397	0.7145	0.5827	0.6882	0.0398	0.0387	0.0383

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-033	0.3238	0.4688	0.4551	0.3326	0.5963	0.0714	0.0379	0.0371	0.0368	0.0368	0.0393	0.0367
JEO-034	0.5468	0.5069	0.5244	0.5871	0.7119	0.7729	0.0386	0.0382	0.0383	0.0384	0.0381	0.038
JEO-035	0.5269	0.5865	0.5261	0.5621	0.5585	0.9907	0.8698	0.0382	0.0378	0.0387	0.0382	0.0377
JEO-036	0.7243	0.8801	0.772	0.8556	0.0383	0.0384	0.038	0.0382	0.0381	0.0382	0.0383	0.0379
JEO-037	0.4995	0.4596	0.2557	0.5973	0.6712	0.4581	0.2212	0.0385	0.038	0.0382	0.0383	0.0374
JEO-038	0.6019	0.5778	0.5245	0.4913	0.5178	0.0379	0.0387	0.0387	0.0382	0.0381	0.0387	0.038
JEO-039	0.496	0.4155	0.2732	0.4871	0.7213	0.6771	0.0388	0.0385	0.039	0.039	0.0493	0.0373
JEO-040	0.5259	0.5762	0.534	0.4985	0.5019	0.5431	0.0375	0.0371	0.0375	0.0378	0.0389	0.0388

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-041	0.3845	0.4985	0.5771	0.6797	0.6823	0.7196	0.7761	0.0372	0.0366	0.0365	0.037	0.0392
JEO-042	0.4168	0.5258	0.4157	0.5132	0.4429	0.5489	0.7419	0.7848	0.0381	0.038	0.0379	0.0381
JEO-043	0.3128	0.4185	0.494	0.55	0.5991	0.4952	0.038	0.0388	0.0383	0.0378	0.0379	0.0375
JEO-044	0.546	0.4725	0.4548	0.5123	0.5042	0.4585	0.0388	0.0382	0.0378	0.0379	0.0382	0.0381
JEO-045	0.3947	0.3848	0.483	0.5807	0.6019	0.267	0.3318	0.0382	0.0379	0.0381	0.0377	0.0375
JEO-046	0.4293	0.4168	0.5345	0.6379	0.6079	0.0379	0.0396	0.0386	0.0386	0.0382	0.0383	0.0382
JEO-047	0.9452	0.6896	0.4585	0.5853	0.8353	0.7065	0.0385	0.0386	0.0393	0.0388	0.049	0.0375
JEO-048	0.6048	0.6806	0.5436	0.0384	0.0387	0.0388	0.0374	0.0379	0.0376	0.0376	0.0388	0.0382

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-049	0.4828	0.8298	0.8076	0.8321	0.0384	0.0389	0.0387	0.0397	0.0381	0.037	0.0387	0.0383
JEO-050	0.3703	0.5719	0.4982	0.5855	0.4888	0.0391	0.0394	0.04	0.0394	0.0387	0.0387	0.0384
JEO-051	0.6995	0.6178	0.2838	0.3026	0.2958	0.3086	0.0381	0.0402	0.0386	0.0394	0.0386	0.0385
JEO-052	0.6486	0.5706	0.5029	0.376	0.0383	0.0389	0.0389	0.041	0.0391	0.0404	0.0389	0.0386
JEO-053	0.3089	0.3212	0.3402	0.9219	0.822	0.5552	0.0398	0.0385	0.0426	0.0417	0.0399	0.0393
JEO-054	0.9105	0.3286	0.4087	0.2871	0.6636	0.0389	0.039	0.04	0.0424	0.0389	0.039	0.0388
JEO-055	0.4576	0.4024	0.3781	0.4498	0.0393	0.0389	0.0413	0.04	0.0403	0.0402	0.0511	0.038
JEO-056	0.5031	0.3262	0.6896	0.3709	0.4307	0.6604	0.0378	0.0372	0.0383	0.0432	0.0392	0.0386

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-057	0.6907	0.2296	0.3218	0.8224	0.4901	0.3983	0.0371	0.036	0.0356	0.0359	0.0365	0.0365
JEO-058	0.795	0.4284	0.3212	0.3904	0.5298	0.404	0.038	0.0377	0.0377	0.0376	0.038	0.0376
JEO-059	0.8127	0.8611	0.8413	0.0377	0.0377	0.0384	0.0383	0.0376	0.0376	0.0375	1.4229	0.0375
JEO-060	0.7013	0.755	0.7178	0.7247	0.0381	0.0381	0.0385	0.0377	0.0376	0.0377	0.0374	0.0375
JEO-061	0.795	0.7769	0.7198	0.9406	0.6975	0.0383	0.0388	0.0373	0.038	0.0373	0.0383	0.0371
JEO-062	0.6783	0.7479	0.7044	0.7215	0.7297	0.7859	0.0388	0.7545	0.0373	0.038	0.038	0.0376
JEO-063	0.7816	0.0382	0.0376	0.0385	0.0391	0.0387	0.0381	0.038	0.0384	0.0391	0.0493	0.0368
JEO-064	0.4718	0.3752	0.4697	0.3266	0.2826	0.0385	0.0372	0.0369	0.0371	0.0376	0.0388	0.0382

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-065	0.7508	0.7734	0.4133	0.5009	0.0373	0.0375	0.0374	0.0364	0.0361	0.0364	0.0367	0.0366
JEO-066	0.5469	0.8407	0.8749	1.0235	0.9419	0.0381	0.0381	0.0378	0.0378	0.0376	0.0378	0.0377
JEO-067	0.0372	0.0376	0.0376	0.0379	0.0374	0.0377	0.0374	0.0376	0.0374	0.0383	0.0378	0.0372
JEO-068	0.8368	0.8736	0.6022	0.9082	1.0246	0.6098	0.0381	0.0383	0.0379	0.0376	0.0379	0.0391
JEO-069	0.8424	0.7702	0.7932	0.0388	0.0382	0.038	0.0381	0.0385	0.0383	0.038	0.0379	0.0373
JEO-070	0.7324	0.9167	0.8657	0.7445	0.0429	0.0394	0.0384	0.0393	0.0381	0.0379	0.0475	0.0378
JEO-071	0.8157	0.9165	0.8221	0.7844	0.0395	0.0387	0.0384	0.0387	0.0393	0.0384	0.0481	0.0371
JEO-072	0.6262	0.7135	0.7308	0.0379	0.0386	0.0384	0.0376	0.0376	0.0373	0.0373	0.0394	0.0384

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-073	0.1838	0.2576	0.6	0.2197	0.6952	0.4893	0.0368	0.0354	0.6647	0.0356	0.0356	0.0365
JEO-074	0.4934	0.9044	0.9908	0.9271	0.9648	0.9059	0.0381	0.0377	0.0375	0.0376	0.0379	0.0493
JEO-075	0.6724	0.7068	0.7048	0.0374	0.0373	0.0381	0.038	0.0377	0.0377	0.0377	0.0381	0.0394
JEO-076	0.6853	0.82	0.7869	0.4452	0.2807	0.6672	0.0386	0.0382	0.0378	0.0378	0.0375	0.0378
JEO-077	0.9613	1.0123	0.5793	0.9385	0.3962	0.3653	0.4362	0.0378	0.0382	0.0374	0.0375	0.0371
JEO-078	0.6451	0.8106	0.8865	0.8148	0.0374	0.0373	0.0375	0.0374	0.0374	0.038	0.0382	0.0373
JEO-079	0.8191	1.1755	1.1374	1.1766	1.2011	1.4012	0.0377	0.038	0.0386	0.039	0.0496	0.0368
JEO-080	0.7207	0.7854	0.8864	0.7593	0.798	0.0386	0.037	0.0368	0.0367	0.0377	0.0384	0.0383

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-081	0.5583	0.5365	0.5384	0.7151	0.8608	0.0376	0.0374	0.0364	0.0364	0.0357	0.0374	0.0371
JEO-082	0.6881	0.038	0.0382	0.0377	0.0381	0.0378	0.0379	0.0378	0.0378	0.0379	0.0378	0.0387
JEO-083	0.6592	0.038	0.0387	0.038	0.0381	0.0377	0.0378	0.038	0.0378	0.0378	0.0379	0.0378
JEO-084	0.5477	0.0381	0.0383	0.0382	0.039	0.0381	0.0383	0.0382	0.038	0.0382	0.038	0.038
JEO-085	0.3758	0.0384	0.0385	0.0384	0.0387	0.0385	0.04	0.0383	0.0381	0.0384	0.0383	0.0385
JEO-086	0.529	0.3841	0.5471	0.1976	0.4238	0.0392	0.2664	0.0381	0.0383	0.0384	0.0388	0.0381
JEO-087	1.46	1.1995	1.3826	0.0384	0.0391	0.0387	0.0382	0.0383	0.0397	0.039	0.0488	0.0371
JEO-088	0.722	0.4182	0.4355	0.4079	0.0386	0.0388	0.0313	0.0266	0.0364	0.0396	0.0386	0.0386

	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08	1.00E-09	1.00E-10	1.00E-11	1.00E-12	1.00E-13	1.00E-14
	1	2	3	4	5	6	7	8	9	10	11	12
JEO-089	0.279	0.3482	0.4856	0.3987	0.4151	0.4533	0.0374	0.0366	0.0366	0.0362	0.0379	0.0371
JEO-090	0.5155	0.038	0.0382	0.0381	0.0381	0.0379	0.0381	0.038	0.038	0.038	0.0381	0.039
JEO-091	0.5725	0.6277	0.0379	0.0381	0.0381	0.038	0.0378	0.0381	0.038	0.038	0.0381	0.0381
JEO-092	0.2602	0.3583	0.352	0.6458	0.4709	0.0382	0.0384	0.038	0.0379	0.0376	0.038	0.0382
JEO-093	0.2518	0.0389	0.0393	0.0393	0.0385	0.0402	0.0401	0.0392	0.0386	0.0376	0.0384	0.039
JEO-094	0.9568	0.9902	0.038	0.0384	0.0389	0.0384	0.0387	0.0381	0.04	0.0384	0.0387	0.0386
JEO-095	0.969	0.8935	0.0387	0.0389	0.0394	0.0391	0.0381	0.0396	0.0397	0.0388	0.0511	0.0378
JEO-096	0.3635	0.2544	0.2542	0.1772	0.0386	0.0391	0.0373	0.038	0.037	0.0423	0.0382	0.0589

Table 3. Spectrophotometric growth data of spores in TSB overnight after serial dilution for samples JEO-001 to JEO-096.

Strain	Approximate Initial Spore Concentration		Strain	Approximate Initial Spore Concentration
JEO-001	1.00E+07		JEO-049	1.00E+06
JEO-002	1.00E+08		JEO-050	1.00E+07
JEO-003	1.00E+08		JEO-051	1.00E+08
JEO-004	1.00E+08		JEO-052	1.00E+06
JEO-005	1.00E+08		JEO-053	1.00E+08
JEO-006	1.00E+08		JEO-054	1.00E+07
JEO-007	1.00E+08		JEO-055	1.00E+06
JEO-008	1.00E+07		JEO-056	1.00E+08
JEO-009	1.00E+08		JEO-057	1.00E+08
JEO-010	1.00E+07		JEO-058	1.00E+08
JEO-011	1.00E+09		JEO-059	1.00E+05
JEO-012	1.00E+09		JEO-060	1.00E+06
JEO-013	1.00E+10		JEO-061	1.00E+07
JEO-014	1.00E+10		JEO-062	1.00E+08
JEO-015	1.00E+08		JEO-063	1.00E+04
JEO-016	1.00E+08		JEO-064	1.00E+07
JEO-017	1.00E+11		JEO-065	1.00E+06
JEO-018	1.00E+08		JEO-066	1.00E+07
JEO-019	1.00E+08		JEO-067	<1000
JEO-020	1.00E+07		JEO-068	1.00E+08
JEO-021	1.00E+11		JEO-069	1.00E+05
JEO-022	1.00E+08		JEO-070	1.00E+06
JEO-023	1.00E+09		JEO-071	1.00E+06
JEO-024	1.00E+10		JEO-072	1.00E+05
JEO-025	1.00E+08		JEO-073	1.00E+08
JEO-026	1.00E+07		JEO-074	1.00E+08
JEO-027	1.00E+09		JEO-075	1.00E+05
JEO-028	1.00E+09		JEO-076	1.00E+08
JEO-029	1.00E+08		JEO-077	1.00E+09
JEO-030	1.00E+08		JEO-078	1.00E+06
JEO-031	1.00E+11		JEO-079	1.00E+08
JEO-032	1.00E+11		JEO-080	1.00E+07
JEO-033	1.00E+07		JEO-081	1.00E+07
JEO-034	1.00E+08		JEO-082	1.00E+03
JEO-035	1.00E+09		JEO-083	1.00E+03
JEO-036	1.00E+06		JEO-084	1.00E+03
JEO-037	1.00E+09		JEO-085	1.00E+03
JEO-038	1.00E+07		JEO-086	1.00E+07
JEO-039	1.00E+08		JEO-087	1.00E+05
JEO-040	1.00E+08		JEO-088	1.00E+06
JEO-041	1.00E+09		JEO-089	1.00E+08
JEO-042	1.00E+10		JEO-090	1.00E+03
JEO-043	1.00E+08		JEO-091	1.00E+04
JEO-044	1.00E+08		JEO-092	1.00E+07
JEO-045	1.00E+09		JEO-093	1.00E+03
JEO-046	1.00E+07		JEO-094	1.00E+04
JEO-047	1.00E+08		JEO-095	1.00E+04
JEO-048	1.00E+05		JEO-096	1.00E+06

Table 4. Calculated spore concentration based on serial dilution growth levels for samples JEO-001 to JEO-096.