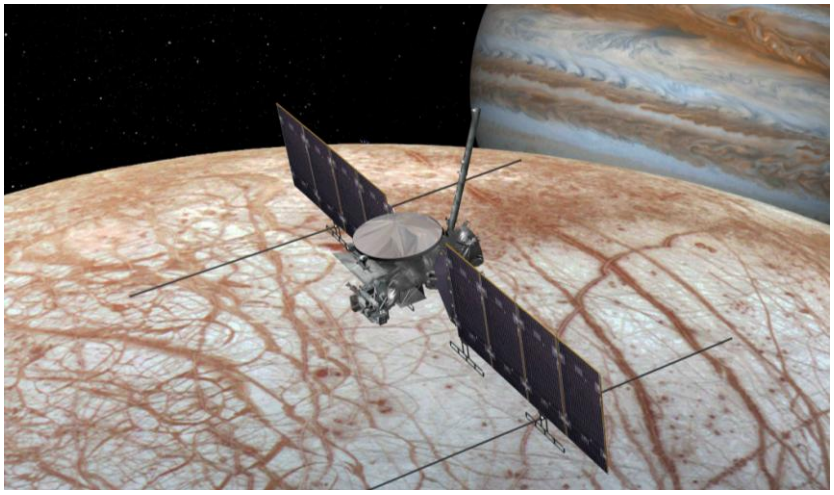
Interactive Effects of Vacuum, Ionizing Radiation, Temperature, and UV Irradiation on Bacterial Survival during Simulated Europan Orbiter and Lander Missions



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C. Proposal Summary

Europa is an icy planetary body orbiting Jupiter that holds the promise of potentially harboring life in its subsurface ocean. Due to tidal forces around Jupiter, it is believed that the iron rocky core might be hot enough to form hydrothermal vents at the core/ocean interface. Recently, NASA has begun to study two mission scenarios to Europa; first an orbiter called the Europa Clipper (EC) (proposed launch in 2023) and a Europa Lander (proposed launch in the late 2020's). Due to concerns of contaminating ice-water niches on Europa (i.e., subsurface ocean and water/brine cavities in the ice crust), the probability for contamination (*Pc*) has been set at 1×10^{-4} for the entire mission. In order to meet that *Pc*, the Planetary Protection Research (PPR) program has requested new research into interactive effects of biocidal space conditions in order to predict and model the inactivation of viable bioburdens that may have been inadvertently launched on the EC spacecraft.

We will propose a series of integrated experiments to characterize the additive and synergistic interactions among four biocidal space conditions on microbial survival. Experiments will focus on (1) interactive effects of vacuum ($<10^{-6}$ Pa) and ionizing radiation (i.e., X-rays, gamma-rays, protons, electrons, and neutrons), (2) interactive effects of vacuum, extreme-temperatures (–120 to 140°C), and solar UV irradiation (from Earth's solar constant to the cis-Jovian environment), and (3) combinations of the above. Experiments with the five ionizing radiation sources will be conducted at U. of Florida facilities in Gainesville and Jacksonville, FL. The interactive studies for vacuum, ionizing radiation, and UV irradiation will be conducted in a Planetary Atmosphere Chamber (Schuerger et al., 2008, Icarus, 194, 86-100).

A set of high-vacuum Radiation Exposure Chambers (REC units) will be built that are capable of holding low-pressures (10^{-6} Pa) and fitted with 1-cm wide aluminum coupons doped with spores/cells of three *Bacillus* spp. or *Deinococcus radiodurans*. Coupons in REC units will be exposed to combinations of the space conditions listed above and assayed for survivors. Also, we will conduct realistic multi-factorial simulations with combinations of vacuum, ionizing radiation, extremes in temperature, and UV irradiation that represent what the EC spacecraft will encounter during a cruise phase to Europa, and its 3-year primary mission in Jovian space.

The overarching goal will be to develop a quantitative EC Microbial Survival (ECMS) model that predicts microbial inactivation kinetics for outbound spacecraft from the interactive effects of the four space conditions listed above. Recently, Schuerger (PI, here), Moores (collaborator, here) et al. (2019, Astrobiology, 19(6) doi: 10.1089/ast.2018.1952) developed a quantitative Lunar Microbial Survival (LMS) model that predicts that almost all spacecraft components and vehicles landed on the Moon have been sterilized by the interactive effects of (in priority) high-temperature, UV irradiation, ionizing radiation, and cis-Lunar vacuum. The Lunar surface is very similar to the interplanetary environment that the EC spacecraft will encounter, except that the ionizing radiation environment in interplanetary space will be higher than that modeled for the Moon. The LMS model will be used to develop the ECMS model for Europan spacecraft populated with new empirical data from biocidal assays with combinations of the four space conditions.

The goal will be to model the EC spacecraft environment, shielding, and interplanetary cruise trajectories to determine if the *Pc* for the mission can be achieved prior to orbital insertion at Europa, or shortly thereafter.

D. Scientific and Technical Section

1. Background

1.1. Introduction

The NASA Decadal Survey (Anonymous, 2013) identified exploration of the Icy Moons of Jupiter and Saturn as a key goal for planetary exploration between 2013 and 2022. During the last 20 years, new studies have elevated the significance of this goal by identifying the presence of subsurface water-oceans on Europa, Ganymede, Callisto, and Enceladus (see reviews by Lunine, 2017; Spohn and Schubert, 2003), and identified active surface plumes of liquid water on Europa (Roth et al., 2014) and Enceladus (Hansen et al., 2006). Furthermore, the NASA Astrobiology Roadmap (Des Marias et al., 2008) outlines 7 primary goals up to 2025 with Goal 2 calling for the search for habitable environments and extant life in our Solar System. Objective 2.2 goes further and specifically identifies Europa as a high target planetary environment to "…model the potential for subsurface habitable environments on icy moons…" The motivation for developing this goal may have been the possible presence of hydrothermal features at the core/mantle and ocean interface in the deep oceans on Europa (Lowell and DuBose, 2005), and the possible presence of liquid cavities in the 15-20 km ice crusts of the icy moons (Walker and Schmidt, 2015) (Fig. 1).

The first planned mission to an icy moon is called the ***Europa Clippe***r (an orbiter; Bayer et al., 2015) that will be launched in 2022-2023; to be followed by a lander targeted for the late 2020's. The key goals of the Europa Clipper (EC) mission are to study the interactions of the core/mantle, ocean, and icy crust to understand the potential habitability of the Europan subsurface (Clark et al., 2011; Bayer et al., 2015) (Fig. 1). There are two key phases of the mission in which the *Science Objectives* include studies into (1) ice-shell and ocean interactions, (2) the composition and chemistry of ice in regards to habitability, and (3) understanding of the geological and impact processes that led to the current surface features. In addition, there are two *Survey Objectives* for the EC mission that include (i) landing site selection and characterization for a future lander, and (ii) to determine the scientific value of surface materials at prospective landing sites.

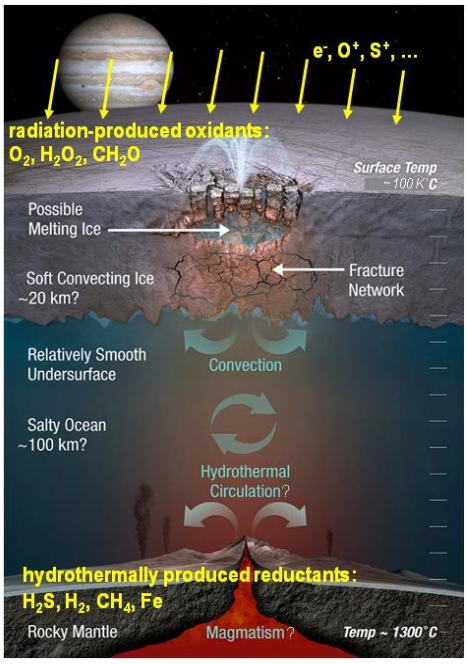

Fig. 1. Cross-section of the Europa ice crust, subsurface ocean, and the core/mantle interactions. Tidal forces around Jupiter squeeze and expand these components and suggest that hydrothermal features may be present at the mantle/ocean interface. (Drawing is courtesy of JPL; artist unknown.)

In order to assure the scientific success of future Europa missions, the Planetary Protection Research program (PPR; C.15, NSPIRES) has identified several key knowledge gaps in planetary protection for missions to icy moons that require new and comprehensive data to prevent the forward contamination of potentially habitable environments. The knowledge gap that we will address in this proposal will be to determine the interactive effects of four biocidal space conditions on bacterial survival in order to predict contamination risks to potentially habitable niches on or in Europa.

1.2. Objectives

The primary goal of the current project will be to study the interactive biocidal effects of space vacuum, ionizing radiation, extreme-temperatures, and UV irradiation on bacterial survival in order to develop a Europa Clipper Microbial Survival (ECMS) model to predict the persistence of bioburdens on the spacecraft during the mission.

According to Bayer et al. (2015), there are two key challenges within the scope of the EC mission that must be effectively addressed prior to launch that include: (1) what are the potential effects of ionizing radiation on spacecraft hardware (not addressed here); and (2) given the high-probability of a liquid ocean and brine cavities in the ice on Europa (Fig. 1), can the forward contamination of these potentially habitable regions be prevented through a combination of pre- and post-launch mitigation processes? The next solar minimum is predicted to occur between 2020 and 2025 in solar cycle 25, and thus, the EC spacecraft will be launched near solar minimum; at relatively low levels of ionizing radiation from the Sun. However, when the EC spacecraft arrives in Jovian space, the ionizing radiation environment will increase dramatically from the intense geomagnetic radiation fields around Jupiter (Nenon et al., 2018a; Reitz, 2008).

Thus, the current proposal will describe how we will study the interactive effects of *four key biocidal space factors* including vacuum [VAC], ionizing radiation [IRAD], extremes in temperature [TEMP], and ultraviolet [UV] irradiation on the survival of three *Bacillus* spp. and one non-spore forming bacterium (see Section 2.6.1). Second, we will create simulations of five locations in space during a cruise phase of the EC spacecraft to Jupiter (see Section 2.6.3) to determine if significant bioburden reductions will occur during transit. And third, we will utilize the new and comprehensive datasets on the biocidal nature of these factors to develop a *Europa Clipper Microbial Survival* (ECMS) model that can be used to predict the inactivation of the launched bioburdens during the course of the EC mission.

Objective-1: What are the interactive effects between VAC ($\leq 10^{-6}$ Pa) and IRAD sources (e.g., protons, neutrons, electrons, X-rays, and γ-rays) for inactivating bacteria? [Years 1 & 2]

Objective-2: What are the interactive biocidal effects of VAC ($\leq 10^{-6}$ Pa) when tested against either TEMP extremes (–120 to +140°C) or UV irradiation extremes (Venus to Jovian space) for inactivating bacteria? [Years 1 & 2]

Objective-3: What are the effects of the space stressors listed above for inactivating spores of of Bacillus subtilis 168 in simulations of a EC cruise phase to Europa? [Year 3]

Objective-4: Construct a Europa Clipper Microbial Survival (ECSM) model for predicting the survival rates of bacterial spores during the cruise phase to Jupiter and during the primary mission in Europa orbit. [Year 3]

1.3. Bacterial Survival on Spacecraft and in Interplanetary Space: A Brief Review

Interplanetary spacecraft are assembled in Spacecraft Assembly Facility (SAF) cleanrooms in which the primary sources of microbial contamination are the human scientists and engineers operating around the vehicles. Using multiple procedures (e.g., culturable assays on diverse media, 16S rDNA sequences of recovered strains, DNA-DNA hybridization, FISH analysis, FAME profiles, high-density 16S microarrays, and metagenomics) a wide diversity of bacteria, viruses, and fungi have been identified from spacecraft SAFs (e.g., La Duc et al., 2007; Probst et al., 2010; Schwendner et al., 2013; Vaishampayan, et al., 2010; Venkateswaran et al.,

2001; Weimaier et al., 2015). Of the diverse species identified in these studies, bacteria comprise the vast majority of recovered strains and are, in general, members of the phyla Actinobacteria, Proteobacteria, and Firmicutes. The phylum Firmicutes represents a key phylum for monitoring spacecraft cleanliness because of their high abundance in SAFs (Smith et al., 2017) and presence of stress-resistant endospores (henceforth spores) in the family Bacillaceae (Horneck et al., 2010). For example, in a study of microbial contamination on the Mars Science Laboratory rover (Smith et al., 2017), 88% of all culturable bacteria were Firmicutes, and 68 % of all isolates in the Firmicutes were in the genus *Bacillus*.

Spores of *Bacillus* spp. have been used extensively to test the survival of hardy bacteria in the space environment, including on Apollo 16, European Retrievable Carrier (EURECA), EXPOSE facility, Long Duration Exposure Facility (LDEF), Foton orbiters, and the International Space Station (ISS), in which spores have survived simulations of interplanetary space, Martian conditions, ionizing radiation, vacuum, and UV irradiation (see reviews by Horneck et al., 2010; Nicholson et al., 2000; Rabbow et al., 2017; Schuerger et al., 2019).

The current project will focus on using *Bacillus spp.* for the interactive experiments on the lethality of VAC, IRAD, TEMP, and UV in order to correlate our results with a wide range of published data on ionizing radiation (e.g., Moeller et al, 2007; 2008), UV irradiation (e.g., Schuerger et al., 2006; 2012), vacuum alone (e.g., Horneck et al., 2012; Schuerger et al., 2003), high-temperature alone (e.g., Kempf et al., 2008; Schubert and Beaudet, 2011), and interactive effects of vacuum and high temperature (e.g., Schuerger et al., 2019; see Section 1.4).

1.4. A Lunar Microbial Survival (LMS) model and its relevance to microbial survival during the Europa Clipper Mission

Recently, Schuerger (PI here), Moores (Collaborator here), et al. (2019) developed a Lunar Microbial Survival (LMS) model that predicts how spacecraft microorganisms might have survived on the Lunar surface since the landing of the Luna 2 in 1959. A brief discussion of the LMS model here is an important step in understanding our approach to the development of an ECMS model for missions to the icy moons of Jupiter.

First, the primary factor in motivating our team to develop the current proposal was a result in the LMS model that showed a dramatic increase in the lethality of vacuum (<10^{-4} Pa) on spores of *Bacillus subtilis* when they were concomitantly exposed to high-temperature (100°C). The effect of each parameter alone yielded a minor loss of viability for vacuum exposed spores (~20% decrease), a moderate loss of viability when spores were exposed to 100°C, but a complete inactivation of all viable spores when the two conditions were combined (> 6 Log reductions) (Fig. 2). This result highlights the key assumption of the current project; namely, that synergistic interactions among diverse biocidal conditions in space are likely to dramatically increase the lethality of the space environment on microbes present on spacecraft surfaces after launch. The result in Fig. 2 is also consistent with other literature that has identified synergistic effects between key biocidal conditions found in space. For example, Bucker and Horneck (1970) demonstrated that vacuum (10^{-3} Pa) increased the

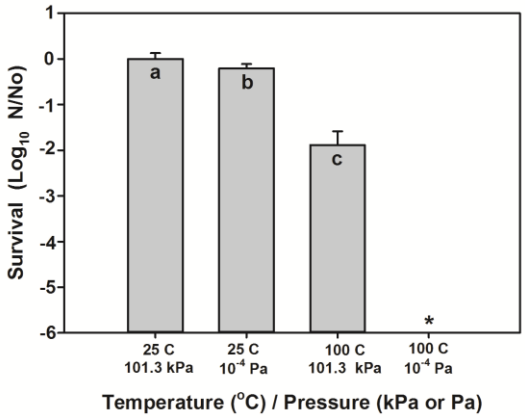

Fig. 2. Spores of the bacterium, *Bacillus subtilis*, were exposed to low pressure (< 10^{-4} Pa), high temperature (100°C), and a combination of both factors in a Moon Simulation Chamber. Each treatment was maintained for 8 hrs. (Schuerger et al., 2019.)

lethality of ionizing radiation in the form of X-rays by 2 orders of magnitude (–2 Logs) compared to a lab-normal pressure of 101.3 kPa; and Hagen et al. (1971) showed that temperature increases from –105°C to 60°C increased the lethality of vacuum (10^{-8} Pa) for both *Bacillus subtilis* and two non-spore forming species by –1 to –2 Logs.

And ***second***, the LMS model predicted that the interactive effects of VAC, IRAD, UV, and high-TEMP would yield extremely high rates of spore/cell inactivation on: (1) external surfaces (exposed to solar UV and heating), (2) shallow internal surfaces (in thermal contact with heated outside surfaces), but (3) not for deeply embedded internal surfaces (not in thermal contact with external heated surfaces) (Table 1). For example, the overall LMS model (i.e., all factors combined) predicts that the Lunar environment creates the potential for up to –231 Logs of biocidal inactivation on external surfaces at the equator per lunation, decreasing to –39 Logs at ± 60° Lat. For the combined factors of VAC + high-TEMP (–188 Logs) exceeded the lethality of high-TEMP alone (–97 Logs), UV-alone (–42 Logs), IRAD-alone (–3 Logs for solar wind particles), and VAC-alone (–0.02 Logs), in decreasing order. The slowest survival rate (–0.02 Logs per lunation) was predicted for deeply embedded internal spacecraft surfaces that were shielded from IRAD, UV, high-TEMP, but not VAC.

Table 1. Lunar Microbial Survival (LMS) model predictions. (Schuerger et al., 2019).

Environmental parameters	LMS model latitudes	Log_{10} reductions in bioburden per lunation predicted by LMS model		
		External surfaces	Shallow internal surfaces	Deep internal surfaces
Overall LMS model	0°	–231	–188	–0.02
	±30°	–154	–116	–0.02
	±60°	–39	–16	–0.02
Cis-Lunar vacuum		–0.02	–0.02	–0.02
UVC + UVB	0°	–42	na	na
	±30°	–37	na	na
	±60°	–23	na	na
Temperature only	0°	–97	–97	na
	±30°	–17	–17	na
	±60°	–0.13	–0.13	na
Vacuum + heat	0°	–188	–188	na
	±30°	–116	–116	na
	±60°	–16	–16	na
Solar wind particles	0°	–3	na	na

In summary, the LMS model is the first quantitative model for microbial survival of Earth-microbes developed for any mission profile in the Solar System that includes four key space stressors. The LMS model predicts fast kill-rates for most microbes in most niches on spacecraft surfaces, except for deeply embedded internal surfaces exposed only to VAC. And the LMS model will be used as the starting point to develop a 1st-order ECMS model for interplanetary spacecraft, in general, and Europa Clipper in particular.

1.5. Europa Clipper Mission & Spacecraft

The Europa Clipper (EC) spacecraft (Fig. 3) will be powered by two high-efficiency solar panels with a wingspan of 50 m. Currently, there are 11 instruments on a 6.4-m tall vehicle with

a 3-m diameter high-gain antenna for data downlink and communications with ground control (see Clark et al., 2011 and Bayer et al., 2015 for more details). The spacecraft will be launched from the Kennedy Space Center in 2022 or 2023 (Clark et al., 2015; JPL–EC Workshop, Nov. 2018, Pasadena, CA) on either NASA's Space Launch System (SLS) or the Space–*X* Falcon Heavy rocket.

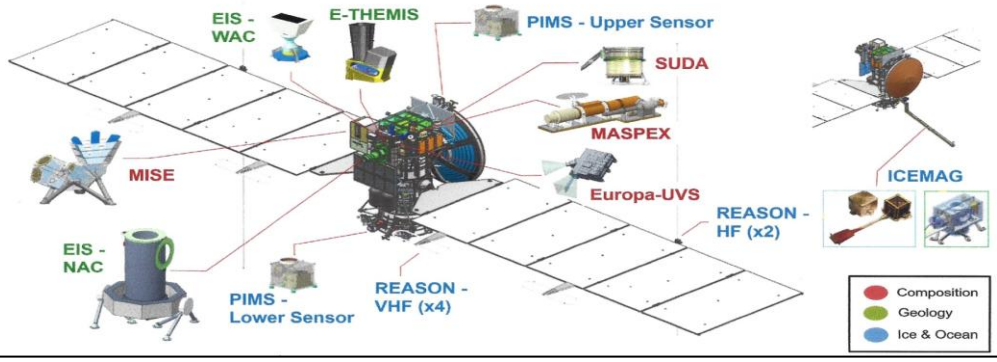

Fig. 3. The Europa Clipper mission is an orbiter with two primary goals: (1) a science campaign to characterize how the internal core, mantle, ocean and ice interact; and (2) a survey campaign to map the surface of Europa for a future lander mission. Instrument abbreviations are defined in Clark et al. (2011).

At the top of the EC spacecraft (Fig. 3) there is a location called the ***EC Vault*** (Fig. 4; closeup) that has extra IRAD shielding to protect sensitive electronics and instruments from the harsh radiation environment in Jovian space (Clark et al., 2011; Bayer et al., 2015). The *Vault* (yellow box in Fig. 4) will be discussed again when we outline the ECMS model (Section 2.7).

The Jovian trapped radiation environment during the EC's primary science mission phase of 3 years will be ~2.7 Mrad (27 kGy) for the overall spacecraft, but will be reduced within the *Vault* to 150 krad (1.5 kGy) (Bayer et al., 2015). Protons and electrons will comprise the majority of the ionizing radiation in the Jovian system (Nenon et al., 2018a) with small contributions from the isotropic space radiation environment including X-rays, gamma-rays (γ-rays), neutrons, solar particle events (SPEs), galactic cosmic rays (GCRs), and the so-called HZE particles (high-charge and high-energy particles; e.g., Fe) (Section 2.2).

And lastly, mission planners are considering two possible trajectories (Fig. 5) of the

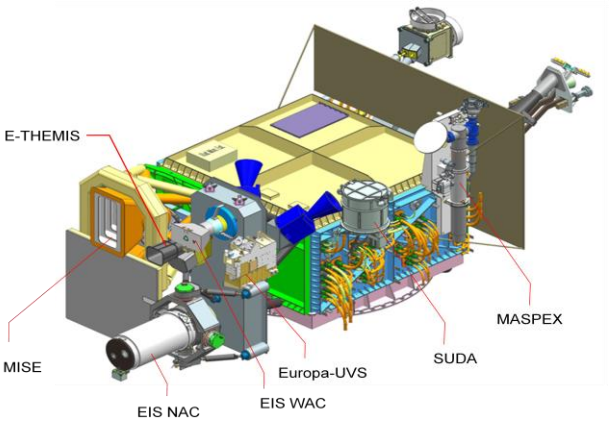

Fig. 4. The Europa Clipper Vault (yellow box) will be the most IRAD-protected locations during the primary mission in Jovian space. [Clark et al., 2011]

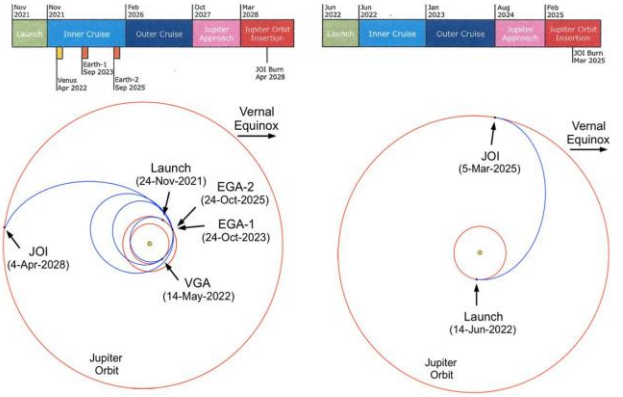

Fig. 5. The *VEEGA* trajectory (left) and the *Direct* trajectory (right) are being considered for the Europa Clipper mission. [Buffington, 2014]

Europa Clipper vehicle; the first is called the *Direct* trajectory (Fig. 5; right; from Buffington, 2014) that would launch in either 2022 or 2023 and arrive ~2.6 years later. The second is called the *VEEGA* trajectory (Fig. 5; left) because it would use Venus-Earth-Earth flybys as gravity assists. The desired trajectory is the *Direct* route because it gets the EC spacecraft to Europa quicker, but the *Direct* trajectory requires the use of the larger Space Launch System, which may not be ready for the 2022 launch window (https://spaceflightnow.com). We will consider both trajectories for designing the biocidal experiments with the four key space factors (see Section 2.6), and for the development of the ECMS model (see Section 2.7).

2. Proposed Research

2.1. Microbial Procedures

The bacteria chosen for the research are as follows: (1) *Bacillus subtilis* 168 (model lab standard with numerous studies published on its tolerance to space conditions [e.g., Horneck et al., 2012] and IRAD [e.g., Moeller et al., 2007; 2008]); (2) *B. pumilus* SAFR-032 (UV-resistant *Bacillus* sp. from the Jet Propulsion Lab; Link et al., 2004), (3) *Bacillus* ATCC 29669 (current NASA standard for a heat-tolerant indicator species; NASA Procedural Requirements NPR 8020.12D; Schubert and Beaudet, 2011); and (4) *Deinococcus radiodurans* R1 (strongly resistant to UV [Dartnell et al., 2010] and ionizing radiation [Paulino-Lima, et al., 2010]).

Spores of the *Bacillus* species will be grown in a liquid-sporulation medium and prepared as described by Mancinelli and Klovstad (2000). Log-phase vegetative cells of *D. radiodurans* will be grown and maintained as described by Dartnell et al. (2010). Spores will be quantified by optical density (OD) to the required concentrations (in general ~2 x 10^7 spores or cells per mL) and 100 µL applied to individual aluminum coupons made of Al-6061 and coated with a chromium-oxide spacecraft treatment called ChemFilm (mil spec C-5541F). The final density of spores/cells per aluminum coupon will be ~2 x 10^6 in a spot ~1-cm in diameter. Schuerger has developed a simple deposition protocol that creates uniform monolayers of individual spores or cells (Fig. 5) on aluminum coupons, which has been described several times (e.g., Schuerger et al., 2003; 2005; 2012).

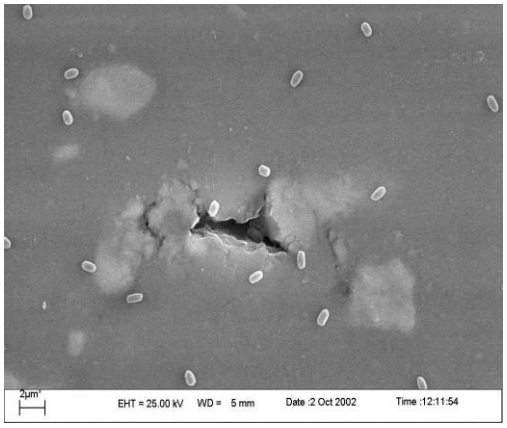

Fig. 5. Spores of the bacterium, *Bacillus subtilis*, adhered to aluminum-6061 coupons. [Adapted from Schuerger et al., 2005.]

Based on the spacecraft microbial surveys discussed above in Section 1.3, spores/cells are most likely to occur on spacecraft surfaces as individual desiccated spores or vegetative cells (non-spore forming species) (Fig. 5). We will verify the deposition protocols at the start of Objective 1 with standard SEM fixation and preparation protocols as shown in the example for Fig. 5 (Schuerger et al., 2005). Monolayers of spores/cells will be exposed to the *four key space factors* including vacuum (VAC; < 10^{-6} Pa), ionizing radiation (IRAD; 100 to 1000 Gy), temperature extremes (–120 to 140°C), and ultraviolet (UV) irradiation (Earth, Venus, Mars, asteroid-belt, and Jupiter solar constants) (see Section 2.6).

After exposure, spores/cells will be removed from the coupon surfaces by the application and peeling of thin layers of polyvinyl alcohol (PVA) films (Tauscher et al., 2006; Raguse et al., 2016). The PVA spore-removal technique will be repeated twice; which has been shown to be effective in removing ~98-99% of the deposited spores/cells (Tauscher et al., 2006). The PVA

"peels" will be placed in 1x phosphate buffered saline (PBS) and assayed with a Most Probable Number protocol described by Schuerger et al. (2003; 2005; 2006). Results will be plotted on Log-Linear scales in which the y-axes will be Log_{10} values of *N/No* for decreasing survivors in which the ending population (*N*) will be divided by starting population (*No*) for each replicate.

2.2. Space radiation

The space radiation environment that will be encountered during the EC mission consists of four main components: (i) trapped particles in the Jovian radiation environment (i.e., primarily charged electrons and protons); (ii) routine Solar Wind Particles (SWPs) dominated by protons (96%), helium ions (4%), and varying small amounts of heavier ions up to iron, (iii) episodic Solar Particle Events (SPEs) (i.e., solar flares), and (iv) isotropic Galactic Cosmic Rays (GCR) composed of various ionized nuclei (Benton and Benton, 2001; Reitz, 2008; Vaniman et al., 1993). However, fluence rates, associated doses, and particle/photonic energies vary wildly with location and time. For example, SWP nuclei normally exhibit energies in the range of 0.3 to 3 keV/nucleon and electron energies from 1 to 100 eV (Vaniman et al., 1993) [eV = electron volts]. The proton flux in the SWP stream can reach up to 3 $x10^8$ particles cm^{-2} s^{-1} and the absorbed dose can go as high as 30 kGy/d at 1 μm depth. Periodically, but unpredictably, SPEs can deliver very high doses of high-energy protons to a target that can approach several GeV (Benton and Benton, 2001).

The intense magnetic field around Jupiter traps very high levels of energetic protons and electrons in much the same way as Earth's magnetic field traps these particles in the Van Allen radiation belts (Benton and Benton, 2019). For example, proton energies around Jupiter can vary between 1.25 and 26.2 MeV in the magnetic equatorial regions but can drop off significantly in magnetic polar directions (Nenon et al., 2018b). And electron energies in the radiation belts of Jupiter can vary between 10 keV and 10 MeV (Paranicas et al., 2001). To avoid these high doses and high energies of protons and electrons, the EC spacecraft will conduct a series of 40-50 eccentric high–inclination flybys of Europa which will take the vehicle mostly into polar directions and into more tenuous radiation environments (Buffington, 2014). Second, the use of the *EC Vault* will help to shield sensitive electronics and instruments from the high fluence rates of ionizing radiation during the mission.

The reference radiation design constraint for the EC spacecraft has been set at 2.7 Mrad (27 kGy) behind 100 mil aluminum for external components of the vehicle, and 150 krad (1.5kGy) for the inside of the *EC Vault* (i.e., behind ~3/8" Al shielding; Bayer et al., 2015; Clark et al., 2001). These values (in Gy) are important to remember while reading the radiation experiments discussed below because research has established that many ionizing radiation sources can kill all spores of *B. subtilis* 168 at ~ 5 kGy (e.g., Moeller et al., 2010). Thus it is plausible that several *lethal doses* (*LD*) (defined as a –10 Log reduction by Schuerger et al., 2019) of ionizing radiation may be accumulated during the lifetime of the EC vehicle.

The microbiology experiments outlined below will adjust the diverse ionizing radiation sources (Table 2) to appropriate dose rates (# of particles hitting the spores per minute) and the particle energies (the energies of individual particles) to reflect the space radiation environment during the cruise phase to Europa and within the Jovian system. It is beyond the scope of any specific project to test all possible combinations of ionizing radiation, other biocidal space factors, several microorganisms, spacecraft hardware, and spacecraft positions within the Solar System. Thus, we will be guided by the logic that the Europa Clipper spacecraft will encounter a series of transitions away from the Sun during its cruise (i.e., decreasing dose rates of SWPs, SPEs, UV, solar-heating), and then second, by the ionizing radiation environment at Jupiter.

2.3. Ionizing Radiation Sources

To develop a comprehensive ECMS model for ionizing radiation, we plan on using a number of IRAD facilities and resources. The experimental approach will be to explore the 100 – 1000 Gy dose range; with particle or photonic energies up to 100 MeV(see Table 2). Initially, the experiments will test the interactive effects of VAC and IRAD alone and in combination; but then be expanded to concurrently test all four biocidal space factors (i.e., VAC, IRAD, TEMP, and UV) in combinations that are aligned to (1) simulating the cruise phase to Jupiter, (2) the Europa radiation environment, and (3) spacecraft shielding.

The experiments will utilize readily accessible IRAD sources within a 2-3 hour driving range for the PI Schuerger and Co-I's Enqvist and Samant. Secondary particles (i.e., bremsstrahlung photons and particles; Paranicas et al., 2002; Reitz, 2008) will be simulated using low-dose X-rays and γ-rays with the programs listed below. We have budgeted ~20 hrs per IRAD source per year for actual beam time. Experiments with HZE particles, and extremely high SPE fluence rates and energies, are beyond the scope of the current project.

Table 2. Ionizing radiation sources, locations, and managers (see Appendix A for photos).

Radiation	Source/Facility	Fluence/dose	Energies	Contact/coordinator
• Protons	UF-Proton Therapy Institute (UF-PTI)	• 1-10 Gy/min	• 1 – 100 MeV	Dr. Z. Li (fee-based.)
• Electrons • X-rays	UF-Shands Hospital	• 4-40 Gy/min • 6-14 Gy/min	• 6 – 20 MeV (*e–)* • 6-18 MeV (X-rays)	Dr. S. Samant (Co-I)
• Neutrons	UF Training reactor (UFTR)	• up to 1 Gy/sec	• thermal neutrons: ~1 MeV	Dr A. Enqvist (Co-I)
• γ-rays	Irradiators; UFTR	• Co-60, Cs-137 up to 1 Gy/sec	• 1+ MeV, 0 – 2 MeV	Dr. A. Enqvist (Co-I)

Protons will be generated by a ProteusPLUS system (model S2C2, IBA Proton Therapy Systems, Reston, VA, USA) located at the University of Florida (UF) Proton Therapy Institute, Jacksonville, FL (managed by Dr. Zuofeng Li). The proton delivery system can be gimbaled in 370-degrees of freedom in most of 7 axes making any sample configuration easy to irradiate with a uniform beam. Electron and X-ray emissions can be achieved from the same instrument (Synergy-Linac System, Elekta, Stockholm, Sweden) that is located at UF Shands Hospital, Gainesville, FL (Co-I Dr. Sanjiv Samant, coordinator). The neutron source is the UF Training Reactor (UFTR) located in the Nuclear Science Building on the UF main campus in Gainesville, FL (Co-I, Enqvist, coordinator). And lastly, two γ-ray sources (cobalt-60 and cesium-137) are available in the Nuclear Science Building (UF main campus) and will be accessible for this research through Co-I Enqvist; who will also coordinate (with UF radiation safety officers) the radiation safety procedures, badging, and training for access at all UF facilities.

Dose verification will be achieved utilizing high-dose ThermoLuminescent Dosimeter (TLD) badges, Optically Stimulated Luminescent Dosimeters (OSLD), or diode/MOSFET dosimeters (Bourland, 2016). Many of the radiation sources are also characterized with facility-appropriate software estimating particle transport and ultimately dose. We propose utilizing the Monte Carlo Codes MCNP, GEANT4, PHITS (Agostinelli et al., 2003; Tatsuhiko et al., 2018; Werner et al., 2018) as needed for various radiation sources both for estimating primary radiation flux and dose, but also to track secondary radiation doses, which might not always be measured

by the dosimeters depending on particle types and radiation type ratios. Additional flux and dose monitoring will be achieved with handheld instrumentation, especially at lower fluence rates at facilities like UFTR where dose scales with power (i.e., flux). TLDs, OSLDs, or diode/MOSFETs will be placed within the Radiation Exposure Chamber (REC units; see below) prior to conducting assays under the IRAD beams.

2.4. Planetary Atmosphere Chamber

A previously described Mars Simulation Chamber (MSC; Fig. 6 here; Schuerger et al., 2008; 2012) will be modified to simulate the higher vacuum of the interplanetary environment, and is renamed here as the Planetary Atmosphere Chamber (PAC) to distinguish between the two operational configurations. The original MSC system was capable of achieving 1 Pa (0.01 mbar) of low pressure using a Varian SH-100 scroll pump. In order to achieve lower pressures of approx. $\leq 10^{-6}$ Pa (10^{-8} mbar), we have budgeted for a Turbo-Pump System (TPS) from Agilent Technologies (Appendix B; March 2019 quote).

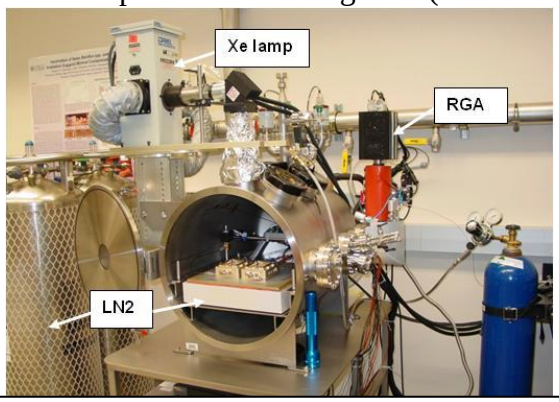

Fig. 6. The PAC system in Schuerger's lab. LN2 = liquid nitrogen system; Xe =xenon UV lamp; and RGA = Residual Gas Analyzer. [Schuerger et al., 2008; 2011.]

Ultraviolet (UV), Visible (VIS), and near-infrared (NIR) irradiation is produced with one, 1000 W xenon-arc lamp that can be easily calibrated to deliver UV fluence rates at specific astronomical units (AU) between Venus and Jupiter. The UV dosage range will be calibrated at the following distances from the Sun: 0.7 AU (Venus; *VEEGA* trajectory), 1 AU (Earth LEO), 1.524 AU (Mars), 3 AU (asteroid belt), and 5.2 AU (Jupiter). This range of distances, and thus, UV intensities will represent a set of cruise phase simulations for the Europa Clipper spacecraft regardless of the trajectory taken. And the temperatures will be maintained at the predicted levels at each AU (see de Pater and Lissauer, 2001).

The PAC system will be able to accurately simulate four key components of the interplanetary environment including: (a) pressures down to 10^{-6} Pa, (b) UVC, UVB, and UVA irradiation from 190 to 400 nm and from 0.7 to 5.2 AUs, (c) temperatures from –150 to 140°C, and (d) the desiccating conditions that are inherently present under high vacuum ($a_w < 0.02$). Circular Al-coupons doped with *Bacillus spp.* spores or *D. radiodurans* cells will be placed in sterilized aluminum sample holders (Fig. 9; Schuerger et al., 2003; 2012) set on top of a LN2 system, and exposed to VAC, TEMP, and UV conditions, alone or in combination (see below).

2.5. Radiation Exposure Chambers (REC units)

Sample holders for experiments with ionizing radiation must be built that are capable of (1) holding an internal pressure of 10^{-6} Pa, (2) holding 16 circular aluminum coupons, and (3) permit the penetration of all ionizing radiation types and UV photons through a transparent window. We will model the new REC units after a system used for the MASE project; called a Trex-Box (Fig. 7; Beblo-Vranesevic et al., 2017). We have previously built very similar pieces of hardware for CH_4 production experiments with UV irradiation of organics (Schuerger et al., 2011) and have a quote from the same machine shop used in the Schuerger et al. (2011) study to build 12 REC units (see Appendix C).

The glass ports in the upper lids of the REC units will be made of 3-mm thick fused silica glass (manufactured by Maier Photonics, Inc., Manchester Center, VT, USA) that have been previously shown to withstand a pressure differential of 101.5 kPa for 7 days without losing internal low pressure or cracking (Schuerger, unpublished). The 12 REC units will be loaded with bacterial samples, pumped down to 10^{-6} Pa, safely stored in a foam-insulated Pelican case (www.thepelicanstore.com), transported to the ionizing radiation sources, and exposed according to the protocols outlined below. After exposure, the REC units will be returned to Schuerger's microbiology lab for processing. This design will permit us to expose 4 replicates of up to 4 bacterial strains at one time (16 total coupons per REC), thus, greatly speeding up the data generated by the interactive experiments described below.

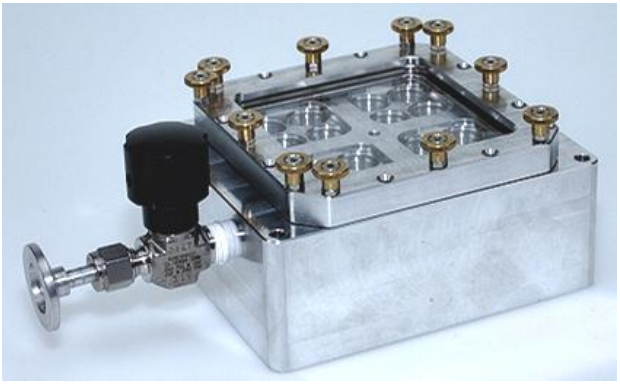

Fig. 7. The MASE ***Trex Box*** used previously by Beblo-Vranesevic et al. (2017) for transferring microbial samples between ionizing radiation sources and microbiology labs under diverse anaerobic conditions. (Photo courtesy of DLR website: https://www.dlr.de.)

2.6. Experiments to Characterize synergistic effects of VAC, IRAD, TEMP, and UV

The Objectives in Section 1.2 list four key biocidal space conditions that will be tested alone or in combination. However, there are 24 possible combinations of these space conditions, and it is beyond the scope of the page limits here to describe each combination in detail. Thus, we will present below three examples that are aligned with the Objectives in Section 1.2.

2.6.1 **Objective 1:** *What are the interactive effects between VAC ($\leq 10^{-6}$ Pa) and IRAD sources (e.g., protons, neutrons, electrons, X-rays, and γ-rays) for inactivating bacteria?* [Years 1 and 2]

The 1st series of experiments will explore the interactive effects of VAC on 5 ionizing radiation sources including X-rays, γ-rays, electrons, protons, and neutrons (Table 1). Spores of the *Bacillus* spp. will be prepared as outlined in Section 2.1, and applied to the coupons so that the monolayers have no aggregates (similar to Fig. 5). Four replicate coupons per species will be placed within sample holders within REC units (Fig. 7), the REC units sealed, pumped down to 10^{-6} Pa (using an independent high-vacuum pump; on-hand), and transported to the IRAD sources. Due to the logistics of having most ionizing radiation sources located in different cities and buildings (Table 1), the primary protocol will be to hold the VAC at $\leq 10^{-6}$ Pa during transport (to be checked and adjusted using a portable high-vacuum pump), expose the cells and spores to dosages of each ionizing source (e.g., 100, 400, 800, and 1000 Gy), and then process all samples for viability as described in Section 2.1. We will select the particle and photonic energies that are averages of the space environment in which the Europa Clipper will be traveling enroute to the Jovian system. For example, in orbit around Jupiter, the energies of the protons range between ~5 and ~100 MeV (Nenon et al., 2018b), and thus, we will use up to 100 MeV as the exposure energies during the VAC + Proton assays.

Treatment scheme: (1) Lab-controls (LAB) that will not be exposed to VAC or IRAD conditions (2) VAC alone (10^{-6} Pa), (3) IRAD alone (each IRAD source done separately), and (4) IRAD +VAC. The LAB samples will be held in non-REC units (see Fig. 9) to reduce the

cost of building four additional REC units; but the LAB controls will be co-transported with the REC units. Once the REC units are exposed to IRAD dosages, all samples will be returned to Schuerger's microbiology lab for processing. This is the basic protocol that all of the experiments described below will follow.

The experimental design will be a Completely Randomized design (i.e., all experiments), and the assays will be conducted twice (n = 8). The statistical approach will be to plot the data on Log-Linear graphs in which the Log_{10} of *N/No* (*N* = post treatment survivors and *No* = Time-zero control survivors) will be on the y-axes. All datasets for each IRAD source will be analyzed by ANOVA + Protected LS-mean Separation tests and by Linear Regression using a PC-based Statistical Analysis System [SAS] (version 9.2 or higher, SAS Institute, Inc., Cary, NC, USA).

Most published results with ionizing radiation against the survival of *Bacillus* spp. spores applied as dried monolayers on spacecraft materials exhibit linear trends with negative slopes and without shoulders (e.g., Moeller et al., 2008; 2012). Thus, we anticipate that the results from the four treatments listed above for each ionizing radiation source applied singularly will yield easily interpretable linear models. If the lethality of the combined effects of VAC and IRAD are synergistic, then the results should be observable as a significantly faster inactivation rate for the VAC + IRAD treatment compared to either VAC-alone or IRAD-alone treatments. If the results indicate either no-interactions, or a minimal additive effect, the separation between each treatment will be narrower (i.e., slopes and y-intercepts of linear models will be similar).

2.6.2 **<u>Objectives 2</u>***: What are the interactive biocidal effects of VAC (≤ 10^{-6} Pa) when tested against either TEMP extremes (–120 to 140°C) or UV irradiation extremes (Venus to Jovian space) for inactivating bacteria?* [Years 1 and 2]

Concurrent with Obj.-1, we will use the PAC system in Schuerger's lab (Fig. 6) to characterize the interactive effects of VAC, TEMP, and UV in a series of assays in which the samples are not exposed to ionizing radiation. The primary justification for the experiments without IRAD was discussed in the Lunar Microbial Survival (LMS) model by Schuerger et al., (2019) (Fig. 2). Namely, Schuerger et al. demonstrated that there was a synergistic interaction between low pressure (10^{-4} Pa) and high-temperature (100°C) when used simultaneously to expose spores of *B. subtilis* HA101. When either parameter was used alone, spore viability remained much higher by comparison. Thus, a basic premise of the research outlined herein is that there are other synergistic interactions between VAC, TEMP, and UV that have not yet been characterized by the astrobiology community.

For example, in Horneck et al. (2012), the combination of VAC and UV during a flight experiment on the ISS added approx. –3 Logs (i.e., a 3 Log decrease in survivors) to the lethality of the UV exposures against *B. subtilis* 168 spores. But the experiments here will probe this relationship further by holding temperatures at a wide range of extremes (e.g., –120, –80, –40, 0, 40, 80, 100, or 140°C while also exposing cells to VAC alone, or with VAC + UV (see below). The goal will be to characterize the interactive effects of VAC, TEMP, and UV irradiation on bacterial survival in space. We expect to develop mathematical relationships from these interactions in the ECMS model phase of the project (Section 2.7), and more accurately predict the combined effects of the space environment on microbial survival.

In addition, once the VAC + TEMP interactive effects are characterized, a subset of the temperatures will be retested in combination with VAC + UV irradiation. The UV dosage range will be simulated at the following distances from the Sun [in astronomical units; AUs]: 0.7 AU (Venus), 1 AU (Earth LEO), 1.524 AU (Mars), 3 AU (asteroid belt), and 5.2 AU (Jupiter). This range of distances, and thus, UV attenuation will represent a cruise phase for the Europa Clipper

or future Europa Lander spacecraft. And TEMP will be maintained at the predicted levels at each AU (see de Pater and Lissauer, 2001).

2.6.3. <u>Objective 3</u>: *What are the effects of the space stressors (e.g., VAC, IRAD, TEMP, UV) for inactivating spores of of Bacillus subtilis 168 in simulations of a EC cruise phase to Europa?* [Year 3]

The number of treatment endmembers will start to exceed the support in time and supplies budgeted herein. Thus, in Obj. 3, we will create simulations of an interplanetary cruise phase scenarios at Venus (0.7 AU), Earth (1 AU), Mars (1.524 AU), the Asteroid belt (3 AU), and Jupiter (5.2 AU). The simulations of the environmental conditions will be based on the modeling work for the Europa Clipper mission (e.g., Bayer et al., 2015; Buffington, 2014; Clark et al., 2011; Nenon et al., 2018a; de Pater and Lissauer, 2001; to mention only a few), and by space radiation studies (e.g., Badhwar et al., 1994; Benton and Benton, 2001; Reitz, 2008). In order to have the treatment combinations reasonable, we will focus on the bacterium *B. subtilis* 168 for these studies. The choice of 168 is based on the wide range of studies published on this strain that involve flight experiments, vacuum, ionizing radiation, UV, and temperature extremes (see Section 2.1; and reviews by Horneck et al., 2010; Nicholson et al., 2000).

Let us assume that the data from Objectives 1 and 2 demonstrate that combination of all four biocidal factors greatly exceeds any individual biocidal factor. Then, we would create simulations at the AU distances from the Sun listed above and expose *B. subtilis* 168 spores to the space conditions in the following order (a) VAC + UV + TEMP conducted simultaneously in the PAC chamber in Schuerger's lab for 3-days, (b) transport the REC units to a series of IRAD exposures (Table 2) at UF (X-rays, γ-rays, electrons, and neutrons), and (c) then at the UF-PTI in Jacksonville, FL. Controls would be built into the experiment such that we could factor out any travel effects, and individual parameter effects. After the final exposure, the samples in the REC units would be returned to Schuerger's lab for processing. But keep in mind, that the experiment (with travel) might take between 10-14 days to complete per iteration; and then there will be 12 REC, units plus controls, multiplied by 4 coupons of *B. subtilis* 168 spores per REC (80+ samples), so another week for processing. And this series would be for only one iteration of Exp-1 (e.g., interplanetary space at 1.524 AU for Mars). The experiment would be repeated at least once. Then the whole series would be conducted for the other AU's listed above.

In summary, Objective 3 is a very complex experimental setup, and we expect that a full year will be required to properly conduct the experiments briefly outlined here. However, the datasets should be the most comprehensive datasets on how a combination of biocidal factors will contribute to the inactivation of bacterial spores during a planetary mission to Europa.

2.7. Europa Clipper Microbial Survival (ECSM) model

<u>Objective 4</u>: *Construct a Europa Clipper Microbial Survival (ECSM) model for predicting the survival rates of bacterial spores during the cruise phase to Jupiter and during the primary mission in Europan orbit.* [Year-3]

The overarching goal of the experiments on the interactive effects of VAC, IRAD, TEMP, and UV is to generate quantitative empirical data that can be used to populate a Europa Clipper Microbial Survival model (e.g., Fig. 8). The ECMS model will be split into two parts: (1) a cruise-phase ECMS model, and (2) a Europa orbit primary mission ECMS model. Both parts will give a complete picture on how the four biocidal space factors will affect the launched

EC bioburdens, and should reveal whether the internal EC surfaces (including the *Vault*) will be sterilized prior to reaching Europa, or shortly thereafter.

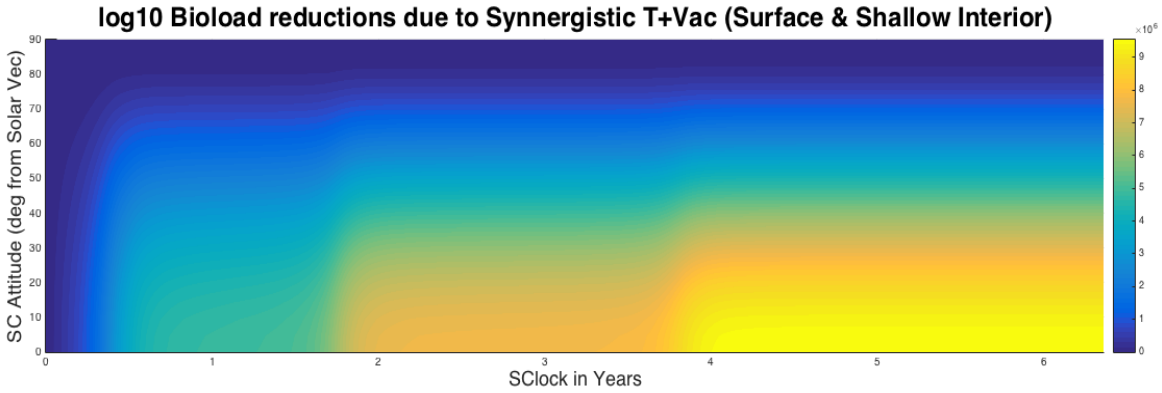

Fig. 8. A preliminary run with a draft ECMS model suggests that surfaces directly facing the Sun on a *VEEGA* trajectory will receive enough solar heating to accumulate a theoretical biocidal dose up to 9 x 10^6 Log_{10} reductions. [Moores and Schuerger, unpublished.]

The ECMS will be based on the Lunar Microbial Survival (LMS) model described in Section 1.4. The LMS model separates the architecture of Lunar spacecraft into (i) external surfaces illuminated by the Sun (hit by VAC, IRAD, high-UV, high-TEMP effects), (ii) shallow internal surfaces in thermal contact with the exterior of the vehicles (hit by VAC and high-TEMP effects), and (iii) deep internal surfaces thermally isolated from the exterior (hit by low-TEMP and VAC-only effects). The LMS model did not attempt to include secondary-particle IRAD effects for deep interior surfaces. Results of the LMS model indicate very high biocidal rates for both external and shallow internal surfaces of landed spacecraft (Table 1), but very slow kill rates for deep interior surfaces (hit by VAC only in the LMS model). The ECMS will divide the EC spacecraft up into these three regions and model them separately during the course of the mission. Furthermore, we believe that the IRAD experiments at low-dosages will provide data to model the effects of secondary particles (i.e., bremsstrahlung photons and particles; Paranicas et al., 2002; Reitz, 2008) and VAC on surviving bioburdens within the *EC Vault*; something not included in the LMS model.

For example, if we assume the EC spacecraft will follow the *VEEGA* trajectory, then Fig. 8 depicts the accumulation of biocidal doses for VAC + high-TEMP on external surfaces (Moores and Schuerger, unpublished). First, notice that each time EC approaches the Sun for the Venus-Earth-Earth gravity assist flybys (i.e., upticks in yellow color at 0.4, 1.75, and 3.8 years), the accumulated lethality (measured in Log_{10} reductions on the right-side axis) approaches the potential for >9 x 10^6 Log_{10} reductions (or > 9 x 10^5 *LD*s) prior to arriving at Europa; essentially sterilizing the external solar-facing surfaces by any definition of the word, sterilize. However, the EC spacecraft will have a very complex architecture in which not all components will be exposed directly to the Sun (Fig. 8; dark-blue dose rates at a 90° angle to the Sun; left axis). Thus, the other biocidal factors of VAC-alone, IRAD (isotropic effects), diverse TEMP + VAC, and reflected UV+VAC must be invoked to reduce bioburdens in off-nadir solar surfaces.

Each of the interactive effects (alone or with observed synergisms) will be integrated into a numerical model to be implemented using MATLAB software (version 9.4, Mathworks, Natick, MA, USA). The ECMS model will divide (1) the spacecraft into external, shallow internal, and deep internal surfaces (*sensu* the LMS model), (2) the cruise and Europa primary mission phases into 30-day intervals (i.e., to align with the LMS model) as, (3) the EC spacecraft

moves away from the Sun, and (4) solar-orientation of the spacecraft. Thus, the TEMP and UV factors will change with time, solar-angle, and distance from the Sun. The factors VAC and IRAD will be assumed to be isotropic and relatively constant during the course of the mission.

The goal of this project will be to accumulate quantitative data so that accurate predications can be made on ***all*** surfaces of the external, shallow-internal, and deep-internal niches of a complex spacecraft like the Europa Clipper enroute to Jovian space via one of two possible trajectories. Results should be applicable towards developing pre-launch planetary protection cleaning protocols, enroute operations to increase the lethality of interplanetary space, and predict if the *EC Vault* will achieve the *Pc* of $< 1 \times 10^{-4}$ prior to orbital insertion.

3. Expected Results and Conclusions

The NASA Procedural Requirement NPR 8020.12D; section 5.4 (see Reference for website; Bayer et al., 2015) states: "*Requirements for flybys, orbiters, and landers to icy satellites, including bioburden reduction, shall be applied in order to reduce the probability of inadvertent contamination (Pc) of an ocean or other liquid water body to less than 1 x 10^{-4} per mission*…." Using the diverse, quantitative, and empirically generated data outlined above, our goal will be to develop a ECMS model to make such predictions for specific times of the flight profile, specific locations on and within the spacecraft (including the *EC Vault*), and for specific mission operations that might be developed to ensure mission safety (i.e., high-inclination flybys of Europa versus stable parking orbits).

The expected outputs of the project are two-fold. First, each series of experiments outlined above for the four key objectives is likely to generate 1-2 peer-reviewed papers in which the survival data are presented for individual and combined effects of the four space conditions tested. Second, the data generated by the VAC, IRAD, TEMP, and UV experiments will then be used to develop a mathematical model (i.e., the ECMS model) on the inactivation processes for spacecraft bacteria over the course of the Europa Clipper mission. The ECMS model will likely to generate at least 2 peer-reviewed papers; one for the EC cruise phase, and a second paper on the primary mission phase around Europa.

The LMS model was based on accumulating *lethal doses* (defined as –10 Log-reductions in bioburdens; Schuerger, Moores, et al., 2019) that were estimated on a ***per lunation*** basis (29.53 days). For the ECMS model, we will break up the intervals into 30-day increments such that mission planners can examine the biocidal nature of the space environment on a month-by-month basis. The ECMS is expected to also predict when the $Pc < 1 \times 10^{-4}$ is reached for all types of locations on and within the EC spacecraft, including the IRAD-shielded *EC Vault*. Such *Pc* predictions in time, using the combined data for biocidal kill curves for VAC, IRAD, TEMP, and UV (alone and in combination) has not been previously developed.

This research is critical and extremely timely for the exploration of icy moons because of the expected near-term development and launch of the Europa Clipper orbiter in 2022-2023. The datasets expected here and the ECMS model should make an immediate impact on the development of the EC orbiter, and will be useable in real-time during the mission to make new and unexpected predictions as operations unfold. And lastly, the results of these experiments may indicate how to proactively increase the lethality in a specific location within the spacecraft or a specific operation in the vehicle. For example, data on the VAC+TEMP interactions might indicate that we can accelerate the sterilization of the *Vault* by increasing the internal temperatures from –40 to + 20°C, with no loss of function and with little extra consumption of power. Second, the VAC+UV data might indicate that by adding a few biocidal UV LEDs (Hollington et al., 2015) to the internal void space within the *Vault* we might help sterilize the

Vault (due to VAC+UV interactions) prior to orbital insertion around Europa. And finally, the VAC+IRAD datasets might demonstrate that the expected low dosage rates of IRAD alone would not sterilize the *Vault*, but interactions between VAC+IRAD+TEMP will.

In summary, the proposed research has an experienced team in microbiology and astrobiology (Schuerger; 25+ yrs), ionizing radiation (Enqvist, Samant, Li; 30+ yrs) and numerical modeling (Moores; 16+ years). Our goal will be to generate new empirical data on the biocidal effects of vacuum, ionizing radiation, extremes in temperature, and UV irradiation in order to develop a quantitative Europa Clipper Microbial Survival model that will provide critical information likely to improve mission success. We anticipate that the ECMS model – like the LMS model (Table 1) – will predict extremely high biocidal kill rates for external and shallow internal surfaces of the EC spacecraft, and may demonstrate that the interactive effects of the four biocidal space conditions will render the internal surfaces of the *EC Vault* sterilized (meeting the $Pc < 1 \times 10^{-4}$ for the mission) prior to orbital insertion, or shortly thereafter. In either case, the Europa Clipper mission specifically, and all icy moon missions in general, will benefit from the expected results from the current project. We hope you agree.

4. Management Approach and Research Timeline

The PI **Andrew Schuerger** (UF) will oversee all aspects of the project including (but not limited to): budgets, experimental design, hands-on experimental work, safety of IRAD operations in conjunction with other lab managers (Table 2), microbiology procedures (with research assistant), statistical analysis, and preparation of all manuscripts. Co-I, **Andreas Enqvist** (UF), will operate the UF Nuclear Training reactor, cobalt-60, and cesium-137 systems on the main campus in Gainesville, FL and assist the team to design the equipment to insert the REC units into the UFTR reactor (for neutrons and γ-rays). Enqvist will also be the safety coordinator for the IRAD sources. All IRAD sources are owned and operated by the University of Florida, and as such, we have institutional support to ensure precise and safe operations of all radiation instruments. Co-I, **Sanjiv Samant** (UF), will coordinate the IRAD experiments using the electron and X-ray source instrument at Shands Hospital in Gainesville, FL. There is one additional radiation specialist (**Zuofeng Li**; UF) who has agreed to assist us in the use of the proton source generator at the UF Proton Therapy Institute in Jacksonville, FL. And finally, Dr. **John Moores** (Collaborator; York U. in Toronto) has agreed to be the lead on developing the ECMS model once the rest of the team sends him the empirical data for the interactive effects of VAC, IRAD, TEMP, and UV on bacterial survival.

Initial experiments will focus on VAC + IRAD experiments (1^{st} priority) and VAC + TEMP+UV experiments conducted concurrently throughout Years 1 and 2. The VAC+IRAD assays will use the REC units to move bacterial spores and cells (held at 10^{-6} Pa) around to the five ionizing radiation sources at UF, but the VAC+TEM +UV assays will be conducted exclusively in the PAC chamber in Schuerger's lab. Furthermore, Schuerger has budgeted to hire a full-time Research Assistant (B.S. or M.S. level) for the research to help with the large amount of samples and microbial processing. Year-3 activities will shift the microbial survival assays to creating cruise-phase simulations for both the *Direct* and *VEEGA* trajectories for the EC spacecraft, with REC units exposed to VAC+TEMP+UV in the PAC chamber and then moved to the IRAD sources in sequence. Concurrently, Year 3 will also include the development of the ECMS model (Obj. 4) from the data derived in Objectives 1, 2, and 3. Completing all four Objectives is ambitious but doable within the budgets and time limits set out herein, and will provide unique data to ensure the success of both the Europa Clipper orbiter and future lander missions.

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