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## Abstract

- Microorganisms can survive harsh conditions, such as low nutrients, desiccation, high temperatures and pH, high pressure and many more. **Extremophiles** are intriguing because of their unique adaptations to severe conditions and capacity of survival.
- We have characterized the **radiation resistance** of extremophilic fungi under **UV<sub>254</sub>** and **simulated Martian UV conditions**. These fungal strains originated from the 1986 Chernobyl nuclear power plant accident and the International Space Station (ISS) HEPA filter.
- Purified conidia (~10<sup>6</sup>/coupon) of 13 exposed fungal isolates were placed in monolayers and air-dried on high-grade aluminum (Al 6061-T6) coupons and exposed to DNA-damaging 254-nm UV irradiation and simulated Mars UV (200 to 400 nm) conditions.
- The surviving fungal isolates were further processed for whole genome, proteome, and transcriptome analyses. The secondary metabolite (SM) profiles of these fungal variants were compiled.
- The results will be used as selection criteria for the upcoming CASIS flight experiment in which selected isolates will be exposed to microgravity utilizing ISS to facilitate natural product production.

## ISSF 21 characteristics

### 1. Phylogeny

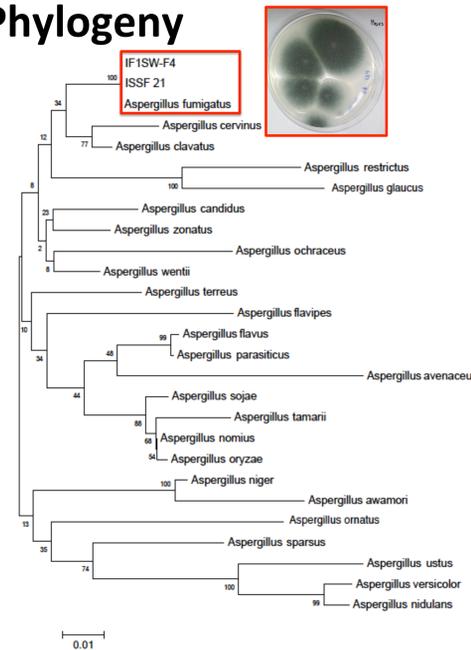


Figure 3. Phylogenetic tree representing *Aspergillus fumigatus* isolated from ISS based on ribosomal Internal Transcribed Spacer (ITS) region. ISSF 21 and IF1SW-F4 were isolated from HEPA filter and Cupola (MT1A) respectively.

### 4. Secondary metabolites profile

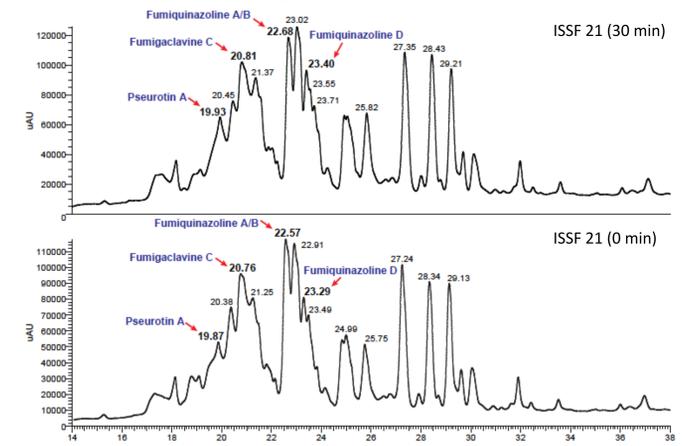


Figure 5. SM profiles of ISSF 21 strains (0 and 30 min Mars conditions) grown on CYA medium

## UV<sub>254</sub> resistance

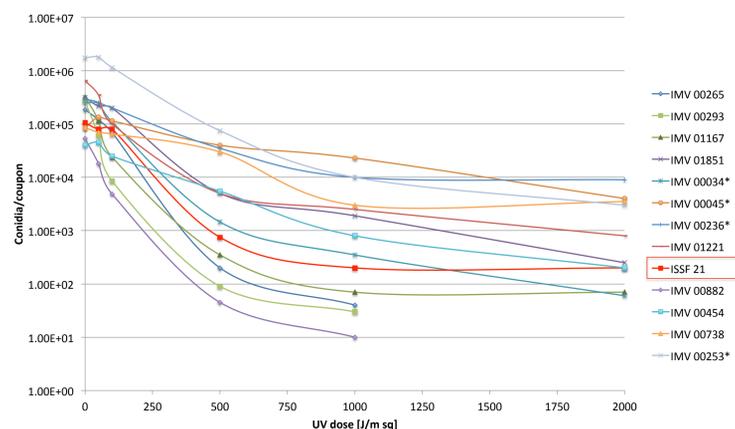


Figure 1. UV<sub>254</sub> characterization of extremophilic fungi from Chernobyl accident site and ISS

### 2. UV<sub>254</sub> resistance

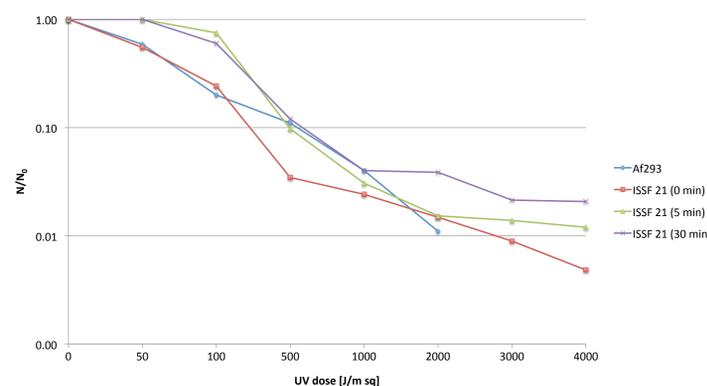


Figure 4. UV<sub>254</sub> resistance of *Aspergillus fumigatus* ISSF 21 strains surviving various Mars conditions (0, 5 and 30 min) and Af293 isolate

## Simulated Mars conditions survival

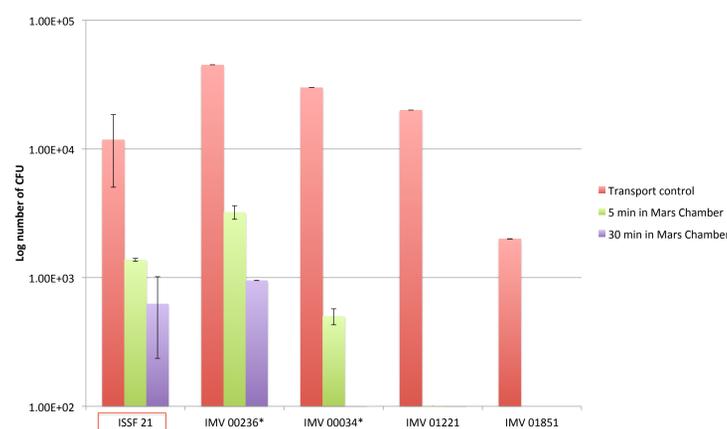


Figure 2. Isolates surviving exposure to simulated Mars conditions

### 3. Virulence genes (WGS)

<i>A. fumigatus</i> 293 type strain gene	Presence in the ISSF 21	Gene name	Gene type <sup>a</sup>	Actual or predicted product
Afu1g17200	+	<i>sidC</i>	NRPS	ferricrocin
Afu1g17740	+	-	NR-PKS	hydroxyferricrocin
Afu2g17600	+	<i>alb1(pkSP)</i>	NR-PKS	YWA1
Afu3g03350	+	<i>side</i>	NRPS	fusarinine C
Afu3g03420	+	<i>sidD</i>	NRPS	triacylfusarinine C
Afu6g09660	+	<i>gliP</i>	NRPS	gliotoxin
Afu6g12050	+	-	NRPS	fumiquinazolines
Afu6g12080	+	-	NRPS	fumiquinazolines
Afu6g13930	+	<i>pyr2</i>	NR-PKS	pyripyropene A
Afu8g00170	+	<i>ftmA</i>	NRPS	fumitremorgins
Afu8g00540	+	<i>psoA</i>	hybrid	pseurotin A

<sup>a</sup> Polyketide synthase (PKS), Non ribosomal peptide synthetase (NRPS), PKS-NRPS (Hybrid), Non reduced polyketide synthase (NR-PKS).

Table 1. Virulence genes present in ISSF 21 original and simulated Mars 30-min exposed strains in comparison to type strain *A. fumigatus* 293. Results based on the whole genome sequencing.

## Conclusions and Significance

- Among 13 Chernobyl and one ISS fungal strains exposed to UV<sub>254</sub>, 10 strains survived the dose of 2000 J/m<sup>2</sup> (Fig. 1).
- When exposed to simulated Martian UV (200 to 400 nm) and atmospheric conditions for 5 minutes (17.6 kJ/m<sup>2</sup>) only 5 strains survived. Extended Mars UV exposure for 30 minutes (105.6 kJ/m<sup>2</sup>) killed all but 2 species, one each from Chernobyl and ISS (Fig. 2).
- When re-exposed to UV<sub>254</sub>, *A. fumigatus* ISSF 21 Mars 30-min strain survived better than any other tested strains (Fig 4).
- Whole genome sequencing of both ISSF 21 strains (0 and 30-min Mars survivor) showed presence of the virulent genes that are reported in the wild type strain Af293 (Table 1). SNPs are under investigation.
- Comparison of SM profiles of ISSF 21 isolate (0-min) with the Mars (30-min) strain showed no significant difference in SM production. Both strains produced toxins like fumiquinazolines A, B and D. The level of the toxins was slightly higher in the Mars 30-min strain than in the ISSF 21 (0-min) isolate (Fig. 5).
- Elaborate characterization revealed that the ISSF 21 strain is a BSL-2 pathogen and hence not included in the CASIS flight project.

### The study is significant for various reasons

- Knowing the fungal resistance to UV<sub>254</sub> and simulated Martian UV conditions will help to understand potential implications of notional forward contamination of extraterrestrial environments during interplanetary missions, especially Mars.
- Exposing fungi to microgravity stress conditions may facilitate unique SM production of interest to the pharmaceutical industry, agriculture and beyond.
- Understanding the “omics” including the pathogenicity of the “problematic fungi” will help to develop countermeasure(s) to eradicate them in the closed habitat.

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