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Proteomic characterization of *Aspergillus fumigatus* isolated from air and surfaces of the International Space Station

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Abstract

The on-going Microbial Observatory Experiments on the International Space Station (ISS) revealed the presence of various microorganisms that may be affected by the distinct environment of the ISS. The low-nutrient environment combined with enhanced irradiation and microgravity may trigger changes in the molecular suite of microorganisms leading to increased virulence and resistance of microbes. Proteomic characterization of two *Aspergillus fumigatus* strains, ISSFT-021 and IF1SW-F4, isolated from HEPA filter debris and cupola surface of the ISS, respectively, is presented, along with a comparison to experimentally established clinical isolates Af293 and CEA10. In-depth analysis highlights variations in the proteome of both ISS-isolated strains when compared to the clinical strains. Proteins that showed increased abundance in ISS isolates were overall involved in stress responses, and carbohydrate and secondary metabolism. Among the most abundant proteins were Pst2 and ArtA involved in oxidative stress response, PdcA and AcuE responsible for ethanol fermentation and glyoxylate cycle, respectively, TpcA, TpcF, and TpcK that are part of trypacidin biosynthetic pathway, and a toxin Asp-hemolysin. This

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Author contributions

AB drafted the manuscript, contributed to sample processing, data analysis and interpretation. JR helped with sample processing and data interpretation. AC and MK conducted protein sample processing, LC/MS analyses, and data processing. KV and CW designed the study, interpreted the data, and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

report provides insight into possible molecular adaptation of filamentous fungi to the unique ISS environment.

Keywords

Aspergillus fumigatus ; proteome; the International Space Station

1. INTRODUCTION

The International Space Station (ISS) is a man-made closed habitat that functions as a platform to study the impact of the distinct space environment, which includes enhanced irradiation and microgravity on humans[1–7], animals[8,9], plants[10–14] and microorganisms[15–19]. Most experiments conducted on board the ISS are precisely planned. Studied organisms are intentionally sent to the ISS to investigate the possible alterations in their physiology, using ground controls for comparison. However, one on-going ISS Microbial Observatory (ISS-MO) experiment focuses on studying hitchhikers that have followed humans and cargo aboard the ISS[20]. Thorough investigation of microbiological characteristics of closed habitats, like the ISS, are indispensable to National Aeronautics and Space Administration (NASA), as manned long-term space flight missions are within reach. A deeper understanding of microbes that coexist in closed habitats with humans remains imperative to astronauts' health and the overall maintenance of closed systems.

Strict scrutiny of the microbiome and mycobiome of the ISS[20–25], Mir[26,27], and Skylab[28], in the past, has revealed prevalence of fungal genera: *Cladosporium*, *Penicillium*, and *Aspergillus* in space environments. These fungi can be both beneficial and detrimental to mankind, as they produce a myriad of commercially useful bioactive compounds[29–34], while also causing allergies[35], infections[36,37] and biodeterioration of habitats[38–40]. *Aspergillus fumigatus*, one of many fungal isolates identified in a recent ISS-MO study[22], is a ubiquitous saprophytic fungus[41]. Its enormous adaptation capacity enables it to not only be omnipresent in the environment, but also to be a successful opportunistic pathogen[42]. *A. fumigatus* causes variety of health conditions spanning from allergies to potentially life-threatening invasive aspergillosis (IA) in immunocompromised individuals[43,44]. Initial characterization of two *A. fumigatus* ISS-isolates, ISSFT-021 and IF1SW-F4, showed no outstanding differences in their genomes and secondary metabolites profiles when compared to clinical isolates CEA10 and Af293, however both isolates were significantly more lethal in a larval zebra fish model of IA[45]. Considering that *A. fumigatus* becomes more virulent in space and therefore potentially more dangerous to astronauts' health, it was pertinent to further investigate molecular changes of ISS-isolated strains.

Presented in this study are the unique differences observed in proteome of two ISS-isolated *A. fumigatus* strains, ISSFT-021 and IF1SW-F4, when compared to Af293 and CEA10. The goal of this study was to understand if the distinct environment of the ISS (low-nutrients, enhanced irradiation and microgravity) alters the proteome of *A. fumigatus*. Due to an existing gap in our understanding of how filamentous fungi molecularly adapt to space

conditions, proteome investigation of these two ISS-isolated *A. fumigatus* strains was prudent.

2. METHODS

2.1. Isolation and identification of *A. fumigatus*.

Procedures to isolate and identify *A. fumigatus* collected from the ISS were described previously[45]. In brief, HEPA filter associated particulates were scraped, resuspended in sterile phosphate-buffered saline (PBS; pH 7.4) and spread onto potato dextrose agar (PDA) plates[22]. Cupola surfaces were sampled with sterile polyester wipes assembled at Jet Propulsion Laboratory (JPL) prior to space flight. After each sampling event on board the ISS, wipes were returned to JPL for subsequent processing. During that process, two of multiple isolates were identified via ITS region and subsequently whole genome sequencing (WGS) as *A. fumigatus*.

2.2. Growth conditions.

Af293, CEA10, ISSFT-021 and IF1SW-F4 were cultivated for 5 days at 30°C on glucose minimal medium (GMM) agar plates (6 g/L $NaNO_3$, 0.52 g/L KCl, 0.52 g/L $MgSO_4 \cdot 7H_2O$, 1.52 g/L KH_2PO_4, 10 g/L D-glucose, 15 g/L agar supplemented with 1 mL/L of Hutner's trace elements) covered with cellophane membrane. Each Petri plate (D=10 cm) was inoculated with $10*10^6$ spores/plate. For supplementary comparison, each strain was also cultured in potato-dextrose (PD, BD Difco, Franklin Lakes, NJ) and Czapek-dox (CD, BD Difco) liquid media for 2 days and 10 days, respectively.

2.3. Protein extraction.

Mycelia and spores from GMM agar plates were collected. The hyphae from the liquid media were collected on Whatman paper filter and washed with cell culture grade water. All samples were stored at −80 °C prior to protein extraction. The lysis buffer consisted of 100 mM triethylammonium bicarbonate (TEAB) with 1:100 Halt Protease Inhibitor Cocktail (Thermo Scientific, Rockford, IL) and 200 μg phenylmethylsulfonyl fluoride (Sigma-Aldrich, St. Louis, MO). The hyphae from the liquid media were first homogenized on ice using a Polytron (Kinematica AG, Bohemia, NY) with a speed setting of 5 for 3–5 times (1 min/time, 30 sec pause). Subsequently, the crude homogenates were subjected to a Precellys 24 homogenizer (Bertin, Rockville, MD) in which each sample was processed inside a 2 mL cryotube with 0.5 mm glass beads three times (at 4 °C, 6500 rpm, 1 min., repeated 3 times with 15 sec pauses in between). Mycelia from GMM were homogenized directly by bead beating due to their small volume. The lysed fungi were centrifuged at 17,000 g for 15 min. Protein concentrations in the supernatants were measured by Bradford assay (Bio-Rad Laboratories, Inc. Hercules, CA).

2.4. Tandem mass tag (TMT) labeling.

200 μg proteins from each group were TCA-precipitated. Obtained protein pellets were washed with ice-cold acetone, and resolubilized in 25 μL TEAB (100 mM) and 25 μL 2,2,2-trifluoroethanol (TFE). Subsequently, proteins were reduced with tris(2-carboxyethyl)phosphine (TCEP, 500 mM), alkylated with iodoacetamide (IAA) and digested

overnight using mass spec grade trypsin/lysC (Promega, Madison, WI) at 37°C. The digested peptides were quantified using the Pierce Quantitative Colorimetric Peptide Assay (Thermo Scientific). 40 μg of peptides from each specific sample was labeled with the Thermo Scientific TMTsixplex Isobaric Mass Tagging Kit (Af293 with TMT^6-126, CEA10 with TMT^6-127, ISSFT-021 with TMT^6-128, and IF1SW-F4 with TMT^6-130) according to the manufacturer's protocol. The TMT^6-131 label was used as a reference that contained an equal amount of peptides from each of the samples. All labeled-peptide mixtures were combined into a single tube, mixed, and fractionated into eight fractions using the Thermo Scientific Pierce High pH Reversed-Phase Peptide Fractionation Kit. The fractionated samples were dried using a SpeedVac concentrator and re-suspended in 1% (v/v) formic acid prior to LC-MS/MS analysis.

2.5. LC-MS/MS analysis.

An Orbitrap Fusion Tribrid mass spectrometer with the Thermo EASY-nLC ion source, 75 μm × 2 cm Acclaim PepMap100 C18 trapping column, and 75 μm × 25 cm PepMap RSLC C18 analytical column was used to analyze the samples. Peptides were eluted at 45°C with a flow rate of 300 nL/min over a 110 min gradient, from 3–30% solvent B (100 min), 30–50% solvent B (3 min), 50–90% solvent B (2 min), and 90% solvent B (2 min). The solvent A was 0.1% formic acid in water and the solvent B was 0.1% formic acid in acetonitrile.

The full MS survey scan (m/z 400–1500) was acquired at a resolution of 120,000 and an automatic gain control (AGC) target of 2×10^5 in the Orbitrap with the 50 ms maximum injection time for MS scans. Monoisotopic precursor ions were selected for fragmentation with charge states 2–7, within a ±10 ppm mass window, using a 70 s dynamic exclusion function. MS^2 scans (m/z 400–2000) were performed using the linear ion trap with the 35% CID collision energy. The ion trap scan rate was set to "rapid", with an AGC target of 4×10^3, and a maximum injection time of 150 ms. Subsequently, ten fragment ions from each MS^2 experiment were subjected to an MS^3 experiment. The MS^3 scan (m/z 100–500) generated the TMT reporter ions in the linear ion trap using HCD at a 55% collision energy, a rapid scan rate and an AGC target of 5×10^3, and a maximum injection time of 250 ms.

2.6. Proteome data processing.

The Proteome Discoverer (version 2.1.0.81, Thermo Scientific) with searching engines Sequest-HT against an *A. fumigatus* Af293 protein database from NCBI containing 9845 non-redundant sequences was used to search all MS/MS spectra. The following parameters: 5 ppm tolerance for precursor ion masses and 0.6 Da tolerance for fragment ion masses were selected. The static modification settings included carbamidomethyl of cysteine residues, whereas dynamic modifications included oxidation of methionine, TMT6plex modification of lysine ε-amino groups and peptide N-termini, and acetyl modification of peptide N-terminus. A false discovery rate (FDR) of 1% for peptides and proteins was obtained using a target-decoy database search. The reporter ions integration tolerance was 0.5 Da while the co-isolation threshold was 75%. The average signal-to-noise threshold of all reporter peaks was greater than 10. The sum of all detected reporter ions of associated peptides from a protein was used to determine the total intensity of a reporter ion for that protein. The ratios between reporter and the reference reporter ions (TMT6–131) were used to estimate

the abundance ratio of each protein. For the statistical analysis, the sum of reporter ion intensities for each protein was Log2 transformed and technical triplicate measurements for each protein were averaged. Only the proteins that were identified with at least one peptide detected in each technical replicate, and quantified in all three technical replicates, were considered for the analysis. One-way ANOVA was performed to identify proteins that are differentially expressed. Proteins with p-value ≤ 0.05 were further evaluated for up- and down-regulation using a cut-off value of ≥ ±1 fold (Log2) change.

3. RESULTS

3.1. Proteome analysis overview.

Proteins with altered abundance in ISS-isolated strains ISSFT-021 and IF1SW-F4, and clinical isolates Af293 and CEA10, were investigated upon extraction of total protein from each strain. Extracted proteins were digested into peptides and labeled using tandem mass tags (TMT), fractionated, and analyzed via LC-MS/MS followed by spectrum/sequence matching using *A. fumigatus* Af293 protein database (NCBI). The abundance ratios of all identified proteins were normalized to Af293, that enabled identification of 553, 464 and 626 increased and 314, 289 and 317 decreased in abundance proteins in CEA10, ISSFT-021 and IF1SW-F4 strains respectively (Table S-1, Figure S-1). When compared to both, Af293 and CEA10, 60 proteins showed increased and 32 decreased abundance in space strains only (fold change (FC) > |2|) (Table S-2). AspGD GO Slim terms[46] were used to study the distribution of differentially abundant proteins in space strains. Analysis of proteins with increased abundance revealed involvement of 14 proteins in carbohydrate metabolic processes, eight in stress responses, five in secondary metabolism and toxins biosynthesis, and two in pathogenesis whereas five, three, one and zero proteins showed respectively decreased abundance in these categories. Proteins associated with cellular amino acid metabolic process (6), lipid cellular homeostasis (3), metabolic processes (2), pathogenesis (2), and translation (2) exhibited increased abundance in space strains only (Figure 1). FungiDB[47] was used to carry out GO term enrichment analysis to gain a general understanding of biological processes possibly affected by unique environment of the ISS. The results revealed that significantly over-represented up-regulated biological processes included secondary metabolic processes (40% of all up-regulated proteins), carbohydrate metabolic processes (23%), and response to chemical (~15%), whereas significantly over-represented down-regulated processes included carbohydrate metabolic processes (15%), response to heat (6%), and mRNA metabolic processes (6%) (Table S-3).

3.2. Secondary metabolism and toxins.

The proteomic analysis revealed altered abundance of proteins involved in secondary metabolism (Table 1). Proteins involved in trypacidin biosynthetic pathway, including emodin O-methyltransferase TpcA (AFUA_4G14580), glutathione S-transferase TpcF (AFUA_4G14530), and dehydratase TpcK (AFUA_4G14470)[48–50], were at least threefold more abundant in ISS-isolated strains than in clinical isolates. Arp1 (AFUA_2G17580), a scytalone dehydratase involved in conidial pigment biosynthesis[51–53] was threefold more abundant, whereas Asp-hemolysin (Asp-HS; AFUA_3G00590)[54] was eight times more abundant in ISS strains.

To confirm observed up-regulation of proteins involved in the trypacidin biosynthesis secondary metabolite profiles of the clinical and ISS isolates were acquired using high performance liquid chromatography-photodiode array detection-mass spectroscopy (HPL-DAD-MS) analysis. Examination of the production yields of monomethylsulochrin, the final intermediate in trypacidin biosynthesis, (trypacidin is not detectable until 7–8 days of growth[50]) showed ~600% and ~200% increased production in ISSFT-021 ad IF1SW-F4, respectively (Figure S-2). Additionally, proteome of clinical and ISS strains was examined in liquid CD and PD media and confirmed significant up-regulation of AspHS (Table S-4). Lastly, observed differences in sporulation capacity between the strains do not seem to be directly correlated with conidiaassociated protein expression levels (Figure S-3), suggesting that the observed variations are due to exposure to space environment rather than different growth rates.

3.3. Stress response.

Among proteins with altered abundance, 11 were involved in the stress response of *A. fumigatus* (Table 2). AFUA_5G11430, a quinone oxidoreductase, and Pst2 (AFUA_1G02820), an NADH-quinone oxidoreductase, involved in oxidative stress response were three and four times more abundant in space strains. Erythromycin esterase, AFUA_1G05850, and 3' exoribonuclease AFUA_2G15980[56] were at least twofold more abundant in ISS strains. Among proteins with decreased abundance several were heat shock proteins including Scf1 (AFUA_1G17370)[57,58], and Awh11 (AFUA_6G12450)[56]. Other down-regulated protein was dehydrin-like protein DprA (AFUA_4G00860) that is known to play a role in oxidative stress response[59].

3.4. Carbohydrate metabolic processes.

Comparative analysis of proteomes of ISS-isolated strains, IF1SW-F4 and ISST-021, with clinical isolates CEA10 and Af293 revealed changes in abundance of proteins involved in carbohydrate metabolic processes (Table 3). Pyruvate decarboxylase PdcA (AFUA_3G11070), which catalyzes the first step in anaerobic conversion of pyruvate to ethanol[60], was about three times more abundant in space strains when compared to clinical isolates. Proteins involved in glycerol metabolism, including glycerol dehydrogenase GldB (AFUA_4G11730), and glycerol kinase AFUA_4G11540[55] were at least 2.5-fold more abundant in ISS-isolated strains. AFUA_5G10540 and AFUA_1G02140, which are homologues of *A. niger* An14g04190 and GdbA (An01g06120)[61], respectively, and involved in glycogen biosynthesis and metabolism were at least twofold more abundant. Protein abundance of AFUA_3G08470, AFUA_4G08880, AFUA_7G_01830, and AFUA_1G14710 involved in glucose metabolism, increased twofold at minimum. Both, the 14–3-3 family protein ArtA (AFUA_2G03290)[62] and malate synthase AcuE (AFUA_6G03540)[63] were threefold more abundant when compared to clinical isolates. At minimum twofold increased abundance of phosphoketolase (AFUA_3G00370) and hexokinase HxkA (AFUA_2G05910)[64] was observed. Among the decreased in abundance proteins were hydrolase Exg17 (AFUA_6G14490)[65] and β−1,3-glucan modifying enzyme Sun1 (AFUA_7G05450)[66].

4. DISCUSSION

The proteome of *A. fumigatus* has been studied under various conditions, including short-[67], and long-term hypoxia[68], following exposure to antifungal agents like amphotericin B[69], and voriconazole[70], and during different developmental stages[58]. However, in this report we present unique proteome differences observed in the two ISS-isolated strains when compared to clinical isolates. To date, there is no report which elucidates the molecular response of filamentous fungi to the distinct environment of the ISS, despite previous reports of their presence on board of the ISS[22] and Mir[27,71]. In-depth understanding of alterations triggered in the proteome of omnipresent *A. fumigatus* remains imperative for astronauts' health, as it is an opportunistic pathogen that affects individuals with impaired immune system functions[41].

Both ISS-isolated *A. fumigatus* strains displayed higher abundance of several proteins involved in trypacidin biosynthesis. Trypacidin is a potent mycotoxin produced by *A. fumigatus* conidia. It has been shown to have cytotoxic activity against A549 alveolar lung cells suggesting its importance during the infection[76]. Further, it has been reported that *A. fumigatus* conidia with disrupted production of trypacidin exhibit higher susceptibility to macrophage clearance[49]. Increased abundance of proteins involved in biosynthesis of trypacidin in both ISS–isolated strains may be possible cause of the reported increased virulence in the larval zebra fish model of both ISS-isolated strains when compared to clinical isolates[45]. Moreover, emodin and questin, precursors of trypacidin, are pigmented anthraquinones that have been reported to have a protective effect against UV in other organisms (*Xanthoria elegans* and *Cetraria islandica*) that produce these types of compounds[50,77]. It is therefore reasonable to assume that up-regulation of trypacidin production may be another adaptation to the enhanced irradiation environment of the ISS, however this hypothesis has to be validated by further experiments.

Further, the level of Asp-HS, which is a known toxin produced by *A. fumigatus*[54,78] was highly increased in both ISS-isolates. While several studies have reported its hemolytic[79] and cytotoxic[80,81] activities in the past, a recent report showed lack of attenuated virulence when Asp-HS gene was deleted[82]. This discrepancy may be strain specific as the studies used different *A. fumigatus* strains to carry out the experiments or related to experimental models used in both studies, as at times results observed for *in vitro* analyses do not correlated with the outcomes observed in *in vivo* studies. The exact role of Asp-HS in the pathogenicity remains to be determined during future studies. Nonetheless, increased abundance of Asp-HS observed in both ISS-isolated strains appears to be a part of the *A. fumigatus* adaptation response to the unique ISS environment.

One of the increased in abundance proteins in ISS-isolated *A. fumigatus* strains was Arp1, which is one of the six enzymes involved in the DHN-melanin production[51–53]. Arp1 disruption resulted in production of reddish pink conidia with induced C3 binding that led to phagocytosis and killing of conidial spores during infection[51,52]. Increased abundance of Arp1 protein may, therefore, be another cause of the previously reported increased virulence in the larval zebra fish model[45]. Additionally, higher AlbA abundance, involved in DHN-melanin production[72], was observed in JSC-093350089 *A. niger* isolated from

the ISS[73]. Seemingly, both ISS-isolated species, *A. fumigatus* and *A. niger*, responded with increased melanin production to enhanced irradiation on board of the ISS. This observation is in agreement with previous reports of increased melanin production in fungi isolated from high-radiation environments such as the Chernobyl Power Plant accident sites or "Evolution Canyon"[74,75]. However, because none of the other enzymes involved in the melanin biosynthesis showed increased abundance when compared to both clinical isolates simultaneously, further assessment of melanin production is necessary to definitively conclude its increased production under ISS conditions.

Proteome analysis of both ISS-isolated *A. fumigatus* strains revealed altered levels of proteins involved in oxidative stress response. ArtA, a regulatory protein, was reported to be increased in abundance in response to incubation with H_2O_2, suggesting its importance in overcoming oxidative stress[62]. Pst2, which is also present in *Candida albicans* and *Saccharomyces cerevisiae*, was shown to be induced in response to oxidative stress[83,84]. Additionally, Pst2 was reported to be a post-translational modification target protein that is ubiquitinated under peroxide stress conditions in *C. albicans*[85]. Interestingly, such post-translational modifications play a role in several cellular processes in eukaryotes, including cell growth regulation and environmental adaptation, suggesting their importance in gaining survival advantage[86]. These data are in agreement with previous reports of induced oxidative stress response in humans, mice and yeast while in space[87–91]. Overall, increased abundance of proteins involved in oxidative stress response was also observed in *Acinetobacter* sp. Ver3 when exposed to UV irradiation[92] and JSC-093350089 *A. niger*[73]. Although increased abundance of several oxidative stress-correlated proteins was observed in this study, it was previously reported that ISS-isolated strains were more resistant than Af293 to H_2O_2 exposure, but less resistant than CEA10, suggesting no adaptation[45].

Among the more abundant proteins in both ISS-isolated strains several were involved in carbohydrate metabolism. Malate synthase AcuE (AFUA_6G03540) is one of the three key enzymes involved in glyoxylate cycle[63], which has been shown to be crucial for fungal growth on C_2 compounds and fatty acids as a sole carbon source[93]. In our earlier study it was documented that these two ISS-isolated strains significantly outgrew both clinical isolates[45]. Further, more abundant PdcA (AFUA_3G11070), a pyruvate decarboxylase, has been shown to be involved in ethanol fermentation[60]. These data therefore suggest possible adaptation of *A. fumigatus* to low-nutrient environment of the ISS[20,22], which is in agreement with changes observed in proteome of *A. fumigatus* during starvation[94]. Similarly, up-regulation of proteins involved in starvation response was observed in ISS-isolated JSC-093350089 *A. niger*[73].

This study presents comparative proteomic analysis of two ISS-isolated *A. fumigatus* strains, ISSFT-021 and IF1SW-F4 when compared to clinically established isolates Af293 and CEA10. Such comparison has enabled the identification of possible adaptation responses to the unique microgravity environment of the ISS, which includes increased abundance of stress response related proteins, and modulation of proteins involved in carbohydrate and secondary metabolism. To our knowledge this is the first report that focused on studying the proteomic changes in filamentous fungus isolated from on board of the ISS. Complex analyses of possible molecular alterations triggered by microgravity and enhanced

irradiation will be pertinent to the future long-term manned space flights, as such an understanding is crucial for astronauts' health and biodeterioration of the closed habitat.

Accession number

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[95] partner repository with the dataset identifier PXD008517.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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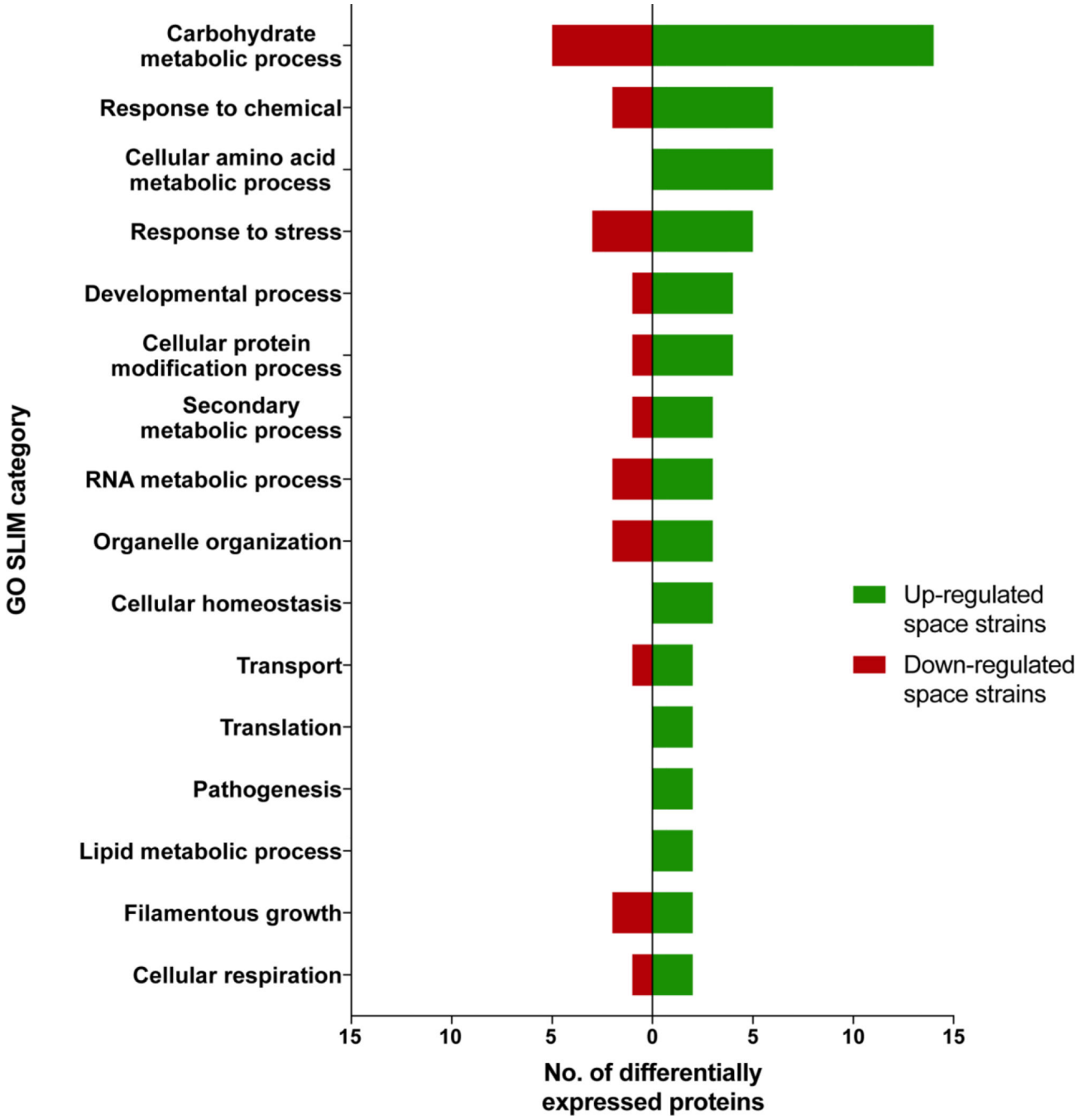
Highlights:

- Proteome characterization of *Aspergillus fumigatus* isolated from the International Space Station (ISS).
- The distinct ISS environment (enhanced radiation, microgravity and scarce nutrients) alters *A. fumigatus* proteome.
- When compared to clinical isolates CEA10 and Af293 the majority of up-regulated proteins are involved in carbohydrate and secondary metabolism, and oxidative stress response.



F1. AspGD GO Slim terms of proteins differentially expressed in space strains when compared to Af293 and CEA10.

Differentially expressed proteins in ISSFT-021 and IF1SW-F4 are categorized into GO Slim categories using AspGD. Categories containing at least 2 up- or down-regulated proteins are presented.

Table 1.

Proteins involved in secondary metabolism and toxin biosynthesis that revealed increased or decreased abundance

ORF	Protein	Putative function/activity	Relative protein abundance*			p-value
			CEA10	ISSFT-021	IF1SW-F4	
AFUA_4G14530	TpcF/GstC	Glutathione S-transferase involved in trypacidin biosynthesis	0.83	3.02	2.46	2.76E-06
AFUA_3G00590	AspHS	Asp-hemolysin; hemolytic toxin	0.00	3.01	3.24	7.48E-09
AFUA_4G14580	TpcA	Emodin O-methyltransferase involved in trypacidin biosynthesis	−0.14	2.95	3.06	6.77E-07
AFUA_4G14470	TpcK	Dehydratase involved in trypacidin biosynthesis	0.27	2.58	2.17	3.04E-03
AFUA_2G17580	Arp1	Scytalone dehydratase involved in conidial pigment biosynthesis	0.58	2.49	2.69	2.24E-07
AFUA_4G00860	DprA	Dehydrin-like protein / oxidative, osmotic and pH stress responses	−3.54	−5.64	−4.62	1.37E-07

* Log2 fold change of CEA10, ISSFT-021, and IF1SW-F4 compared to Af293 ($P < 0.05$)

Table 2.

Proteins involved in stress response that revealed increased or decreased abundance

ORF	Protein	Putative function/activity	Relative protein abundance*			p-value
			CEA10	ISSFT-021	IF1SW-F4	
AFUA_3G08470		Glucose-6-phosphate 1- dehydrogenase Glutathione S-transferase	1.41	3.06	3.33	8.66E-07
AFUA_4G14530	TpcF/GstC	involved in trypacidin biosynthesis	0.83	3.02	2.46	2.76E-06
AFUA_4G11730	GldB	Glycerol dehydrogenase	1.29	2.83	3.70	5.34E-07
AFUA_5G11430		Quinone oxidoreductase	0.95	2.55	2.51	1.45E-09
AFUA_2G03290	ArtA	14–3-3 family protein	0.67	2.21	2.82	1.90E-07
AFUA_1G02820	Pst2	NADH-quinone oxidoreductase	−0.37	2.10	2.55	9.33E-06
AFUA_2G15980		3’ exoribonuclease family protein; pre-miRNA processing	0.02	1.24	2.20	4.73E-03
AFUA_1G05850		Erythromycin esterase	−0.16	1.22	2.48	6.61E-03
AFUA_1G17370	Scf1	Heat shock protein	−2.95	−5.23	−4.26	4.25E-07
AFUA_6G12450	Awh11	Heat shock protein	−4.05	−5.81	−5.12	5.46E-05
AFUA_4G00860	DprA	Dehydrin-like protein / oxidative, osmotic and pH stress responses	−3.54	−5.64	−4.62	1.37E-07

*Log2 fold change of CEA10, ISSFT-021, and IF1SW-F4 compared to Af293 ($P < 0.05$)

Table 3.

Proteins involved in carbohydrate metabolism that revealed increased or decreased abundance

ORF	Protein	Putative function/activity	Relative protein abundance*			p-value
			CEA10	ISSFT-021	IF1SW-F4	
AFUA_3G11070	PdcA	Pyruvate decarboxylase involved in ethanol fermentation pathway	1.95	3.42	3.43	9.50E-08
AFUA_4G11540		Glycerol kinase	1.52	3.18	2.94	5.99E-05
AFUA_3G08470		Glucose-6-phosphate 1- dehydrogenase	1.41	3.06	3.33	8.66E-07
AFUA_4G11730	GldB	Glycerol dehydrogenase	1.29	2.83	3.70	5.34E-07
AFUA_1G14710		Beta-glucosidase	1.63	2.70	3.52	4.34E-05
AFUA_5G10540		1,4-alpha-glucan branching enzyme activity, glycogen biosynthesis	0.91	2.23	3.06	1.39E-07
AFUA_2G03290	ArtA	14–3-3 family protein	0.67	2.21	2.82	1.90E-07
AFUA_6G03540	AcuE	Malate synthase	0.38	2.10	2.16	1.22E-07
AFUA_1G02140		Glycogen debranching enzyme	0.87	1.98	3.18	1.08E-03
AFUA_3G00370		Phosphoketolase	0.51	1.88	2.84	3.50E-05
AFUA_4G04680		FGGY-family carbohydrate kinase	0.81	1.82	2.61	1.73E-05
AFUA_4G08880		Glucose-6-phosphate 1-epimerase	−0.32	1.46	2.61	7.00E-05
AFUA_7G01830	Ugp1	UTP-glucose-1 -phosphate uridylyltransferase	0.00	1.35	2.11	4.43E-05
AFUA_2G05910	HxkA	Hexokinase	−0.01	1.20	2.12	8.68E-07
AFUA_7G05450	Sun1	Beta-1,3-glucan modifying enzyme	−0.61	−1.87	−2.15	4.13E-02
AFUA_1G06910		Arabinogalactan endo-1,4-beta- galactosidase activity	−0.43	−1.53	−2.04	9.05E-06
AFUA_6G05030		Polysaccharide deacetylase	−0.09	−1.21	−1.23	1.53E-03
AFUA_6G14490	Exg17	O-glycosyl hydrolase	−0.21	−1.41	−1.33	4.68E-05
AFUA_1G03140		Glycosyl hydrolase	−0.31	−1.31	−1.44	1.39E-06

*Log2 fold change of CEA10, ISSFT-021, and IF1SW-F4 compared to Af293 ($P < 0.05$)