The International Space Station Environment Triggers Molecular Responses in Aspergillus niger

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Due to immense phenotypic plasticity and adaptability, Aspergillus niger is a cosmopolitan fungus that thrives in versatile environments, including the International Space Station (ISS). This is the first report of genomic, proteomic, and metabolomic alterations observed in A. niger strain JSC-093350089 grown in a controlled experiment aboard the ISS. Whole-genome sequencing (WGS) revealed that ISS conditions, including microgravity and enhanced irradiation, triggered non-synonymous point mutations in specific regions, chromosomes VIII and XII of the JSC-093350089 genome when compared to the ground-grown control. Proteome analysis showed altered abundance of proteins involved in carbohydrate metabolism, stress response, and cellular amino acid and protein catabolic processes following growth aboard the ISS. Metabolome analysis further confirmed that space conditions altered molecular suite of ISS-grown A. niger JSC-093350089. After regrowing both strains on Earth, production of antioxidant—Pyranonigrin A was significantly induced in the ISS-flown, but not the ground control strain. In summary, the microgravity and enhanced irradiation triggered unique molecular responses in the A. niger JSC-093350089 suggesting adaptive responses.

Keywords: Aspergillus niger, International Space Station, metabolome, proteome, genome

INTRODUCTION

The International Space Station (ISS) is a research facility orbiting at an approximate altitude of 250 miles that is utilized to study physiological, psychological, and immunological responses of humans living in isolation (Mehta et al., 2004; Crucian et al., 2013; Cucinotta, 2014; Benjamin et al., 2016; Ombergen et al., 2017). However, the distinct ISS environment, which includes microgravity and enhanced irradiation, affects the metabolism of all living organisms aboard the ISS including humans. There is a growing body of research that focuses on molecular characterization of animal (Ijiri, 2003; Tavella et al., 2012), plant (Link et al., 2003; Driss-Ecole
et al., 2008; Kittang et al., 2014), and microbial (Rabbow et al., 2003; Benoit et al., 2006) responses to the conditions encountered in the ISS. Among the most studied microorganisms are various species of bacteria (Klaus et al., 1997; Weng et al., 1998; Nickerson et al., 2000; Vaishampayan et al., 2012), yeast (Takahashi et al., 2001; Purevdorj-Gage et al., 2006; Altenburg et al., 2008; Liu et al., 2008; Crabbé et al., 2013), and black fungi (Onofri et al., 2008, 2012, 2015). However, there are few reported studies that characterize the molecular responses of filamentous fungi (Romsdahl et al., 2018, 2019; Blachowicz et al., 2019b).

Filamentous fungi are producers of a myriad of bioactive compounds or secondary metabolites (SMs). These SMs often confer environmental advantage, which facilitate survival in hostile niches despite not being directly essential for survival (Keller et al., 2005; Fox and Howlett, 2008; Brakhage and Schroeckh, 2011; Rohls and Churchhill, 2011; Brakhage, 2013). SMs span from potent bioactive molecules in the drug discovery processes (Borel et al., 1995; Elander, 2003; Mulder et al., 2015) or other branches of the industry (Vandenberge et al., 2000; Rodriguez Couto and Toca Herrera, 2006; Piscielli et al., 2010; Dhillon et al., 2011) to health hazardous toxins (Barnes, 1970; Shephard, 2008; Hof and Kupfahl, 2009; Eaton and Groopman, 2013). Altered production of various SMs is one potential mechanism of fungal adaptation to extreme environments. For example, increased production of melanin, a pigment with UV protective properties, was observed in fungi isolated from Chernobyl nuclear power plant (Dadachova et al., 2008) and “Evolution Canyon” (Singeravelan et al., 2008). One such highly melanized fungal species is *Aspergillus niger*.

Industrially important *A. niger* (Schuster et al., 2002) has been isolated from various ecological niches, including decaying leaves (Nikolcheva et al., 2003), common households (Adams et al., 2013; Barberán et al., 2015), and the ISS (Checinska et al., 2015). The *A. niger* strain JSC-093350089 isolated from the surface of the US compartment of the ISS was previously characterized using multi-omics techniques. Performed analyses revealed genetic variance typical for the *A. niger* clade, increased abundance of proteins involved in starvation response, oxidative stress, and cell wall modulation (Romsdahl et al., 2018), and alteration in SM production levels when compared to well-studied *A. niger* ATCC 1015 strain (Romsdahl et al., 2019). However, definite ascribing of observed molecular alterations to the ISS environment was not possible, since the strains were not grown in microgravity using a controlled experiment with ground counterparts. Nevertheless, in-depth characterization of ISS-isolated JSC-093350089 *A. niger* provided insight into potential space-induced molecular phenotypes.

This study is the first report of the multi-omics characterization of *A. niger* JSC-093350089 grown aboard the ISS and compared to ground controls. To study the impact of the enhanced irradiation and microgravity on JSC-093350089, the strain was transported to and grown aboard the ISS. Upon return to Earth, ISS-grown samples, along with ground controls, were immediately processed for metabolomic, proteomic, and genomic analyses with the aim of obtaining important insights into the adaptive responses of *A. niger* to space conditions. In addition, ISS-grown samples were regrown on Earth to identify any conserved molecular alterations.

### MATERIALS AND METHODS

#### Isolation and Identification of *Aspergillus niger*

Procedures to isolate and identify *A. niger* collected from the ISS were described previously (Romsdahl et al., 2018). In brief, sterile swabs soaked in saline solution were used to sample the ISS surface and transported to Earth. Particles retrieved from the swab were spread into potato dextrose agar (PDA) plates and any growing colonies were purified, collected, and further analyzed. One of the collected isolates was identified as *A. niger* via ITS region sequencing, which was subsequently confirmed via whole-genome sequencing (WGS).

#### Growth Conditions

JSC-093350089 was cultivated on glucose minimal medium (GMM) agar plates (6 g/l NaNO\(_3\), 0.52 g/l KCl, 0.52 g/l MgSO\(_4\)·7H\(_2\)O, 1.52 g/l KH\(_2\)PO\(_4\), 10 g/l D-glucose, and 15 g/l agar supplemented with 1 ml/l of Hutner’s trace elements) covered with a cellophane membrane. Each of 10 prepared Petri plates (D = 10 cm) was inoculated with 1 × 10\(^7\) conidia/plate. Subsequently, plates were sealed with 3 M™ Micropore™ Surgical Tape (VWR International, Radnor, PA, United States) and placed in four Biological Research in Canister (BRIC) systems (three and two plates/BRIC). BRICs were divided into two groups, which were exact mimics, and transferred to 4°C. The exact timeline of the experiment is presented in [Supplementary Figure 1](#SupplementaryFigure1). The whole experiment lasted 42 days from preparing the payload by a science team prior to launch till the handout of the ISS-grown samples back to the science team after the flight. The first group of BRICs was sent to the ISS and continuously kept at 4°C (1–20 days) prior to being transferred to ambient temperature for the active growth phase ~22°C for 12 days (21–32 days). After that time BRICs were stored at 4°C before returning to Earth (33–42 days). Upon arrival to Earth, BRICs were turned over to the science team for the downstream analyses, which commenced immediately. The second group of BRICs, treated as controls, was kept on Earth at Kennedy Space Center (KSC) and mimicked the ISS experiment timeline with roughly 2 h of delay. BRICs containing control samples were shipped from KSC to research team along with the ISS-grown samples. Lastly, for an additional secondary metabolite analysis, 1 × 10\(^7\) conidia/plate of the ISS- and ground-grown JSC-093350089 were grown on GMM medium at 28°C for 5 days.

#### Genomic DNA Extraction and Whole-Genome Sequencing

Mycelia and conidia were collected from ground- and ISS-grown JSC-09335008 GMM agar plates. DNA was extracted using the Power Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California, United States) following the manufacturers protocol. Extracted DNA was checked for quality using Qubit 2.0.
FluReseq Nano DNA Library Preparation Kit (Illumina, San Diego, California, United States) followed by WGS at the Duke Center for Genomic and Computational Biology. Samples were sequenced using a HiSeq 4,000 Illumina Sequencer generating 101 base long reads.

Genetic Variation Identification
Illumina sequence reads were trimmed using Trimmomatic v 0.36 (Bolger et al., 2014) and checked for quality using FastQC v 0.11.5 (Andrews, 2010). The genome and annotation files for A. niger CBS 513.88, (Pel et al., 2007) were downloaded from the FungiDB web portal (Stajich et al., 2012). Reads were mapped to CBS 513.88 the reference genome using the Burrows-Wheeler Aligner (BWA) software package v 0.7.12 (Li and Durbin, 2009) and further processed with SAMtools v 1.6 to generate sorted BAM files (Li et al., 2009). SNPs and INDELS were identified using GATK v 3.7 (DePristo et al., 2011). Duplicates were marked using Picard-tools MarkDuplicates1 to remove PCR artifacts. Sequence reads containing putative INDELS were realigned using GATK’s IndelRealigner to generate an updated BAM file. Variants within each sample were called using GATK’s Haplotype Caller. GATK’s VariantFilteration was used to filter each VCF file based on stringent cutoffs for quality and coverage {SNPs: QD < 2.0, MQ < 40.0, QUAL < 100, FS > 60.0, MQRankSum < −12.5, SOR > 4.0, ReadPosRankSum < −8.0; Indels: QD < 2.0, FS > 200.0, MQRankSum < −12.5, SOR > 4.0, InbreedingCoeff < −0.8, ReadPosRankSum < −20.0}, so that only high-quality variants remained.

Protein Extraction
Myceia and conidia from GGM agar plates were collected and stored at −80°C prior to protein extraction. Proteins were extracted with the lysis buffer consisting of 100 mm triethyrammonium bicarbonate (TEAB) with 1:100 Halt Protease Inhibitor Cocktail (Thermo Scientific, Rockford, IL) and 200 μg/ml phenylmethylsulfonyl fluoride (Sigma-Aldrich, St. Louis, MO, United States). Myceia and conidia were homogenized by bead beating using Precells 24 homogenizer (Bertin, Rockville, MD). The lysed fungal material was centrifuged at 17,000×g for 15 min and the protein concentration in the supernatants was measured by the Bradford assay (Bio-Rad Laboratories, Inc. Hercules, CA, United States).

Tandem Mass Tag (TMT) Labeling
A 100 μg proteins from each sample were precipitated in 20% trichloroacetic acid (TCA) at 4°C. Protein pellets were washed with ice-cold acetone and re-suspended in 25 μl TEAB (100 mM) and 25 μl 2,2,2-trifluoroethanol (TFE). Proteins were reduced with 1 μl of tris(2-carboxyethyl)phosphine (TCEP, 500 mM), alkylated with iodoacetamide (IAA, 30 mM), and digested with 2.5 μg/sample of trypsin (Promega, Madison, WI, United States) overnight at 37°C. The digested peptides were quantified using the Pierce Quantitative Colorimetric Peptide Assay (Thermo Scientific, Waltham, MA, United States). 40 μg of peptides from each specific sample was labeled with the Thermo Scientific TMT Sixplex Isobaric Mass Tagging Kit (JSC-E1 (ground 1) with TMT®-128, JSC-E2 (ground 2) with TMT®-130, JSC-S1 (ISS 1) with TMT®-129, JSC-S2 (ISS 2) with TMT®-131) according to the manufacturer’s protocol. All labeled-peptide mixtures were combined into a single tube, mixed, and fractionated using the Thermo Scientific Pierce High pH Reversed-Phase Peptide Fractionation Kit. While this kit usually uses eight fractions with step elution of up to 50% acetonitrile, ninth fraction was added eluting at 100% acetonitrile. Nine fractionated samples were dried using a SpeedVac concentrator and re-suspended in 1% (v/v) formic acid prior to liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis.

LC-MS/MS Analysis
The samples were analyzed on an Orbitrap Fusion Tribrid mass spectrometer with an EASY-nLC 1,000 Liquid Chromatograph, a 75 μm × 2 cm Acclaim PepMap100 C18 trapping column, and a 75 μm × 25 cm PepMap RSLC C18 analytical column, and an Easy-Spray ion source (Thermo Scientific, Waltham, MA, United States). The peptides were eluted at 45°C with a flow rate of 300 nl/min over a 110 min gradient, from 3 to 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 400–1,500) was acquired at a resolution of 120,000 and an automatic gain control (AGC) target of 2 × 10^6 in the Orbitrap with the 50 ms maximum injection time for MS scans. Monoisotopic precursor ions were selected with charge states 2–7, ± ± 10 ppm mass window, and 70’s dynamic exclusion. The MS2 scan (m/z 400–2000) was performed using the linear ion trap with the 35% collision-induced dissociation (CID) energy. The ion trap scan rate was set to “rapid,” with an AGC target of 4 × 10^6, and a 150 ms maximum injection time. Ten fragment ions from each MS2 experiment were subsequently selected for an MS3 experiment. The MS3 scan (m/z 100–500) was performed to generate the TMT reporter ions in the linear ion trap using higher-energy collisional dissociation (HCD) at a 55% collision energy, a rapid scan rate and an AGC target of 5 × 10^6, and a maximum injection time of 250 ms.

Proteome Data Analysis
All MS data (MS1, MS2, and MS3) were searched using the Proteome Discoverer (version 2.2.0.388, Thermo Scientific) with the Sequest-HT searching engines against an Aspergillus niger CBS 513.88 database containing 10,549 sequences (NCBI). The searches were performed with the following parameters: 2 maximum missed cleavage sites, 6 minimum peptide length, 5 ppm tolerance for precursor ion masses, and 0.6 Dalton tolerance for fragment ion masses. The static modification settings included carbamidomethyl of cysteine residues, and

1https://broadinstitute.github.io/picard/
RESULTS

Genome Variation in the ISS-Grown JSC-093350089 Aspergillus niger

The genomes of ISS-flown and ground-grown JSC-093350089 were compared to identify occurring genetic variations. Obtained reads were aligned to the CBS 513.88 reference genome and single-nucleotide polymorphisms (SNPs) present in the ground control were filtered. This revealed presence of 375 SNPs and 620 INDELs that occurred because of the exposure to conditions aboard the ISS (Table 1). All identified genetic variations are summarized in Supplementary Tables 1 and 2, presenting SNPs and INDELs, respectively. Distribution of non-synonymous point mutations among genes is presented in Table 2. Interestingly, about 80% of these mutations occurred in chromosome VIII and 13% occurred in chromosome XI, while the remaining 7% were distributed among other chromosomes (Supplementary Figure 2A). The majority of missense point mutations (75%) were observed within genes of unknown function. However, several characterized genes containing missense SNPs have DNA-binding activity (An06g01180, An08g11890, and An12g00840), DNA polymerase activity (An08g11520), protein kinase and transferase activity (An08g12110), phospholipase activity (An08g12250), and chromosome anchoring RacA protein binding activity (An12g06420; Table 2). Additionally, mutations like one stop lost, one start gained and one 5 prime untranslated region (UTR) mutation were observed. Most of the observed SNPs (~55%) and INDELs (71%) were located in intergenic regions (Table 1). Interestingly, unlike SNPs, INDELs were distributed throughout all chromosomes. However, similarly to SNPs, the highest number of INDELs was found in chromosomes VIII and XII. Among observed INDELs 109 caused framseshift, 14 lead to disruptive intrame deletion, and a few caused start lost and stop gained (Supplementary Table 2; Supplementary Figure 2B).

SM Statistical Analysis

To compare the yields of produced SMs in ISS-grown, ground-grown, and regrown samples, the area under the electrospray ionization curve (ESI) was integrated for each compound. SM data collected from three independent biological replicates of ISS- and ground-grown, and regrown JSC-093350089 were used for testing statistical significance of production yields of identified SMs by Welch’s corrected t-test. The data are presented as column charts with corresponding error bars. Data analysis was conducted using GraphPad Prism version 7.
Proteomic Characterization of ISS-Grown JSC-093350089 *Aspergillus niger*

Differentially expressed proteins in ISS-grown JSC-093350089 strain were investigated following the extraction of total protein from two biological replicates of ISS-grown and ground control counterpart strains. Due to the low yields of extracted proteins, biological replicates were combined and divided into two parts that were then TMT labeled and subtracted to analysis via LC–MS/MS followed by spectrum/sequence matching using *A. niger* CBS 513.88 protein database (NCBI). Protein abundance ratios in ISS-grown JSC-093350089 were normalized to Earth-grown counterparts, which enabled identification of 70 up- and 142 downregulated proteins (fold change (FC) > 2, p < 0.05) in response to space conditions (Supplementary Tables 3 and 4, respectively). AspGD Gene Ontology (GO) term enrichment analysis was conducted using FungiDB (Stajich et al., 2012), which revealed that significantly over-represented upregulated biological processes included carbohydrate metabolic processes (28% of all upregulated proteins) and stress response (10%), whereas significantly over-represented downregulated processes included cellular amino acid metabolic processes (13%), proteasomal ubiquitin-independent (10%) and dependent processes (10%), and proteasomal protein catabolic processes (10%); Supplementary Table 5).

The majority of differentially expressed proteins in ISS-grown JSC-093350089 *A. niger* were involved in carbohydrate metabolism (Table 3). Interestingly, eight of these genes, including cellobiohydrolases A and B (An07g09330 and An01g11660), XlnA 1,4-β-xylanase (An03g00940), and D-xylene reductases YxrA and XdhA (An01g093740 and An12g00030) were regulated by XlnR. XlnR is a transcriptional regulator involved in degradation of polysaccharides, xylan, cellulose, and D-xylene (Hasper et al., 2000). β-glucanases An11g01540 and An02g00850, which are involved in carbon starvation response in *A. niger* (Nitsche et al., 2012), were at minimum 3-fold upregulated in ISS-grown strain. α,1,2-mannosidases An08g08370, An13g01260 were at least 3.5-fold upregulated, whereas pyruvate decarboxylase PdcA (An02g00820) was nearly 3-fold upregulated. Pyruvate kinase KPiA (An07g08990), pyruvate dehydrogenase Pda1 (An07g09530), and isocitrate lyase AcUD (An01g09270) were at least 2-fold less abundant in ISS-grown samples. Several proteins involved in the stress response were differentially expressed in ISS-grown JSC-093350089 (Table 4). Proteins exhibiting at least 2-fold upregulation included cell wall organization protein EcmA (An04g01230) and An16g07920, whose orthologs play a role in salt stress response. Downregulated stress response proteins included heat shock protein An06g01610, DNA-binding protein HtaA (An11g11300), and quinone reductase An12g06300. Lastly, a variety of proteins involved in cellular amino acid processes (Table 5), and protein catabolic processes (Table 6) were downregulated.

### Table 2 | Single-nucleotide polymorphisms (SNPs) in ISS-grown JSC-093350089 when compared to ground control.

| Function | Gene     | Base mutation when compared to ground control | Type of mutation |
|----------|----------|-----------------------------------------------|------------------|
| RNA polymerase II transcription factor activity, sequence-specific DNA binding | An06g01180 | An06_G279214A | 5 prime UTR |
| RNA-directed DNA polymerase activity and role in RNA-dependent DNA replication | An08g11520 | An08_G2725926T | Missense |
| An08_T2819644G | Stop lost |
| An08_C2733499T | Unknown function |
| DNA-binding activity | An08g12110 | An08_G2726382A | Missense |
| Protein kinase and transferase activity | An08g12250 | An08_G2725657T | Missense |
| Phospholipase | An12g00840 | An12_G2229742C | Missense |
| DNA-binding, RNA polymerase II transcription factor activity | An12g00840 | An12_G2229742C | Missense |
| RacA binding protein, polarized cell growth | An12g06420 | An12_G2229742C | Missense |
| Unknown function | An08g08380 | An08_G2818780C | Missense |
| Unknown function | An08g11220 | An08_G2818780C | Missense |
| Unknown function | An08g11230 | An08_G2818780C | Missense |
| Unknown function | An08g11540 | An08_G2818780C | Missense |
| Unknown function | An08g11550 | An08_G2818780C | Missense |
| Unknown function | An08g11570 | An08_G2818780C | Missense |
| Unknown function | An08g11650 | An08_G2818780C | Missense |
| Unknown function | An08g11670 | An08_G2818780C | Missense |
| Unknown function | An08g11830 | An08_G2818780C | Missense |
| Unknown function | An08g11840 | An08_G2818780C | Missense |
| Unknown function | An08g11860 | An08_G2818780C | Missense |

(Continued)
TABLE 2 | Continued

| Function          | Gene       | Base mutation when compared to ground control | Type of mutation |
|-------------------|------------|---------------------------------------------|------------------|
| Unknown function  | An08g11870 | An08_G28209864A                            | Missense         |
|                   |            | An08_C2821036T                             |                  |
|                   |            | An08_T2821061T                             |                  |
|                   |            | An08_T2821094C                             |                  |
|                   |            | An08_A2821104C                             |                  |
|                   |            | An08_C2821119A                             |                  |
|                   |            | An08_T2821648G                             |                  |
|                   |            | An08_C2821664T                             |                  |
|                   |            | An08_T2821693A                             |                  |
|                   |            | An08_G2821697A                             |                  |
|                   |            | An08_A2821958T                             |                  |
|                   |            | An08_G2822640C                             | Missense         |
|                   |            | An08_G2821995A                             |                  |
| Unknown function  | An08g11880 | An08_G2824016C                             | Missense         |
|                   |            | An08_C2827842C                             |                  |
|                   |            | An08_G2828992G                             |                  |
| Unknown function  | An08g11940 | An08_A2835378G                             | Missense         |
|                   |            | An08_T2838585A                             | Missense         |
|                   |            | An08_T2838568C                             | Missense         |
|                   |            | An08_G2837183G                             | Missense         |
| Unknown function  | An08g11960 | An08_A2838318G                             | Missense         |
|                   |            | An08_G2839738A                             | Missense         |
| Unknown function  | An08g12230 | An08_C2911588T                             | Missense         |
|                   |            | An08_T2911676C                             | Missense         |
|                   |            | An08_G2912446C                             | Missense         |
|                   |            | An08_T2912451G                             | Missense         |
|                   |            | An08_A2913614T                             | Missense         |
|                   |            | An08_T2911894A                             | Splice region    |
| Unknown function  | An08g12230 | An08_G2913874C                             | Start gained     |
|                   |            | An08_A2916240C                             | Missense         |
| Unknown function  | An08g12240 | An08_T2912451G                             |                  |
|                   |            | An12_T1429831C                             |                  |
|                   |            | An12g05800C                                |                  |

**DISCUSSION**

It is critical to study molecular changes occurring in living organisms to understand the adaptation mechanisms allowing for surviving in extreme environments. One of such scientifically intriguing environments is the ISS, which is characterized by the presence of enhanced irradiation and microgravity. Due to its uniqueness, the ISS is under constant microbial monitoring, which allows for the isolation of wide array of microorganisms that often-become subjects of scientific investigations (Checinska et al., 2015; Knox et al., 2016; Checinska Sielaff et al., 2017; Venkateswaran et al., 2017; Bijlani et al., 2021). However, like in the case of the *A. niger* strain JSC-093350089 (Romsdahl et al., 2018, 2020), these investigations are more of a descriptive nature as definitive ascribing of observed molecular changes requires precisely controlled experiments. Therefore, to further investigate the differences in JSC-093350089 that were observed when compared to a “terrestrial” strain, the isolate was sent to the ISS in a planned experiment. Genomic, proteomic, and metabolomic alterations occurring in ISS-grown samples were analyzed following sample return and compared to ground-grown counterparts.

Genome analysis of ISS-grown JSC-093350089 revealed the introduction of SNPs and INDELS in response to space conditions. Interestingly, the majority of observed non-synonymous SNPs and INDELS were located within chromosomes VIII and XII, which suggests that only selected regions of the genome undergo positive selection to confer selective advantage while adapting to the space environment. This is in agreement with previous reports of space-induced genetic variations, as ISS-grown *Aspergillus nidulans* (Romsdahl et al., 2019) and spaceflight-grown *Staphylococcus aureus* (Guo et al., 2015) both exhibited genetic mutations that occurred in specific clustered regions of the genome. Although the functions of many genes containing non-synonymous SNPs were unknown, several of these genes possessed transposable element and DNA-binding activity. One such gene, An08g11520, was an analogue of transposon I factor and has RNA-directed DNA polymerase activity, which is consistent with genetic changes observed in transposable element genes in both *A. nidulans* (Romsdahl et al., 2019) and *S. aureus* (Guo et al., 2015). Alterations in transposable element genes likely influence their activity and lead to the introduction of variations within the genome in response to environmental stress (Capy et al., 2000; Muszewska et al., 2017). The results from this study further underscore the significant role of transposable elements in adaptation to the spacecraft environment. Future studies should investigate the functions of uncharacterized genes containing non-synonymous SNPs, as such knowledge may provide key information on how fungi adapt to space conditions. Noteworthy, when radiation-adapted strain of *Exophiala dermatitidis* and the non-radiation-adapted control strain were exposed to Polonium-210, a mostly transcriptomic rather than genomic response to radiation was observed in radiation-adapted strain. This suggests that strains previously exposed to irradiation respond to subsequent exposures in a unique way (Malo et al., 2021). Based on the observations reported for *E. dermatitidis*, it is plausible that *A. niger*
JSC-093350089 strain response to the ISS environment was also unique, as it was previously isolated from the ISS and likely radioadapted itself. However, such assumption may not be confirmed in the current study, as there is no available not-radioadapted control for the *A. niger* JSC-093350089 strain. Future studies should be warranted to investigate whether *A. niger* JSC-093350089 strain's response to the ISS environment changes with consecutive exposures to the ISS environment when compared to the *original* ISS isolate. Finally, it has been previously reported that in *Aspergillus* genome intragenic regions...
TABLE 3 | Differentially expressed proteins involved in carbohydrate metabolism.

| ORF     | Protein   | CAZy Family | Function / Activity                     | Relative protein abundance* | p-value  |
|---------|-----------|-------------|----------------------------------------|----------------------------|----------|
| An03g00940 | XlnA/XynA | GH10        | 1,4-β-xylosidase                       | 2.22                       | 2.66E-03 |
| An01g11660 | CbB       | GH7, CBM1   | Cellobiohydrolase B                    | 2.00                       | 2.49E-03 |
| An03g00500 |          | GH30        | 1,6-β-glucosidase                      | 1.97                       | 6.91E-03 |
| An11g01540 |          | GH16        | β-glucanase                            | 1.93                       | 4.20E-03 |
| An08g08370 |          | GH92        | α-1,2-mannosidase                      | 1.93                       | 5.22E-03 |
| An13g01260 |          | GH92        | α-1,2-mannosidase                      | 1.83                       | 4.16E-03 |
| An15g04900 |          | AA9, CBM1   | β-1,4-glucanase D                     | 1.77                       | 2.18E-02 |
| An11g03340 | AamA      | GH13        | acid α-amyrase                         | 1.71                       | 8.95E-04 |
| An02g00850 |          | GH16        | β-glucanase                            | 1.70                       | 4.28E-02 |
| An11g01120 |          |             | Erythrose reductase                    | 1.65                       | 7.94E-04 |
| An15g07800 | AglC      | GH13        | 4-α-glucanotransferase                | 1.61                       | 2.64E-03 |
| An03g00960 | AxlA      | GH62        | α-L-arabinofuranosidase               | 1.60                       | 9.66E-03 |
| An02g06820 | PdcA      |             | Pyruvate decarboxylase                 | 1.57                       | 5.76E-03 |
| An02g11150 | AglB      | GH27        | α-galactosidase II                    | 1.37                       | 2.89E-03 |
| An08g01710 | AbtC      | GH51        | Arabinofuranosidase                   | 1.36                       | 4.94E-04 |
| An14g02760 | EglA      | GH12        | β-1,4-glucanase                       | 1.27                       | 6.70E-05 |
| An14g02070 | CEnc      |             | Acetylxyylan esterase                 | 1.27                       | 8.67E-03 |
| An05g02410 |          | GH2         | Glycosidase hydrolase                  | 1.10                       | 1.77E-02 |
| An07g09330 | CbhA      | GH7         | Cellobiohydrolase A                   | 1.07                       | 1.44E-03 |
| An01g03740 | XynA      | –           | D-xylene reductase                    | –1.08                      | 2.59E-02 |
| An12g00030 | XdhA      | –           | D-xylulose reductase                  | –1.10                      | 1.72E-02 |
| An07g08990 | PkiA      | –           | Pyruvate kinase                       | –1.12                      | 2.92E-03 |
| An18g06500 |          |             | Phosphomannomutase                    | –1.14                      | 6.84E-03 |
| An12g03070 | GlaB      | GH15        | Glucoamylase                          | –1.38                      | 7.87E-03 |
| An11g02550 |          |             | Phosphoenolpyruvate carboxykinase     | –1.51                      | 3.44E-03 |
| An15g01920 | McoA      | –           | 2-Methylocitrate synthase             | –1.55                      | 2.18E-02 |
| An01g02970 | AcdU      | –           | Isocitrate lyase                      | –1.59                      | 9.83E-03 |
| An15g03550 |          | GH43        | Hydrolase                             | –1.60                      | 9.18E-04 |
| An07g08530 | Pda1      | –           | Pyruvate dehydrogenase                | –2.19                      | 2.00E-03 |

*p-Log2 fold change of ISS-grown JSC-093350089 compared to Earth-grown counterpart (p < 0.05).
production of all SMs, which is the opposite production pattern observed during the initial characterization of the metabolome of JSC-09335008 when compared to a “terrestrial” strain (Romsdahl et al., 2020). This discrepancy may be related to the fact that metabolomic profile of ISS-isolated JSC-09335008 was compared to the well-studied ATCC 1015 strain, rather than a “proper” JSC-09335008 ground control. Further investigation of the space environment-induced SM profile of the JSC-09335008 strain should be conducted to confirm whether decreased production of pestalamide B, nigernazine B (alkaloid), and nigragillin (alkaloid) are important biological adaptations. Given that sending experiments to the ISS is not readily available, it will be critical to use more easily accessible microgravity simulators, like High Aspect Ratio Vessels—RPMs, or random positioning machines—RPMs to gain more insights in the space-induced phenotype of A. niger JSC-09335008 strain.

Finally, to gain insight into observed differences in acquired SM profiles between current and previous study (Romsdahl et al., 2020) further experiment was conducted. Both ISS- and ground-grown JSC-09335008 were regrown in the same conditions (28°C for 5 days) as used in the previous study, which resulted in observing similar trends in SM production. After regrowing at 28°C, the ISS-grown JSC-09335008 produced higher yields of all SMs when compared to the regrown ground control, including approximately 60% increased production of the antioxidant pyranonigrin A. Pyranonigrin A was previously proposed to have a radioprotective nature, as pyranonigrin A-deficient JSC-09335008 strain was more sensitive to UVC exposure than the wild type JSC-09335008 strain (Romsdahl et al., 2020). Interestingly, pyranonigrin A production was not detected in the ISS- and ground-grown samples following the
experiment on the ISS where growth temperature was about at 22°C. Due to this temperature-dependent discrepancy in observed SM profiles, the most important question to address is whether pyranonigrin A truly provides A. niger protection while in space. Such protection could potentially have various biotechnological applications for use of pyranonigrin A, including within human space programs and cancer therapies. Therefore, studies confirming pyranonigrin A potential as a radioprotective agent should be warranted. Finally, future studies should examine production of pyranonigrin A under various temperatures aboard the ISS, as well as its protective nature within the space environment to definitively answer this question.

**TABLE 6 |** Differentially expressed proteins involved in protein catabolic process.

| ORF       | Protein | Function / Activity                                      | Relative protein abundance* | p-value   |
|-----------|---------|---------------------------------------------------------|----------------------------|-----------|
| An02g07210 | PepE    | Acid aspartic protease                                   | −1.21                      | 1.27E-04  |
| An14g06800 | Pre10   | 20S CP alpha subunit of the proteasome                   | −1.27                      | 1.08E-03  |
| An04g01800 | Pre14   | Hypothetical protein                                     | −1.53                      | 2.50E-03  |
| An02g07040 | Scil    | 20S CP alpha subunit of the proteasome                   | −1.84                      | 4.37E-03  |
| An02g03400 | Pup2    | 20S CP alpha subunit of the proteasome                   | −1.84                      | 5.62E-03  |
| An11g04820 | Endopeptidase |                                                  | −1.95                      | 1.20E-02  |
| An07g02010 | Pre8    | 20S CP alpha subunit of the proteasome                   | −1.99                      | 2.83E-03  |
| An11g06720 | Pre9    | 20S CP alpha subunit of the proteasome                   | −1.99                      | 9.48E-03  |
| An18g06880 | Role    | in proteasomal ubiquitin-independent protein catabolic process | −2.03                  | 4.65E-03  |
| An04g01870 | Pre17   | Endopeptidase activator activity                          | −2.05                      | 5.99E-03  |
| An19g06700 | Pre7    | 20S CP beta subunit of the proteasome                    | −2.08                      | 2.59E-03  |
| An11g01760 | Pre2p   | Protein similar to proteasome 20S subunit Pre2p           | −2.10                      | 9.96E-04  |
| An13g01210 | Endopeptidase |                                                  | −2.12                      | 4.55E-03  |
| An15g00510 | Pre5    | 20S CP alpha subunit of the proteasome                   | −2.13                      | 3.01E-03  |
| An02g10790 | Pre6    | 20S CP alpha subunit of the proteasome                   | −2.18                      | 6.04E-03  |

*Log2 fold change of ISS-grown JSC-093350089 compared to Earth-grown counterpart (p < 0.05).

**FIGURE 2 |** Secondary metabolite production of ISS-grown JSC-093350089 when compared to ground controls. (A) Secondary metabolite profiles of ISS- and ground-grown JSC-093350089 when grown on GMM. (B) Metabolite quantification showing the percent change for each metabolite in relation to ground-grown JSC-093350089; significance was determined using Welch’s t-test. *** means and indicates statistical significance.
This study is the first report of the multi-omics response of *A. niger* to space conditions during a controlled experiment, which enhances our understanding of its space-induced phenotype. Such understanding may be translated to development of protective measures for both astronauts and the spacecraft during future manned space explorations, as *A. niger* is ubiquitous fungus present in many human-occupied closed habitats (Checinska et al., 2015; Blachowicz et al., 2017). Lastly, a thorough understanding of the space-induced secondary metabolomic alterations of industrially important *A. niger* may result in creating a potent producer of compounds of interest during space voyages.

**DATA AVAILABILITY STATEMENT**

Raw WGS reads for JSC-093350089 ISS- and ground-grown are available in the NCBI SRA, under accession numbers SAMN25997338 and SAMN25997339 and BioProject accession number PRJNA807647. Proteomics data is accessible through Massive with the dataset identifier MSV000088986.

**AUTHOR CONTRIBUTIONS**

AB drafted the manuscript, contributed to sample processing, and conducted data analysis and interpretation. JR contributed to sample processing and data interpretation. AC and MK conducted protein sample processing, LC–MS analyses, and data processing. SM and JS contributed to genome analysis. TT designed the study and drafted the manuscript. KV and CW conceptualized the project, coordinated the flight experiment, designed the study, interpreted the data, and drafted the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.893071/full?supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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