Integrated proteomic and metabolomic analysis to study the effects of spaceflight on Candida albicans

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Research article

Keywords: Spaceflight, Metabolomics, Proteomics, Candida albicans

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Abstract

Background Candida albicans is an opportunistic pathogenic yeast, which could become pathogenic in various stressful environmental factors including the spaceflight environment. In this study, we aim to explore the phenotypic changes and possible mechanisms of Candida albicans after exposure to spaceflight conditions.

Results The effect of Candida albicans after carried on the "SJ-10" satellite for 12 days was evaluated by proliferation, morphogenic, environmental resistance and virulence experiment. The result showed that the proliferation rate, biofilm formation, antioxidant capacity, cytotoxicity and filamentous morphology of Candida albicans were increased in the spaceflight group compared to the control group. Proteomics and metabolomics technologies were used to analyze the profiles of proteins and metabolites in Candida albicans under spaceflight conditions. Proteomic analysis identified 564 up-regulated proteins involved in ribosome, DNA replication, base excision repair and sulfur metabolism in the spaceflight group. And 345 down-regulated proteins related to metabolic processes were observed. The metabolomic analysis found 5 different expressed metabolites. The combined analysis of proteomic and metabolomic revealed the accumulation of cysteine and methionine in Candida albicans after spaceflight.

Conclusions Mechanisms that could explain the results in the phenotyping experiment of Candida albicans were found through proteomic and metabolomic analysis. And our data provide an important basis for the assessment of the risk that Candida albicans could cause under spaceflight environment.

Background

In recent years, with the development of space technology, space microbial safety has become a research hotspot. It has been known that microorganisms such as bacteria and fungi have widely existed in the International Space Station[1]. *Candida albicans* is a common conditional pathogen that usually parasitizes on human skin, mouth, urinary tract, and reproductive system [2]. While the pathogenicity of *Candida albicans* may change with the changes in the external environment[3]. It has been reported that microorganisms including *Candida albicans* proliferate more rapidly in the International Space Station [4; 5], thereby multiplying the risk of onboard cross-contamination, colonization, and infection. Worse, space environment can potentially alter microbial physiology and virulence[6]. And the immunological investigations of the astronauts recorded several dysregulations, including lymphocyte proliferation, cytokine production[7] and redistribution of leukocyte subsets[8]. Together, the presence of *Candida albicans* poses a potential threat to astronauts' health. However, little is known about the molecular mechanism changes of *Candida albicans* under the space environment.

A comprehensive understanding of the molecular communication will therefore provide new insights into this molecular mechanism. With the development of omics technology, high-throughput molecular identification and quantitation became available. Proteomics[9] could explore the expression of all the proteins of *Candida albicans* under different environmental conditions. Metabolomics[10] is the study of

the composition and variation of metabolic groups, thereby revealing the overall metabolic response and dynamic changes under different conditions. Thus, the results of integrated omics analysis could be useful for understanding the molecular mechanism of *Candida albicans* with the rapid proliferation and enhanced toxicity under the space environment.

In this study, we took an integrative proteomic and metabolomic approach to identify the differentially expressed molecules of *Candida albicans* carried by the SJ−10 satellite. The result of proteomic and metabolomic could reflect the phenotypic changes of *Candida albicans.* To our knowledge, this is the first multi-omics approach to study *Candida albicans* under spaceflight environment.

Results

Effect of *Candida albicans* under space environment

After exposure to the spaceflight environment, *Candida albicans* were recovered in the SDB medium and their survival was evaluated by OD_{600} measurement. The same measurements were applied towards non-exposed control *Candida albicans,* which

were cultured at the ground. The result showed that the growth lag period of the spaceflight group was shorter than that of the control group (Fig1A). And the proliferation rate of the spaceflight group was faster than control group ($P<0.05$). As shown in Fig1B, the surface of *Candida albicans* in spaceflight group was rougher and the wrinkles at the edges were increased when compared to control group. The amount of biofilm formation was significantly increased in spaceflight group (Fig1C). Increased filamentous forms and budding with tight cell connections were observed in spaceflight *Candida albicans* through SEM, while grape-like clusters with sparse cell connections were observed in control group (Fig1D). The environmental resistance evaluation result showed that the survival rate of spaceflight group was higher than that of control group in SDB containing hydrogen peroxide (Fig1E). However, no significantly different were observed between two groups of *Candida albicans* in resistance to acid, alkali, alcohol, and salt (Supplementary Figure S1). The result of virulence experiment (Supplementary Figure S2) showed that spaceflight group had a lower maximum and a more significant decrease of the normalized cell index (NCI) compared to control group, which indicates that spaceflight group has stronger cytotoxicity. All in all, the proliferation rate, biofilm formation, antioxidant capacity and cytotoxicity of *Candida albicans* were increased after exposure to spaceflight environment.

Proteomic analysis of *Candida albicans*

In total, 3670 proteins were identified and 3499 proteins were quantified in the spaceflight group and control group of *Candida albicans* by TMT (Supplementary Table S1). Proteins with ratios spaceflight /control greater or lower than 1.2-fold change with P value < 0.05 were considered to be significantly changed. This resulted in 564 significantly up-regulated and 345 significantly down-regulated proteins in the spaceflight group compared with the control group (Supplementary Table S2), which were shown in

Figure 2A. Quantitative proteome data of *Candida albicans* samples were used for hierarchical clustering(Fig 2B) and Principal Component Analysis (PCA) (Fig2C). The result showed that spaceflight group and control group are clearly separated, which reflected the significant change of protein expression in *Candida albicans* after exposure to spaceflight environment.

To identify biological function changes in *Candida albicans* under spaceflight environment, KEGG enrichment analysis was performed with the differentially expressed proteins by DAVID. The significantly enriched KEGG pathways (ranked by P value) in up-regulated or down-regulated proteins were shown in Figure 2D. Among the 564 up-regulated proteins in spaceflight group, pathways including ribosome and DNA replication were enriched, which explains the increased proliferation rate of spaceflight *Candida albicans.* In addition, base excision repair was also enriched, which related to DNA damage repair and may explain the increased antioxidant capacity of the spaceflight group. Sulfur metabolism was enriched in up-regulated proteins. While metabolic processes such as carbon metabolism, biosynthesis of secondary metabolites, glyoxylate and dicarboxylate metabolism, propanoate metabolism, phenylalanine metabolism, tyrosine metabolism, and fatty acid degradation were significantly enriched in the down-regulated proteins. This reflected the complex metabolic regulation in spaceflight *Candida albicans.*

Metabolomic analysis of Candida albicans

To investigate the effect of spaceflight on metabolism, we used ultra-performance liquid chromatography mass spectrometry (UPLC/MS)-based metabolomics approach to untarget quantify the metabolites in *Candida albicans.* After comparing two groups of *Candida albicans* with OPLS-DA, 5 significantly different abundance features (P<0.05, VIP>1) were identified (Supplementary Table S3). Those features were further annotated with public databases. Out of these five metabolites, 5'-Methylthioadenosine and adenylsuccinic acid were significantly up-regulated in spaceflight *Candida albicans* (Fig3A). While LysoPE (18:3(9Z,12Z,15Z)/0:0), LysoPE (16:1(9Z)/0:0) and LysoPE (18:2(9Z,12Z)/0:0) were significantly down-regulated in spaceflight *Candida albicans (*Fig3B).

Integrated analysis of proteome and metabolome data in *Candida albicans*

To integrate the result of proteome and metabolome data, we transformed protein ID and metabolites name to KEGG ID and KEGG compound, respectively. After mapping molecular objects to KEGG pathway, three pathways including purine metabolism, cysteine and methionine metabolism, and alanine, aspartate glutamate metabolism were found to be regulated by both differentially expressed proteins and differentially expressed metabolites (Supplementary Figure S3). Among those three pathways, adenylsuccinic acid, which was up-regulated in spaceflight group, was mapped both in alanine, aspartate glutamate metabolism, and purine metabolism pathway. Consistent with the metabolome result, adenylosuccinate synthetase, which catalyzes IMP and L-aspartate to generate adenylsuccinic acid, was also up-regulated (P = 0.0001, fold change = 1.17) in spaceflight group from proteome data. 5'-

Methylthioadenosine (MTA) is a naturally occurring sulfur-containing nucleoside, which is a major by-product of polyamine biosynthesis involved in cysteine and methionine metabolism pathway[11]. We found MTA was up-regulated in the spaceflight group with metabolomics analysis. While S-methyl−5'-thioadenosine phosphorylase (MEU1), which involved in the breakdown of MTA and responsible for the first step in the methionine salvage pathway after MTA has been generated from S-adenosylmethionine[12], was significantly up-regulated in spaceflight group of proteomics.

Discussion

Candida albicans is an opportunistic pathogenic yeast[13], which usually exists as a commensal organism but can become pathogenic in immunocompromised individuals under a variety of conditions[14]. Long term spaceflight with microgravity and motionless may have adverse effects on the immune system of astronauts[7]. Meanwhile, Jiang et al[15] reported that simulated microgravity promoted the growth rate of Candida albicans significantly. Altenburg et al[16] found that the growth of Candida albicans in simulated microgravity results in an increase in filamentous forms of the organism which is consistent with enhanced pathogenicity. Those reports indicated that the existence of Candida albicans in the space environment may be threated to the health of astronauts. And the specific mechanisms of Candida albicans' increased proliferation and pathogenicity need further study. In this study, the combination of proteomic and metabolomic approach was used to study the effects of spaceflight on Candida albicans. To our knowledge, this is the first report of Candida albicans under spaceflight conditions with proteomic and metabolomic analyses.

We explored the phenotypic change of Candida albicans after exposure to the spaceflight environment. Increased proliferation rate and filamentous morphology of Candida albicans in spaceflight groups were observed, which were consistent with the previous report[15; 16]. In addition, we found that the biofilm relative formation, antioxidant capacity, and cytotoxicity of Candida albicans were increased in spaceflight group. Crabbé et al [17] reported that genes involved in oxidative stress resistance were up-regulated in spaceflight cultured Candida albicans with microarray analysis, which was consistent with our result. However, Hammond et al[18] reported that the virulence of Candida albicans to kill wild type Caenorhabditis elegans was reduced under spaceflight environment. The difference between their results and ours may because their experiment was done in the International Space Station with 48h, while our spaceflight environment was low earth orbit flight for 12 days. The difference in space flight time and environment may have different influences on the microorganism.

Integrated analysis of proteomic and metabolomic of Candida albicans revealed that spaceflight may have an influence on cysteine and methionine metabolism pathway. Although MTA, which is synthesized from methionine, was up-regulated in the spaceflight group. The expression of MEU1, which involved in the methionine salvage pathway by breakdown MTA, was increased in the spaceflight group. This may reduce the consumption of methionine. While bifunctional cysteine synthase (MET15)[19], which has cysteine synthase activity and may synthase cysteine from serine, was increased in spaceflight group.

Meanwhile, aspartate aminotransferase (AAT21) and aspartate transaminase (AAT1), which may play roles in catalyzes cysteine[20],were down-regulated in spaceflight group. So we concluded that cysteine and methionine that in the spaceflight group of *Candida albicans.* Besides, methionine and cysteine are sulfur-containing amino acids, and proteins involved in sulfur metabolism such as sulfite reductase subunit alpha (MET10), sulfite reductase beta subunit, sulfate adenylyltransferase (MET3) and adenylyl-sulfate kinase (MET14) were simultaneously up-regulated in spaceflight group. Interestingly, many research reported[21; 22] that most genes in methionine and cysteine biosynthesis pathway were up-regulated during biofilm formation. In addition, Li et al[23] showed that the development of *Candida albicans* was enhanced by addition of methionine and cysteine. The result inferred from proteomic and metabolomic analysis was consistent with the observed influence on phenotypic of *Candida albicans* after spaceflight.

Conclusions

We explored the effect of spaceflight on *Candida albicans* by combining proteomic and metabolomic analyses in this study. To our knowledge, this is the first proteomic and metabolomic study on *Candida albicans* after spaceflight. Increased proliferation rate, biofilm formation, antioxidant capacity, cytotoxicity, and filamentous morphology were observed in the spaceflight group of *Candida albicans* compared to the control group. Proteomic analysis identified 3670 proteins and showed that spaceflight samples and control samples could be separated with proteomic data. Enrichment analysis with differentially expressed proteins indicated that proteins in ribosome, DNA replication, base excision repair, and sulfur metabolism were significantly up-regulated, while proteins in many metabolic processes were significantly down-regulated. Metabolomic analyses found five different expressed metabolites. The combined analysis of proteomic and metabolomic revealed the accumulation of cysteine and methionine in *Candida albicans* after spaceflight. This study showed that proteomic and metabolomic are useful tools that could explain the phenotypic changes of *Candida albicans.*And the data in this study will facilitate the future mechanism exploration and disease prevention of *Candida albicans.*

Materials And Method

Strains and growth conditions

*Candida albicans (*CMCC(F)98001)were purchased from China General Microbiological Culture Collection Center. Sabouraud-dextrose broth (SDB) was purchased from Oxoid. *Candida albicans* was cultured in exponential phase (OD_{600} = 1) and inoculated into SDB. After incubated at 30°C for 30 h, half of the samples were cultured in space for 12 days carried by the "SJ−10" satellite. And the rest samples were cultured at the ground as control. Strains were preserved by adding glycerin and stored at −20°C.

Growth curves of *Candida albicans*

Candida albicans in the spaceflight group and control group were recovered on SDB medium at 30°C overnight, respectively. Samples were inoculated into SDB liquid medium and incubated at 150 rpm at 30°C. The growth curves of *Candida albicans* were recorded by using a spectrophotometer. The initial OD_{600} measurement is measured every 2 hours at the beginning of the lag phase and is measured every 1 h in the exponential phase.

Colony wrinkles and biofilm formation assay

Preserved strains of *Candida albicans* (5μL) were inoculated into SDB solid medium and incubated at 30°C for 5 h. Colony wrinkles of *Candida albicans* were observed with a microscope.

Preserved strains of *Candida albicans* (20μL) were added into 2 mL SDL liquid medium in glass tubes and incubated at 150 rpm at 30°C until the stationary phase. OD_{600} was measurement after incubation and the glass tubes were washed two times with deionized water gently. Glass tubes were added with 5 mL 0.1⬜ crystal violet solution and dyeing for 15 min after drying 1h at 60°C. After washed with deionized water gently, 5 mL dimethyl sulfoxide was added into the glass tubes and OD_{570} was measured 1h later. Biofilm formation was calculated as $100×OD_{570}/OD_{600}$.

Morphology of *Candida albicans*

Preserved strains of *Candida albicans* (1μL) were recovered with 5 mL SDB liquid medium at 150 rpm at 30°C overnight. Then coverslips were placed in a solution mixed with sample and SDB liquid medium (1:2). After incubation 8h at 30°C, coverslips were fixed with 5% glutaraldehyde solution at 4°C overnight. Then samples were sent to China Academy of Chinese Medical Sciences for scanning electron microscope (SEM) analysis. Briefly, samples were fixed with tannic acid for 1h. Then samples were dehydrated by a graded series of ethanol (50%, 70%, 90%, and 100%) for 15 mins at each step, and transferred to tert-butanol for 30 mins. In the end, the samples were dehydrated with dryer and coated with gold-palladium. The morphology of *Candida albicans* was observed in Hitachi S−3400N SEM.

Environmental resistance assay

Preserved strains of *Candida albicans* (1μL) were inoculated into SDB solid medium and incubated at 30°C overnight. Recovered samples were inoculated into SDB liquid medium and incubated at 150 rpm at 30°C until the exponential phase (OD_{600} = 1). Then samples were added into five SDB liquid mediums (1:100) containing HCL (pH 3.5), ammonia (pH 9.5), 3% ethanol, 0.0003% hydrogen peroxide or 450mmol/L NaCl, respectively. While normal SDB liquid medium was used as a background. After incubated 7h at 200 r/min at 30°C, samples were diluted with PBS at three volume ratios (10^{-3}, 10^{-4} and 10^{-5}). And 200μL diluted samples were evenly spread to SDB solid medium, respectively. The number of colonies was calculated after incubation at 30°C. The survival rate was calculated as the mean number of colonies from mixed SDB divide by the mean number of colonies from normal SDB.

Virulence of *Candida albicans*

LoVo cells were maintained in DMEM-F12 growth medium containing 10% FBS at 37°C in a CO_2 incubator. Cells were harvest with trypsin and adjusting concentration to $2×10^5$ cells/mL. *Candida albicans* was recovered with SDB liquid medium at 150 rpm at 30°C overnight. After centrifugation at 4000 rpm for 5 min, the precipitation was resuspended with PBS. After centrifugation again, the precipitation was resuspended with DMEM-F12 medium at the concentration of 4.5×107 CFU/mL. Then 100 μL *Candida albicans* were added into 200 μL LoVo cells. Samples were detected with real time cell analyzer (RTCA) in a CO_2 incubator for 10h at 37°C.

Sample preparation for proteome and metabolome analysis

Candida albicans in spaceflight group and control group were recovered on SDB solid medium at 30°C overnight, respectively. Recovered samples were inoculated into SDB liquid medium and incubated at 150 rpm at 30°C and grown to an OD_{600} of 1. Samples were harvested by centrifugation at 4000 rpm for 5 min at 4°C. The precipitate was collected and washed with PBS three times. All samples were stored at −80°C until use.

Proteome analysis

Samples were resuspended with lysate buffer (7 M urea, 2 M thiourea, 40 mM DTT, 1mM PMSF[24]) and were sonicated (1s/1s intervals, 80W power) for 3 mins. Cell debris was removed by centrifugation at 3000 g for 5 min at 4°C. Protein concentration was determined by Bradford assay and aliquoted to store at −80°C. For each group, 100 μg of proteins were mixed with 120 μL reducing buffer (10 mM DTT, 8 M Urea, 100 mM triethylammonium bicarbonate (TEAB), pH 8.0) in Amicon® Ultra−0.5 Centrifugal Filter (10 kDa)[25] and incubated at 60°C for 1 h. Then iodoacetamide was added to the solution with the final concentration of 50 mM and incubated for 40 min at room temperature in the dark. After centrifugation at 12000 rpm for 20 min, samples were washed three times with TEAB and digested with trypsin (Promega, Madison, WI, USA) (enzyme to protein ratio 1:50) at 37°C overnight. Digested peptides were labeled with TMT reagents (Thermo Fisher Scientific) according to the manufacturer's instructions. For 6-plex TMT, spaceflight group samples were labeled with TMT tags 126, 127, 128, and control samples were labeled with TMT tags 129, 130, and 131, respectively. Equal amounts of TMT-labeled peptides were mixed and dried, then resuspended in buffer A (2% acetonitrile, 98% water with ammonia at pH 10) and fractionated to 15 fractions with 1100 HPLC System (Agilent Technologies, USA).

Peptides were redissolved with 0.1% formic acid (FA) and analyzed on a Q-Exactive HF mass spectrometer (Thermo Fisher Scientific, USA) coupled with a nanospray Flex source (Thermo Fisher Scientific, USA). Samples were loaded and separated by a C18 column (15 cm × 75 μm) on an EASY - nLC^{TM} 1200 system (Thermo Fisher Scientific, USA). The flow rate was 300 nL/min and linear gradient was 90 min. The mass spectrometer was operated in the data-dependent mode with positive polarity at

electrospray voltage of 2 kV. Full scan MS spectra (m/z 300–1600) were acquired in the orbitrap with the resolution as 70 K, the automatic gain control (AGC) target was 1e6 and the maximum injection time was 80 ms. The top 10 intense ions were isolated for HCD MS/MS fragmentation. In MS2, the resolution was 17500 and the AGC target was 2e5. Fragmentation was performed with normalized collision energy (NCE) of 32% and dynamic exclusion duration of 15 s.

The mass spectrometry (MS) raw data were analyzed with Proteome Discoverer software (version 2.2) using the Sequest search engine to search against the UniProt *candida albicans* database. The following parameters were applied: precursor mass tolerance was 10 ppm; fragment tolerance was 0.02 Da; the dynamic modifications were oxidation (M); the static modification was carbamidomethyl (C) and TMT labeling of amines and lysine; a maximum of two missed cleavages were allowed. Peptides with FDR < 0.01 (based on the target-decoy database algorithm[26]) were used for protein grouping.

Metabolome analysis

Metabolites were extracted with 300 µL methanol and ultrasonic oscillation for 10 min. The supernatant was collected after centrifugation at 12000 rpm for 10 min at 4°C. UPLC-QTOF/MS (Agilent Technologies, USA) was implemented for metabolites detection. The experimental quality was evaluated by quality control (QC) samples. Features alignment, picking, and identification were performed by Progenesis QI (Waters, Nonlinear Dynamics, Newcastle, UK). By combining the univariate and multivariate statistical analysis, significantly changed features (P value<0.05, VIP >1) were acquired. Those features were further annotated by Progenesis QI with public databases including Metlin, LIPID MAPS, PubChem, YMDB (Yeast Metabolome Database) and KEGG.

Statistical analysis

Perseus software[27] was used to calculate the differentially expressed proteins with unpaired Student's t-test. SIMCA (Version 14.1) was used for statistical analysis of metabolomics data with OPLS-DA (orthogonal projections to latent structures discriminant analysis). For other results, SPSS software package (Version 16.0) was used for statistical analysis with unpaired Student's t-test. The data are presented as means ± SD. P < 0.05 was defined as statistical significance.

Abbreviations

AAT1: aspartate transaminase; AAT21: aspartate aminotransferase; AGC: automatic gain control; MET3: sulfate adenylyltransferase; MET10: sulfite reductase subunit alpha; MET14: adenylyl-sulfate kinase;MET15:bifunctional cysteine synthase; MEU1: S-methyl–5'-thioadenosine phosphorylase; MS: mass spectrometry; MTA: 5'-Methylthioadenosine; NCE: normalized collision energy; NCI: normalized cell index; OPLS-DA: orthogonal projections to latent structures discriminant analysis; PCA: Principal Component Analysis; RTCA: real time cell analyzer; SDB: Sabouraud dextrose broth; SEM: scanning

electron microscope; UPLC/MS: ultra-performance liquid chromatography mass spectrometry; YMDB: Yeast Metabolome Database

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The mass spectrometry raw data were deposited to the iProX (http://iprox.org).

URL: https://www.iprox.org/page/PSV023.html;?url = 1573459747156q3cJ

Password: 1j0Q

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by National Significant Science Foundation (2015ZX09J15102), 1226 Engineering Health Major Project (AWS16J016), 1226 Engineering Health Key Project (BWS17J028).

Authors' contributions

JW performed the bioinformatics analysis and wrote the paper. YL and GZ performed the experiments and analyzed the data. JG, JL, XW and CX conceived the experiments and participated in discussion data. TL designed the experiments and revised the manuscript. All authors have read and approved the manuscript.

Acknowledgements

We thank Shanghai LuMing Biological Technology Co. Ltd. for proteome detection and data analysis.

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Figures
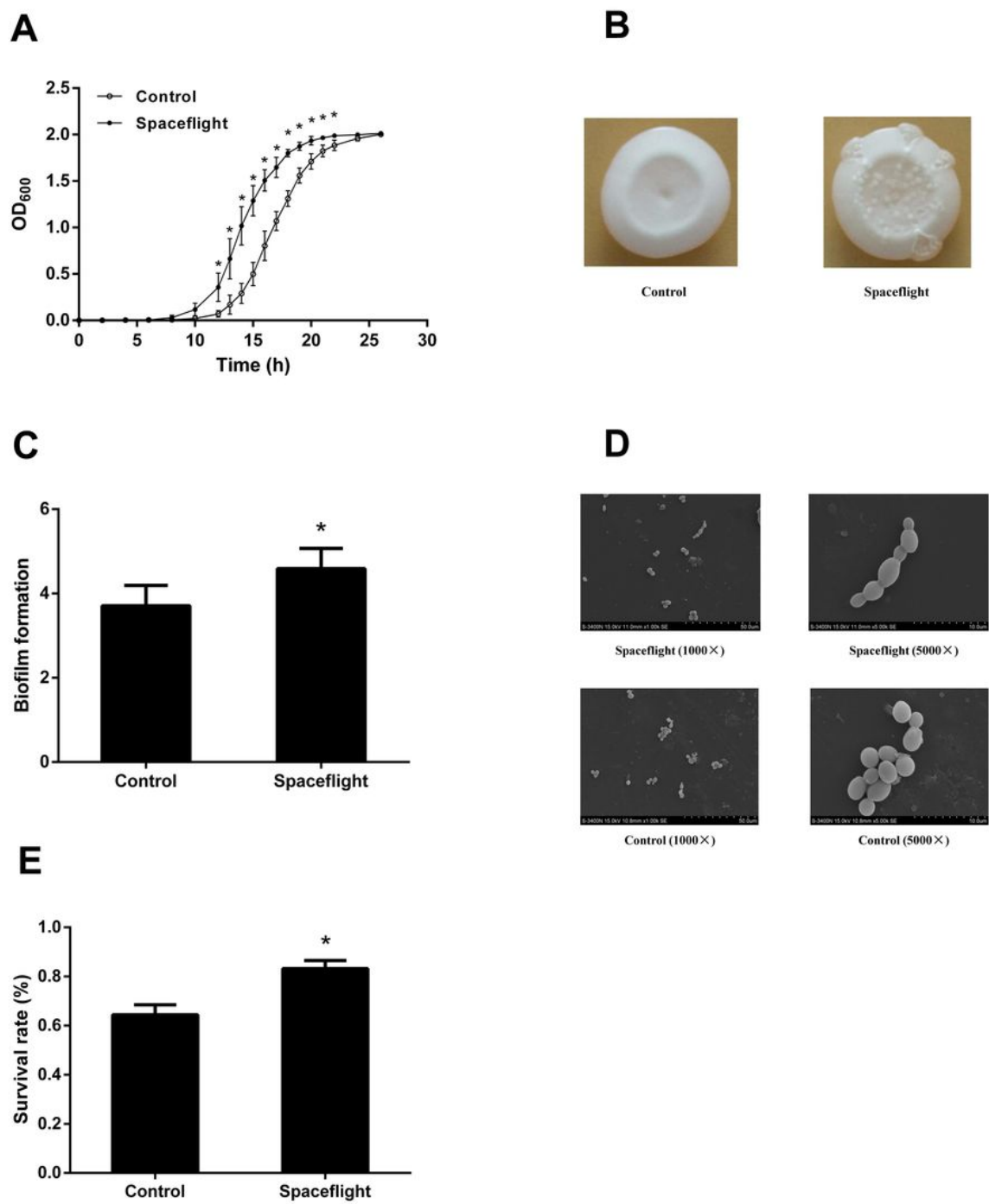
Figure 1

Effect of Candida albicans under spaceflight environment. (A)The growth curves of Candida albicans under spaceflight environment compared with control. (B) Colony wrinkles of Candida albicans after exposured to spaceflight (right) compared with control (left). (C) The relative biofilm formation of

Candida albicans. (D) Cell morphology of Candida albicans under normal and spaceflight conditions. (E) Survival rate of Candida albicans exposed to 0.0003% hydrogen peroxide under spaceflight compared with the control group. *: P < 0.05.
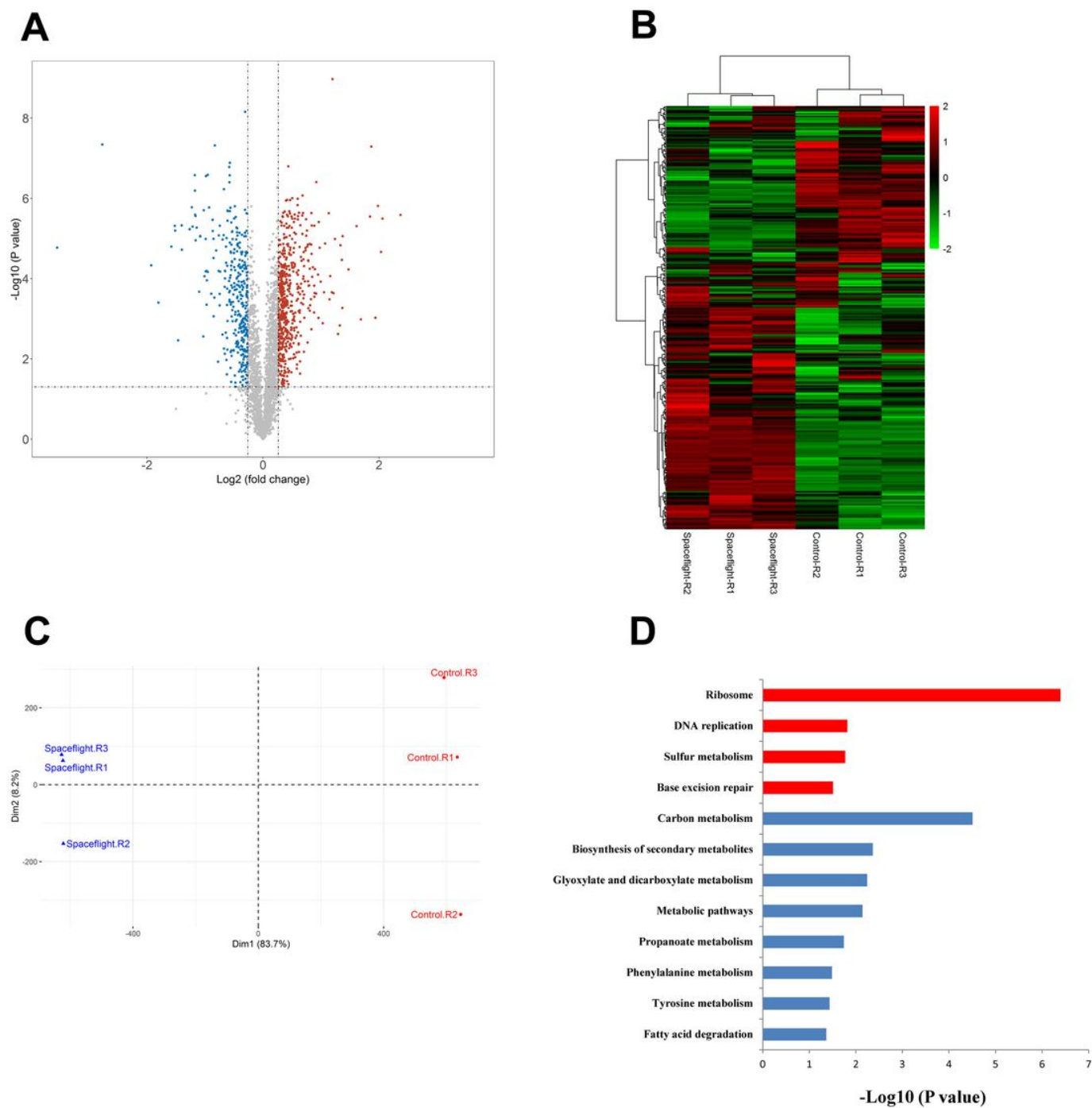
Figure 2

Proteomic analysis of Candida albicans. (A)Volcano plot of differentially expressed proteins. The red points represented 564 proteins that were up-regulated in spaceflight group (P < 0.05; fold change ≥ 1.2). The blue points represented 345 proteins that were down-regulated in spaceflight group (P value<0.05;

fold change ≤ 0.83). (B) Hierarchical clustering of Candida albicans proteomes. The heat map represented the Z scores of all proteins quantified in TMT technology. (C) PCA analysis of Candida albicans proteomes. (D) Enriched KEGG pathways in up-regulated (red) and down-regulated (blue) proteins of spaceflight group. The x-axis shows the enrichment significance presented with −log10 (p-value).
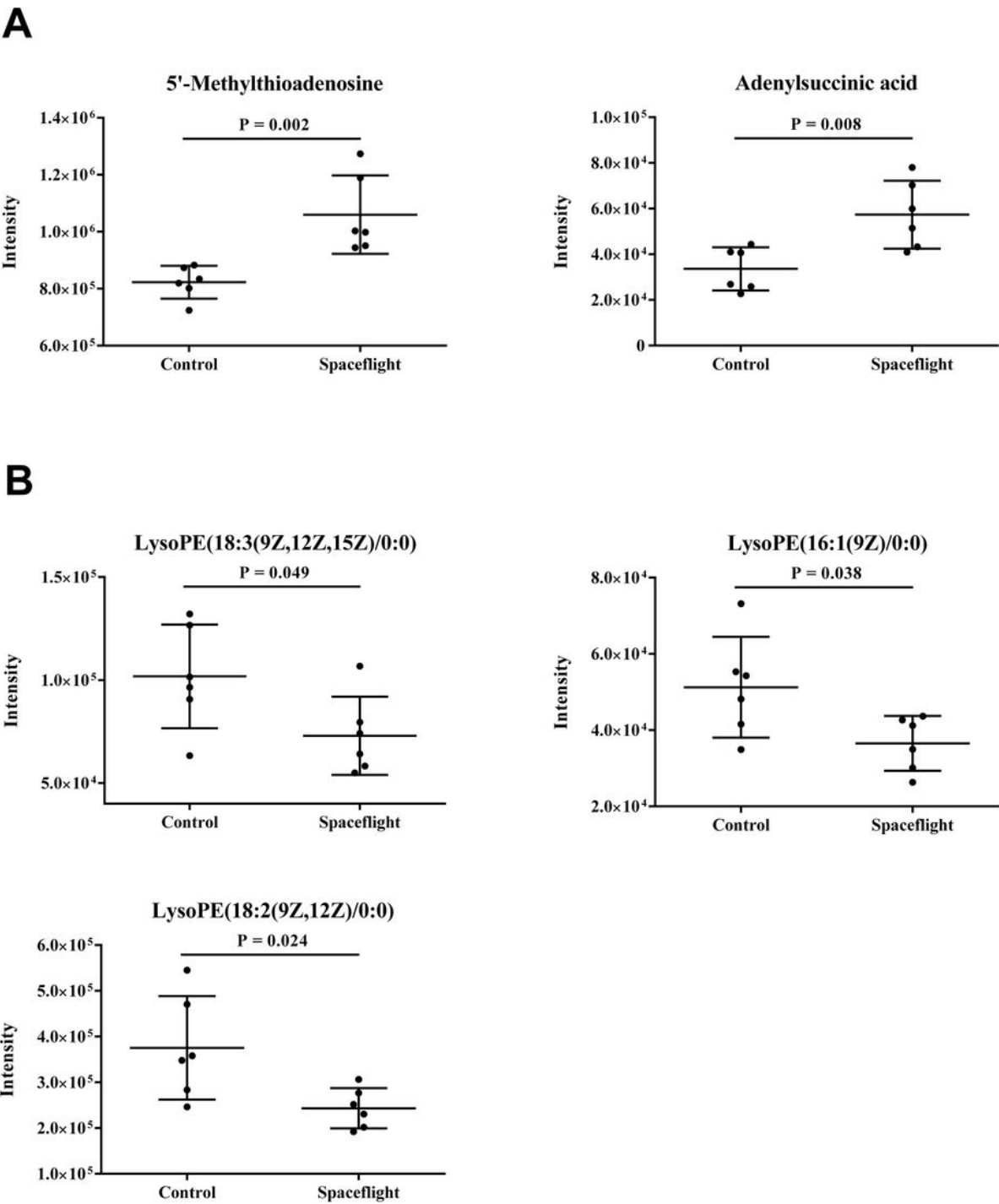
Figure 3

Differential metabolites of Candida albicans. (A) Scatter plots of two up-regulated metabolites in the spaceflight group. (B) Scatter plots of three down-regulated metabolites in the spaceflight group. P value was calculated with unpaired Student's t-test.