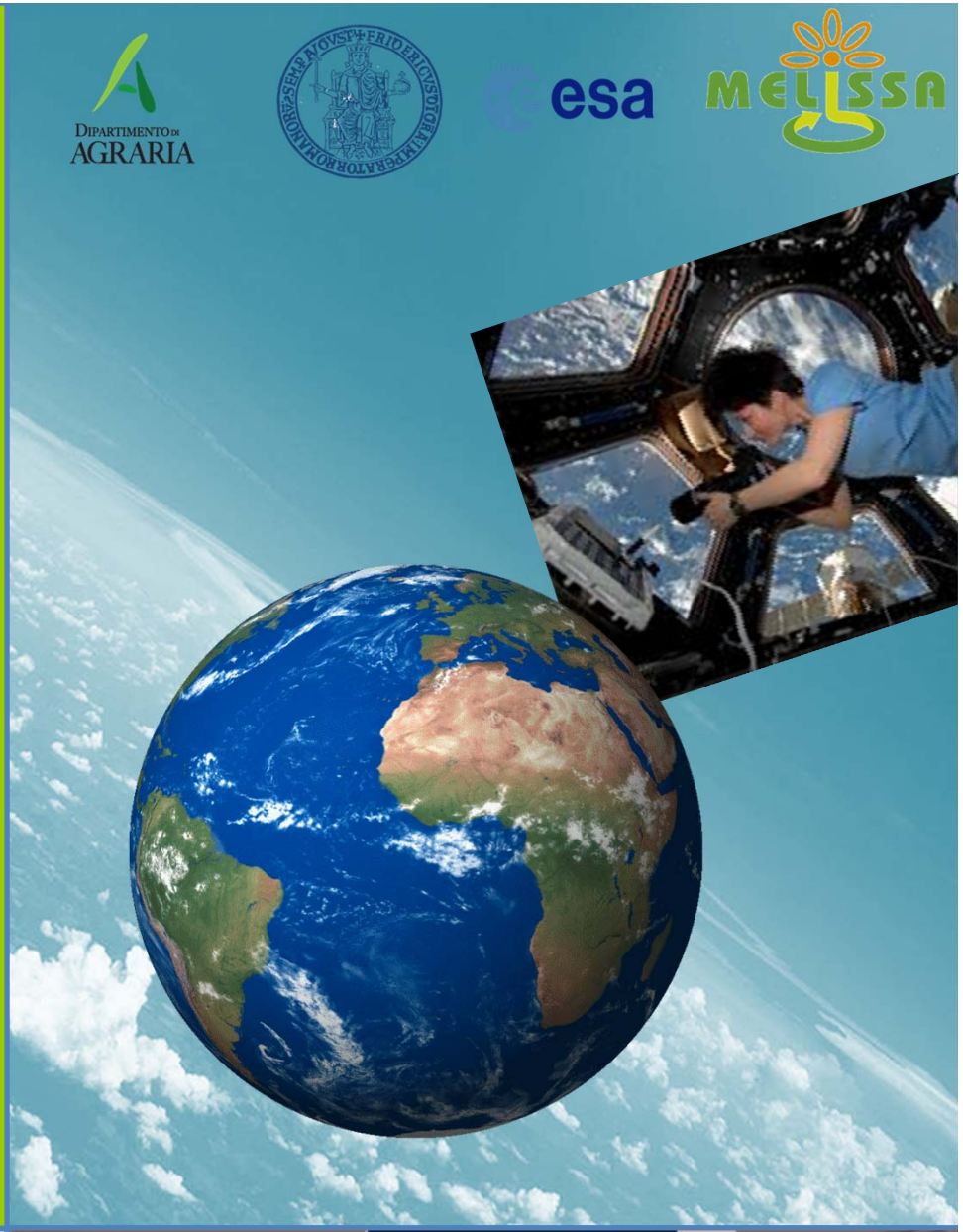
Metabolic, transcriptional and proteomic changes of the probiotic *Lactobacillus reuteri* DSM17938 under simulated microgravity



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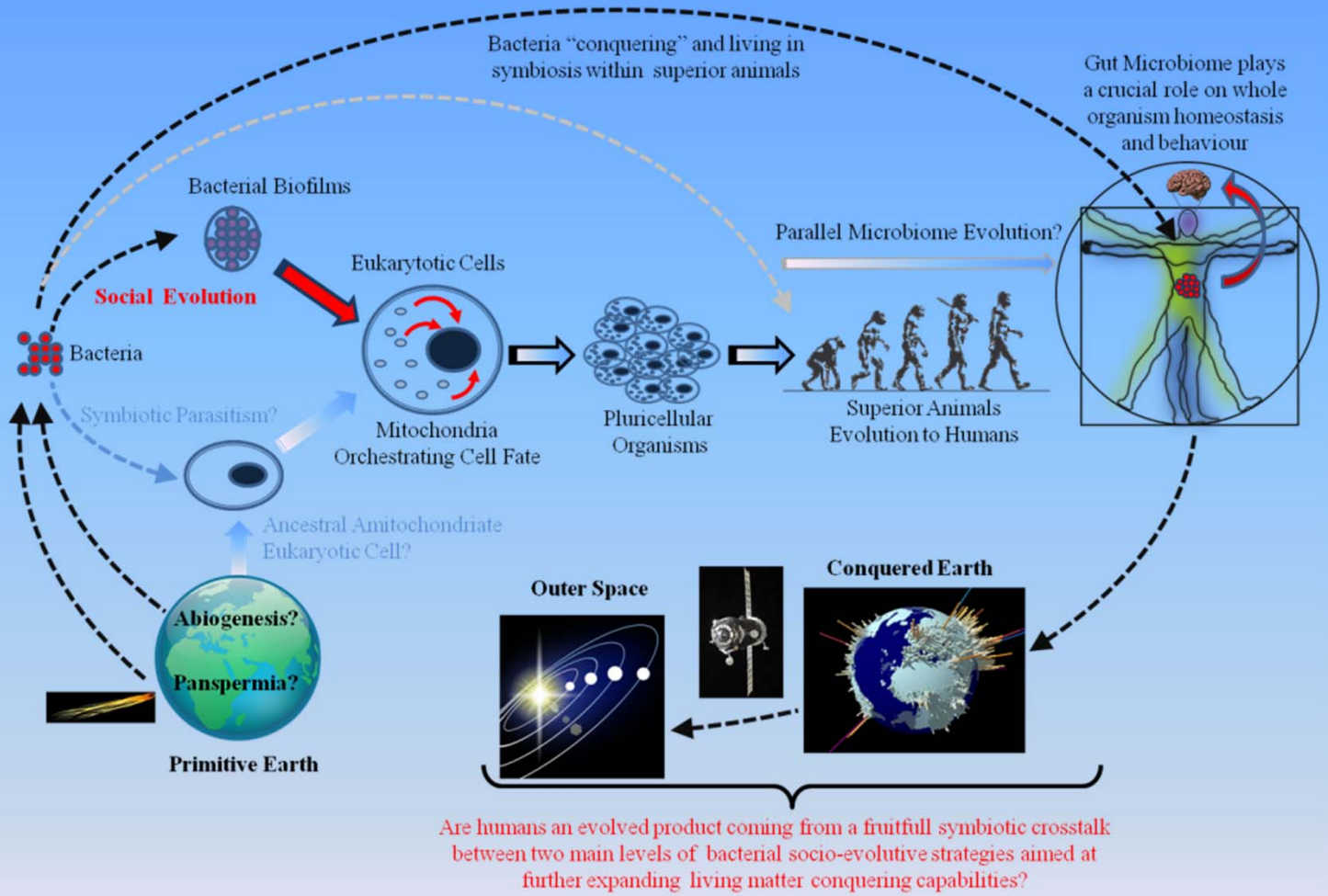
Bacteria in the Life



By Julio L. Padròn Velàzquez, Are humans a “clothed mass of microbes” engaged in a sort of panspermia? Hypothesis 2016, 14(1): e7, doi:10.5779/hypothesis. v14i1.479

Bacteria in the extraterrestrial Life

Proceedings of the Second Lunar Science Conference, Vol. 3, pp. 2721–2733
The M.I.T. Press, 1971.

Surveyor III: Bacterium isolated from lunar-retrieved TV camera

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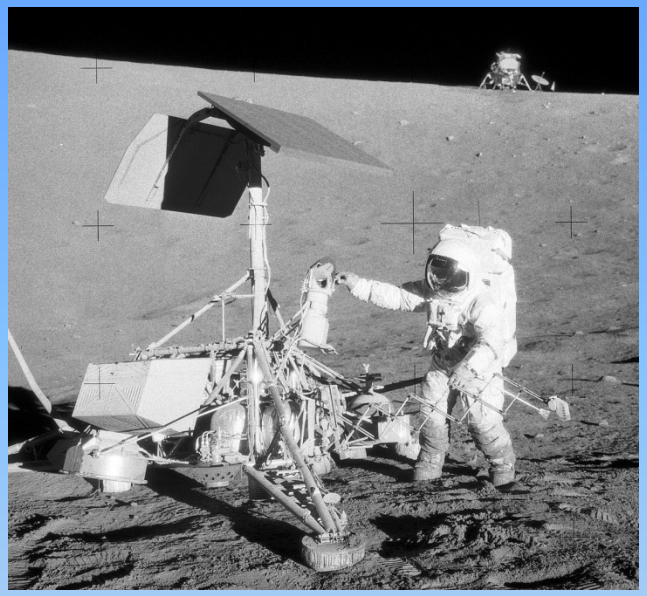
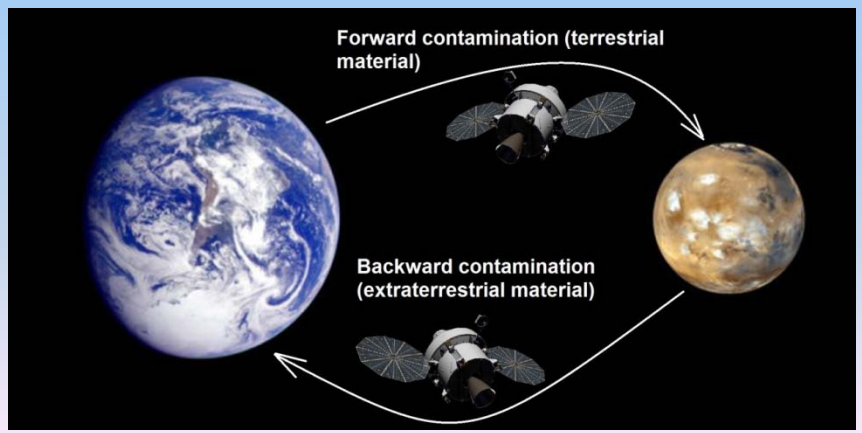
(*Received* 9 *February* 1971; *accepted in revised form* 31 *March* 1971)

Abstract—Selected components of the unmanned Surveyor III spacecraft which had remained on the lunar surface for 2½ years were collected and returned to earth by the crew of Apollo 12. A bacterium, *Streptococcus mitis*, was isolated from a sample of foam taken from the interior of the retrieved TV camera. The available data suggests that the bacterium was deposited in the camera prior to the Surveyor III spacecraft launch. The authors suggest that lyophilizing conditions existing during prelaunch vacuum testing and later on the lunar surface may have been instrumental in the apparent survival of this microorganism.

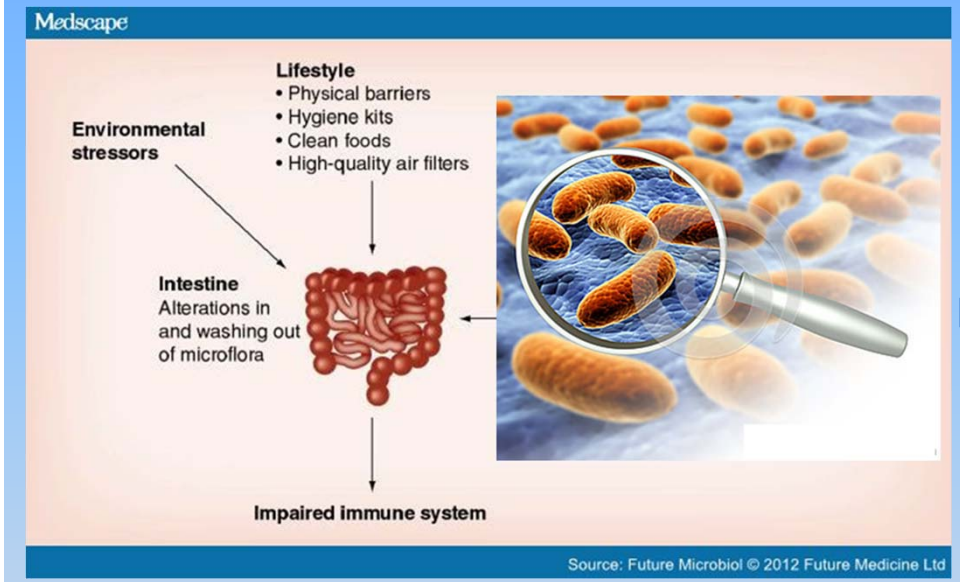


What is the truth about the Moon Microbes?



The Microbiota and probiotic bacteria

Spaceflight has several disrupting events that could lead to astronaut intestinal changes

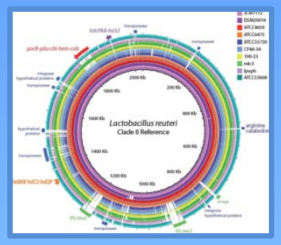


The human gut microbiota is implicated in human health and disease

Any natural food supplement that could help to maintain an healthy microbiota may be a major benefit for astronauts

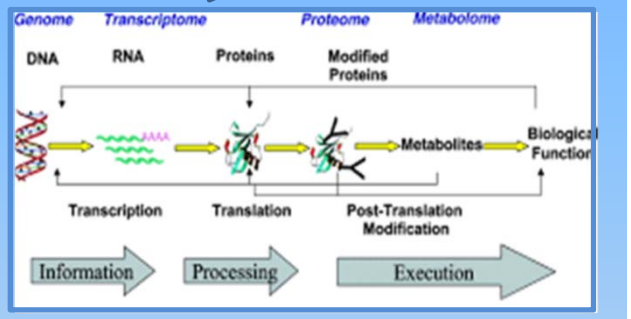
Aim of the work

Cultivation of microorganisms in spacecraft for probiotic biomass production



Lactobacillus reuteri DSM17938

Metabolic, transcriptomic and proteomic analysis

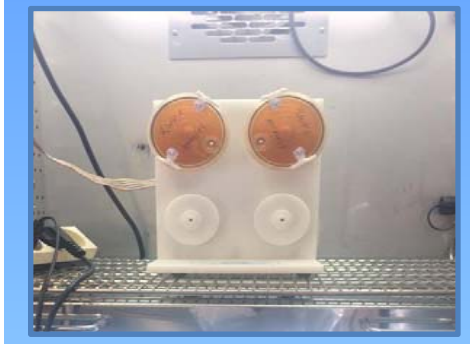


Discover the probiotic abilities, linked to the expression of some genes and proteins and study if they could be compromised in microgravity condition

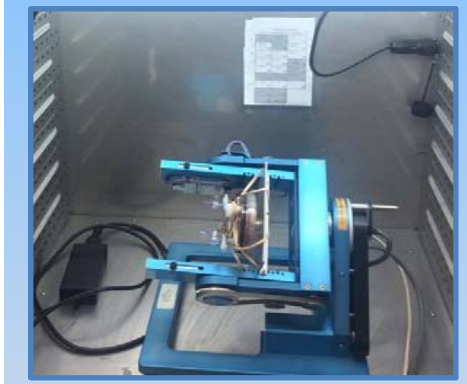
Study of adaptation mechanisms of *L. reuteri* to microgravity conditions

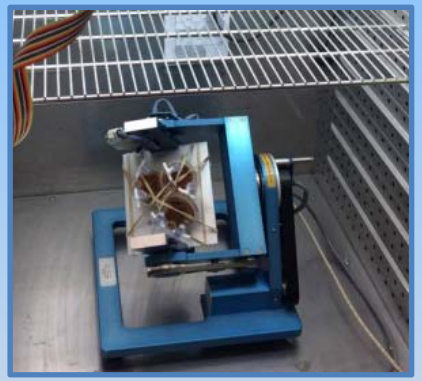
Experimental plan

Rotating Wall Vessel (RWV)



Random Positioning Machine (RPM)

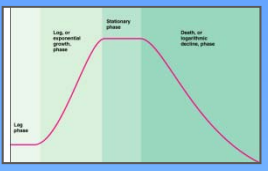




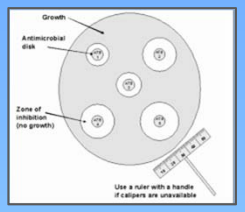
Random Positioning Machine (RPM) and Rotating Wall Vessel (RWV) provided by the SCK-CEN , Institute of Environment Health and Safety, Mol (Belgium)

Activities

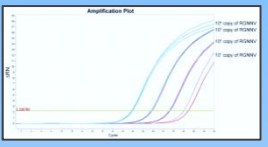
1) Growth kinetics over 72 h through measurements of absorbance at 600 nm



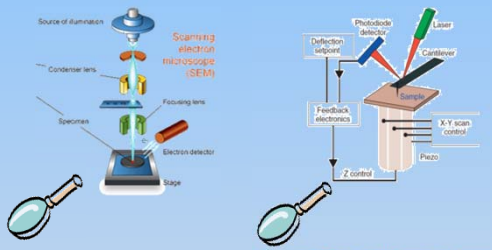
2) Reuterin production assay



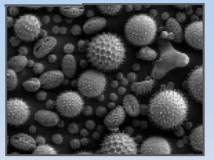
3) RNA isolation , reverse transcription and RT-qPCR

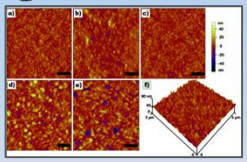


4) Simulated gastrointestinal passage assay

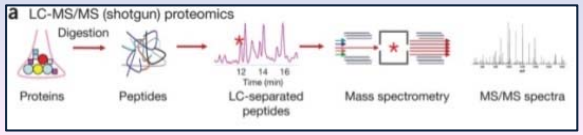


5) SEM and AFM observations



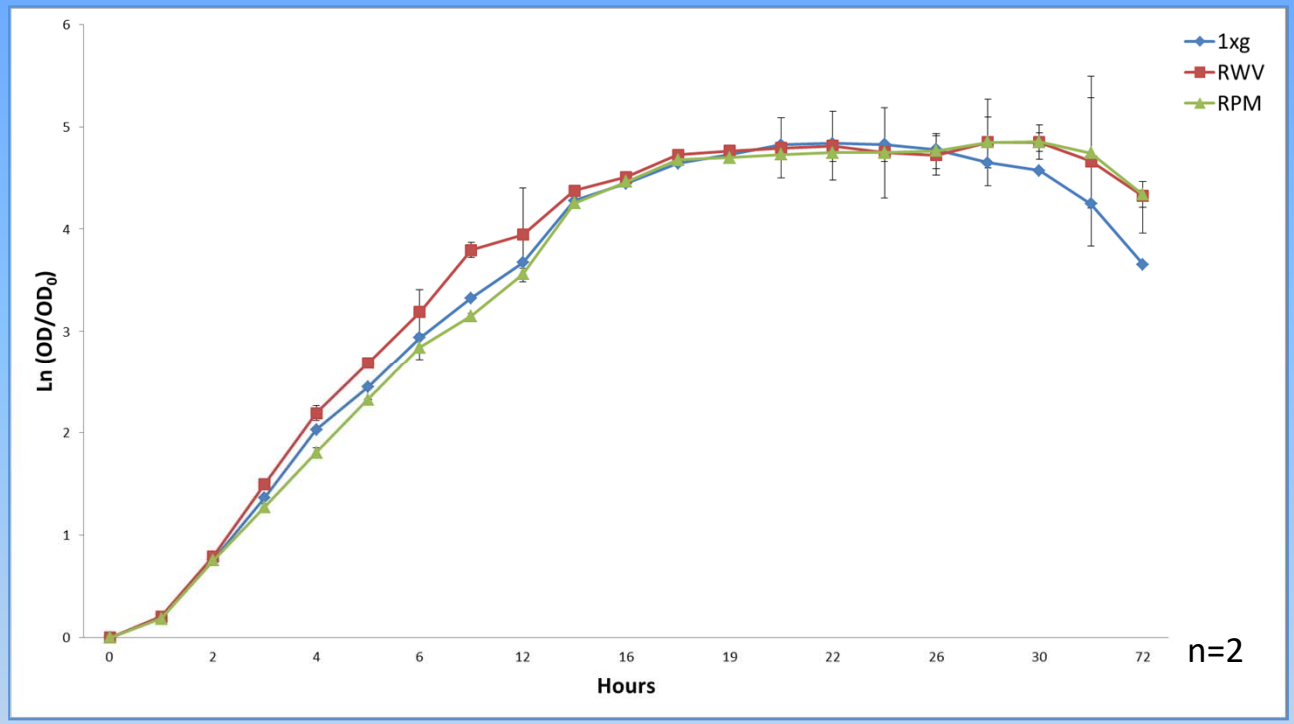


6) Protein identification by Orbitrap mass spectrometry and analysis of metabolic pathways



GROWTH KINETICS

RESULTS



Baranyi and Roberts model

Samples (n=2)	Avg ± Std Dev of maximum growth rate
1xg	0.498±0.037 h^{-1}
RWV	0.556±0.023 h^{-1}
RPM	0.472±0.016 h^{-1}

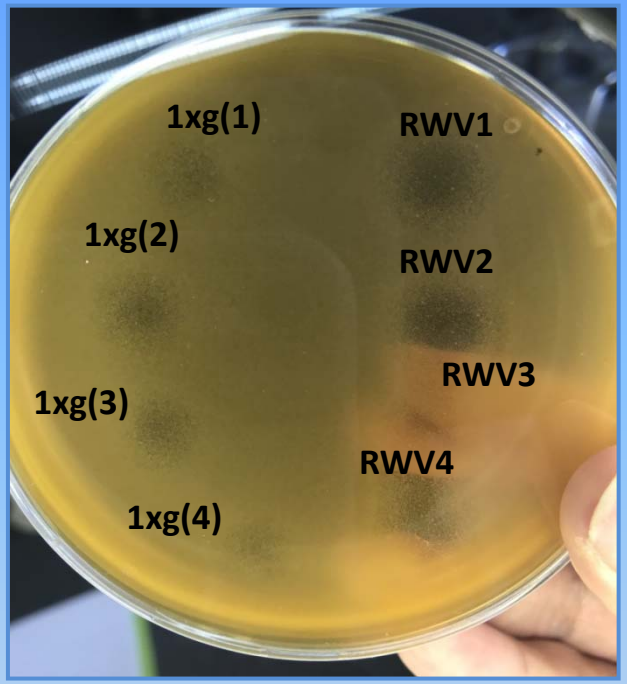
Cultures grown in RWV, RPM and 1xg did not show significantly different growth rate in all phases

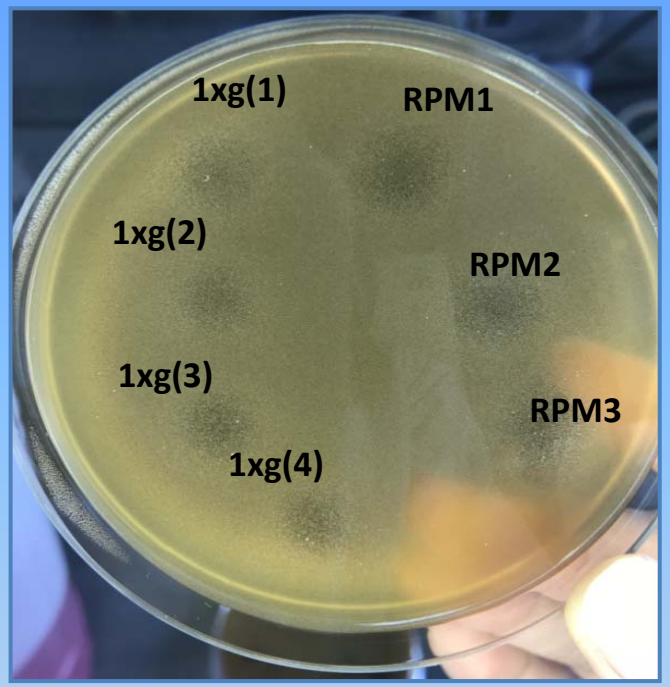
Samples collection for the subsequent tests:

7 h -> for gene expression and proteomic analysis;
15 h-> for gene expression, proteomic analysis, gastrointestinal assay and antimicrobial test;
24 h-> for gene expression

REUTERIN PRODUCTION

RESULTS





After the two treatments, the liquid phase of *L. reuteri* grown in RWV and RPM exhibited higher antibacterial activity against *S. aureus* in comparison to the control

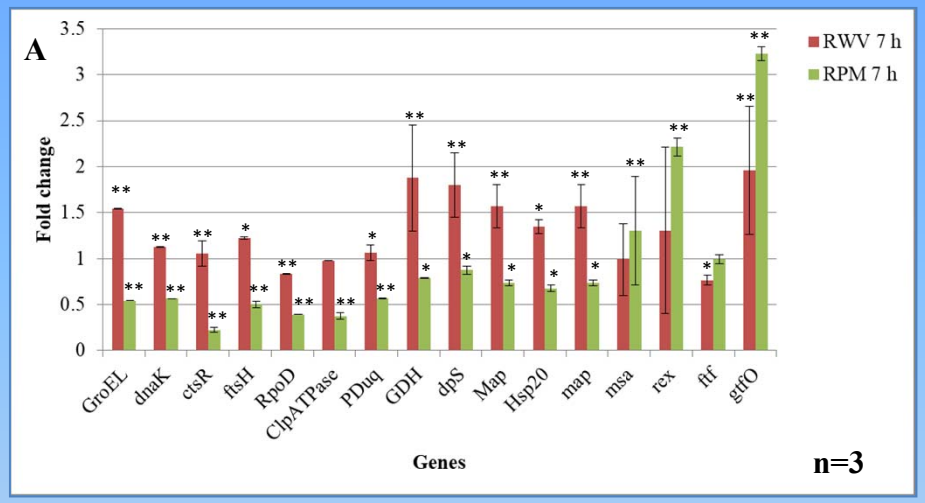
Samples (n=4)	Average ± Std Dev of Inhibiton halo
1xg	22±0.8mm
RWV	32±0.6 mm*
RPM	31±0.8mm*

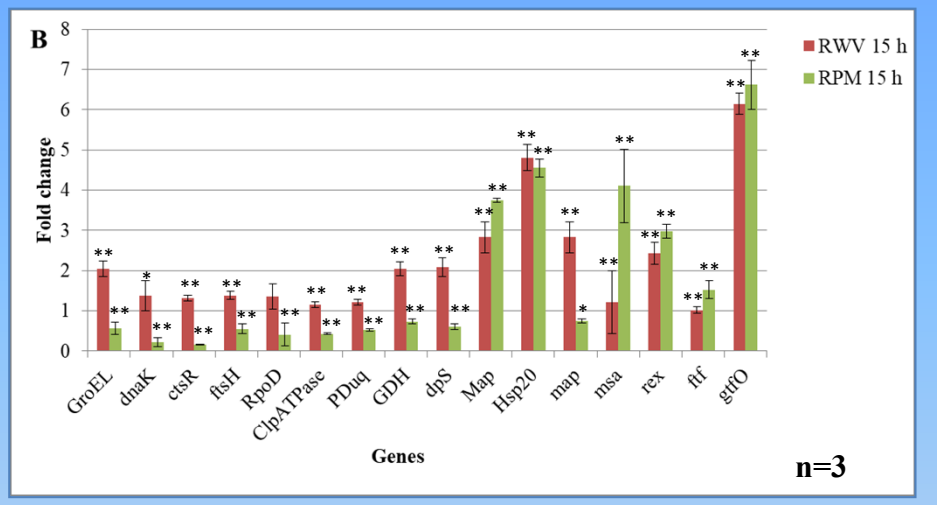
* Pvalue<0.05 using t-Test analysis

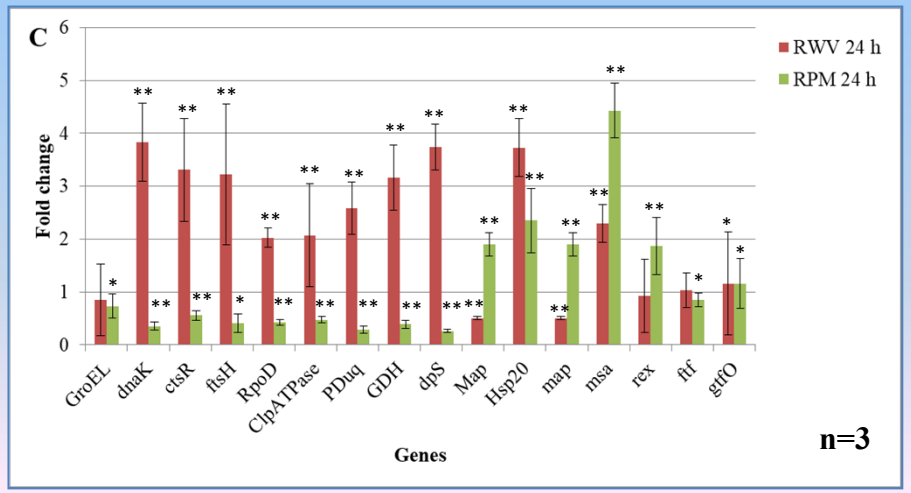
GENE EXPRESSION

RESULTS

Real time qPCR analysis after 7 h (A), 15h (B) and 24h (C) of treatment in RWV and RPM







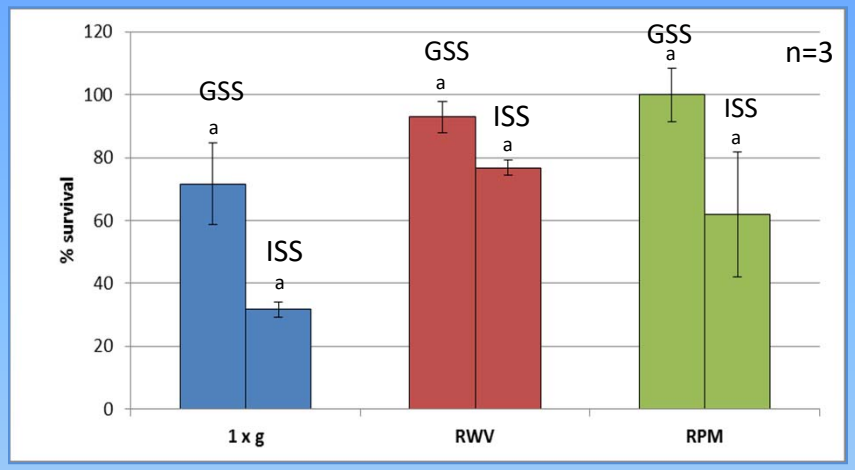
RT-qPCR results showed a significant fold change in the expression level of most of the key stress genes in comparison with the control

* p values < 0.05,
** p value < 0.01using t-Test analysis

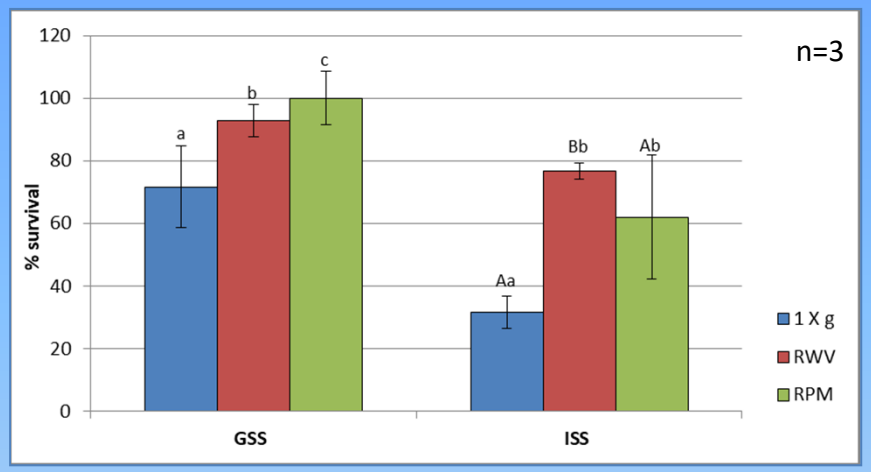
GASTROINTESTINAL PASSAGE

RESULTS

GSS= gastric simulating solution
ISS= intestinal simulating solution



Different letters indicate that mean values between GSS and ISS treatments within single group are significantly different (P < 0.05) as determined by t-test



Different letters indicate that mean values of GSS and ISS treatments between groups are significantly different (P < 0.05) as determined by t-test

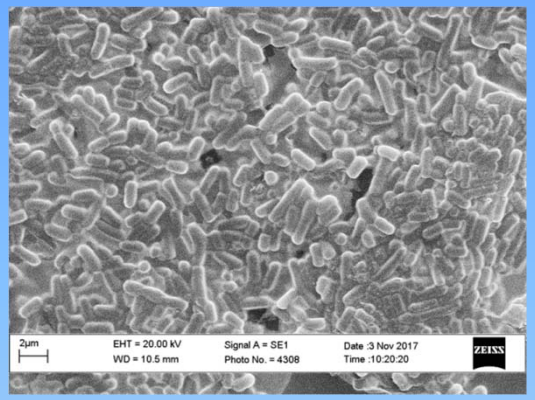
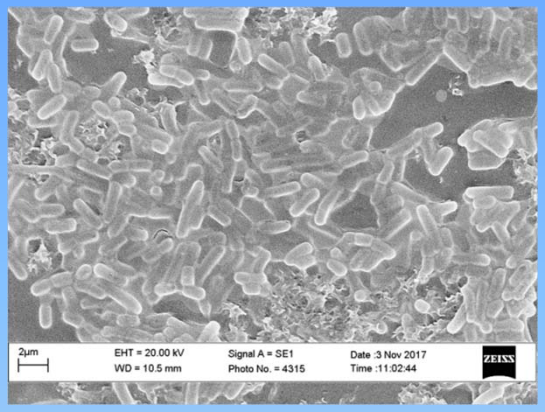
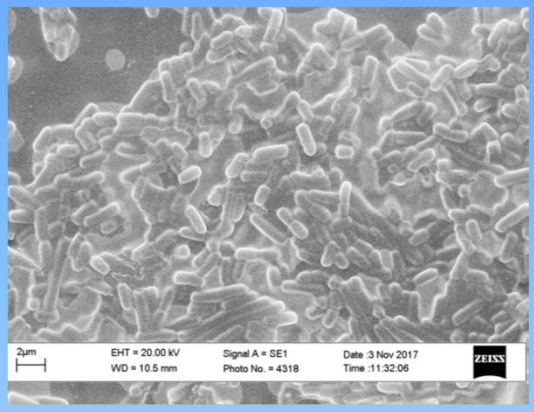
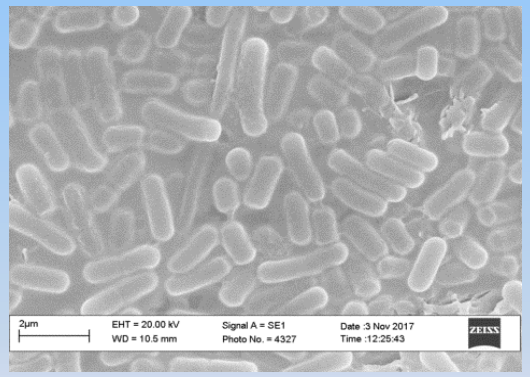
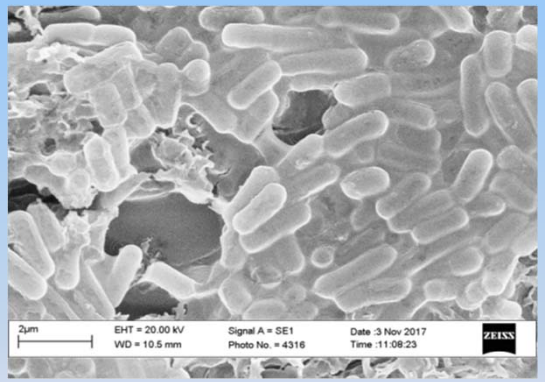
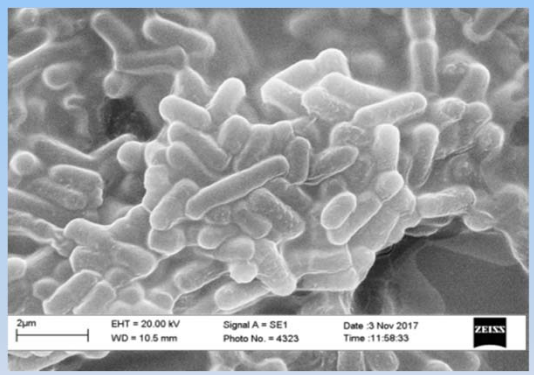
ANOVA	T_0	GSS	ISS
Between Groups	9.8522E-11**	0.000556**	0.010676*
In 1xg Group	0.0613519		
In RWV Group	0.0123332*		
In RPM Group	0.0599454		

* p values < 0.05, ** p value < 0.01 using ANOVA test

GSS treatment determines higher survival rate of cells grown under RPM;
ISS treatment determines higher survival rate of cells grown under RWV

SCANNING ELECTRON MICROSCOPE OBSERVATIONS

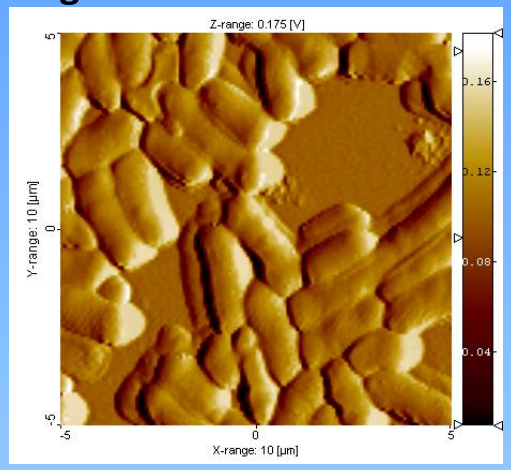
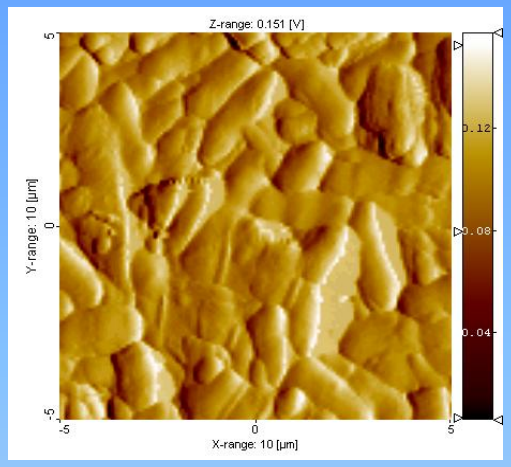
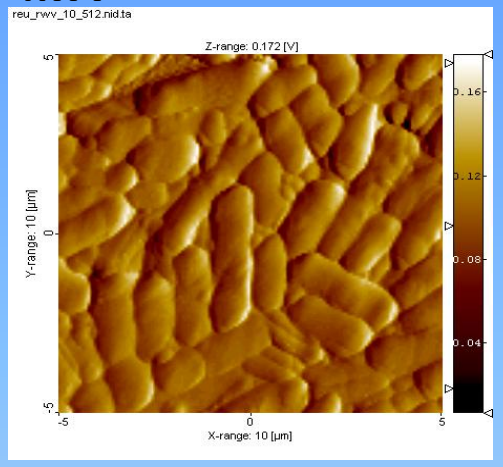
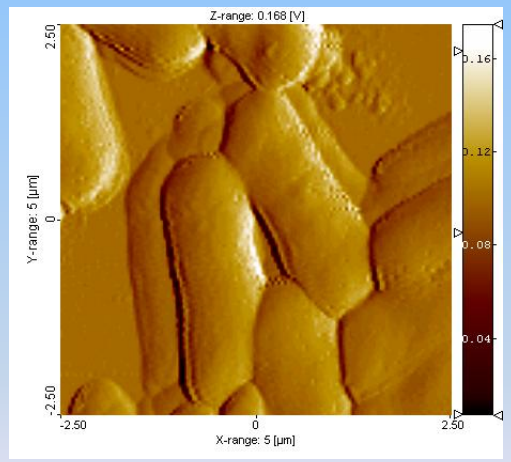
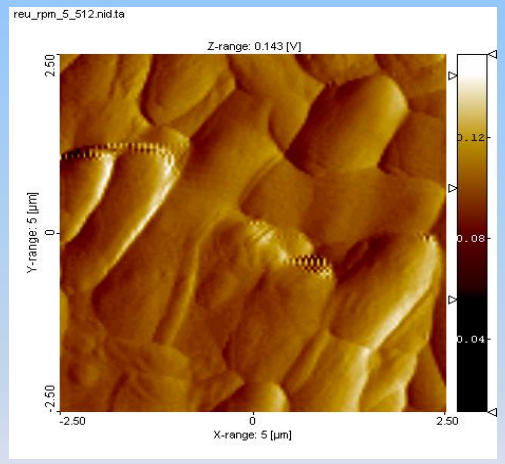
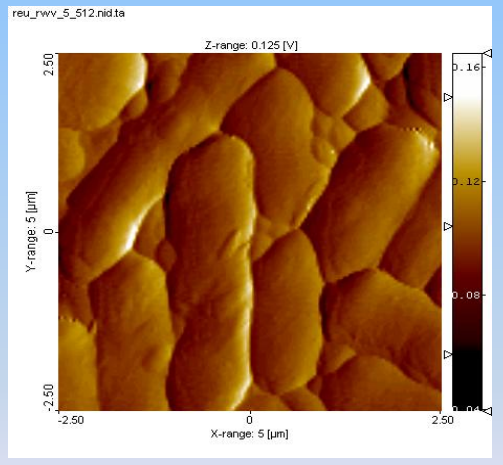
RESULTS

	1xg	RPM	RWV
10 kX			
25 kX			

Cells grown in microgravity showed no differences in shape and size but higher tendency to form aggregates

ATOMIC FORCE MICROSCOPE OBSERVATIONS

RESULTS

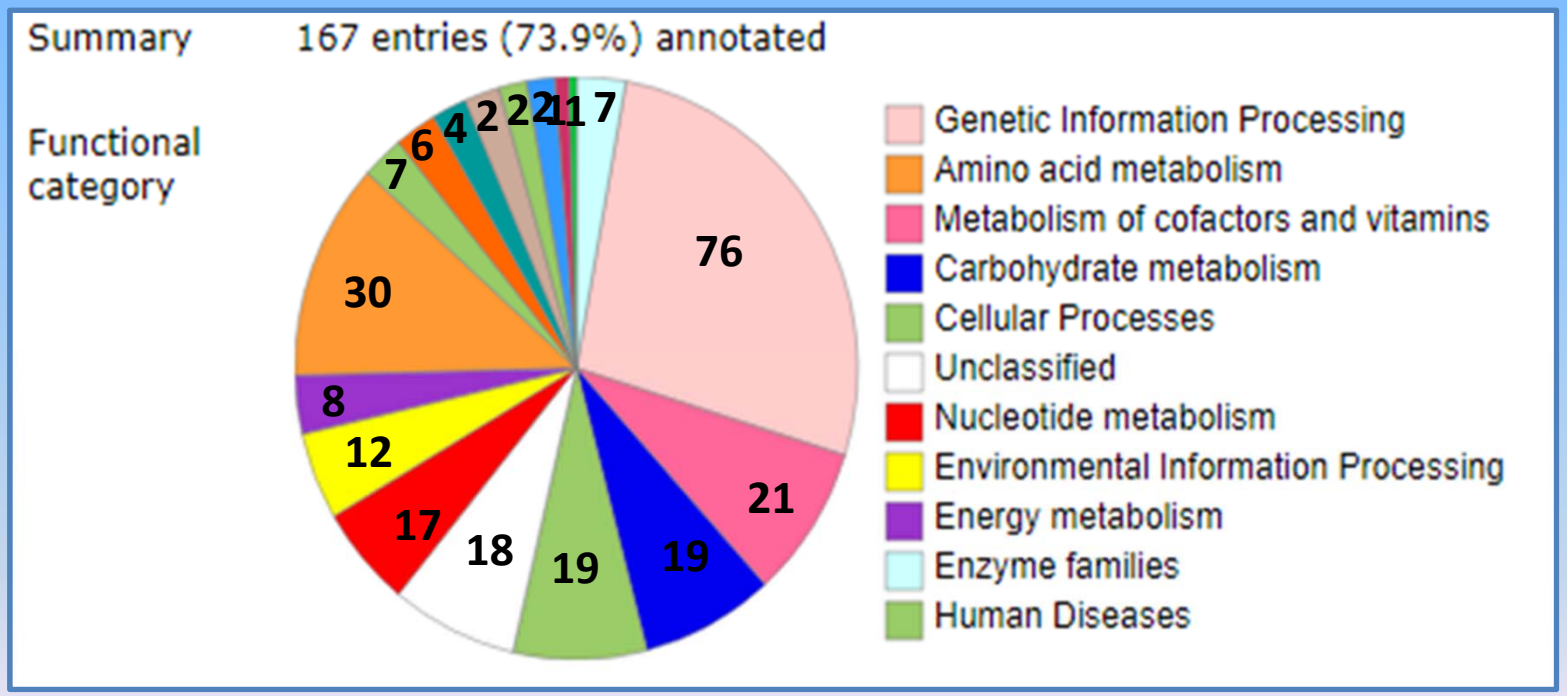
	1xg	RPM	RWV
10 µM			
5 µM			

More investigations are needed for studying roughness parameters

PROTEOMIC ANALYSIS

RESULTS

1083 identified proteins → 226 differentially expressed proteins → 167 annotated proteins

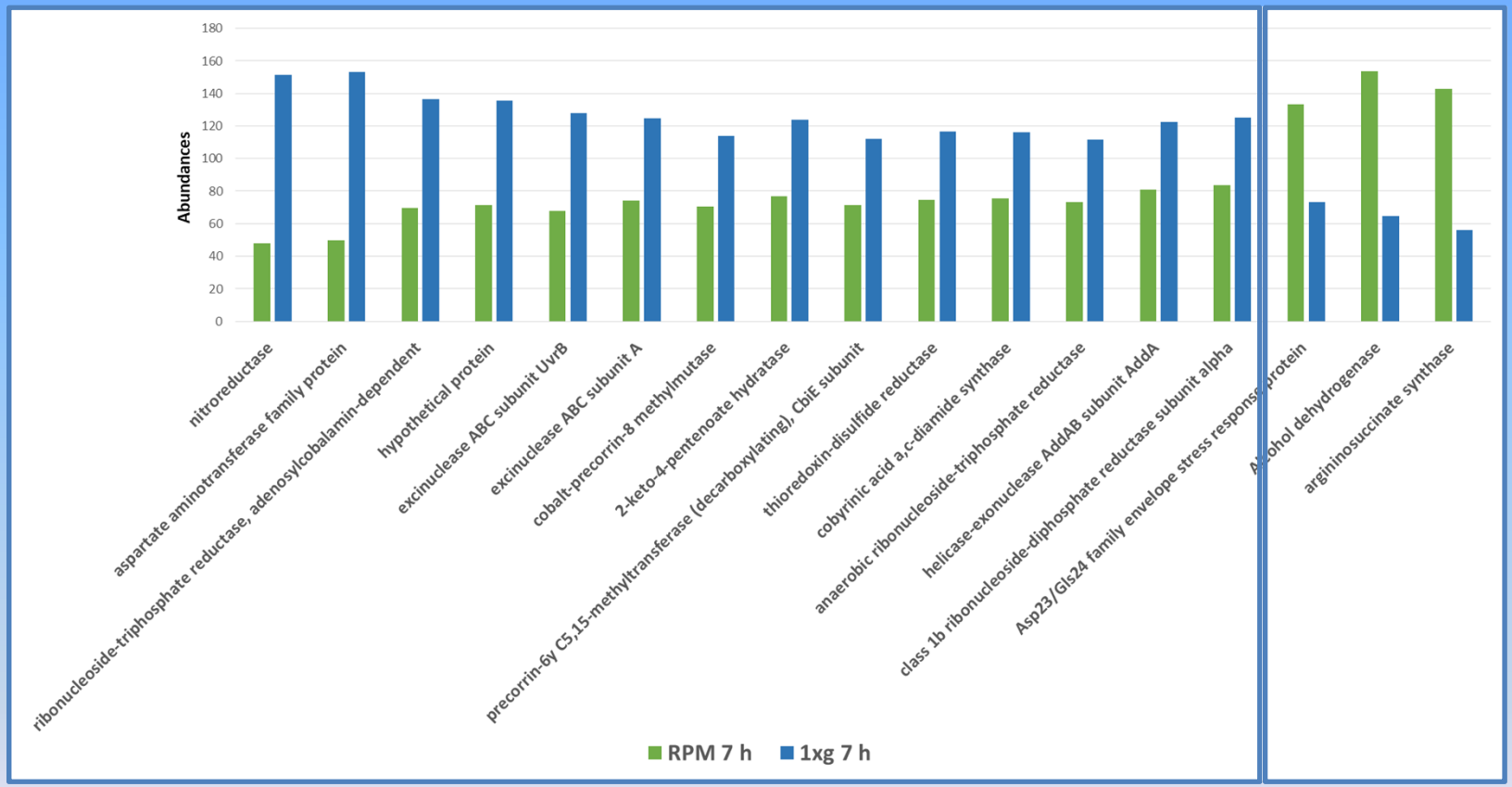


PROTEOMIC ANALYSIS

RESULTS

Differentially expressed proteins after 7 h treatment in RPM

- 15 underexpressed
- 3 overexpressed



KEGG analysis

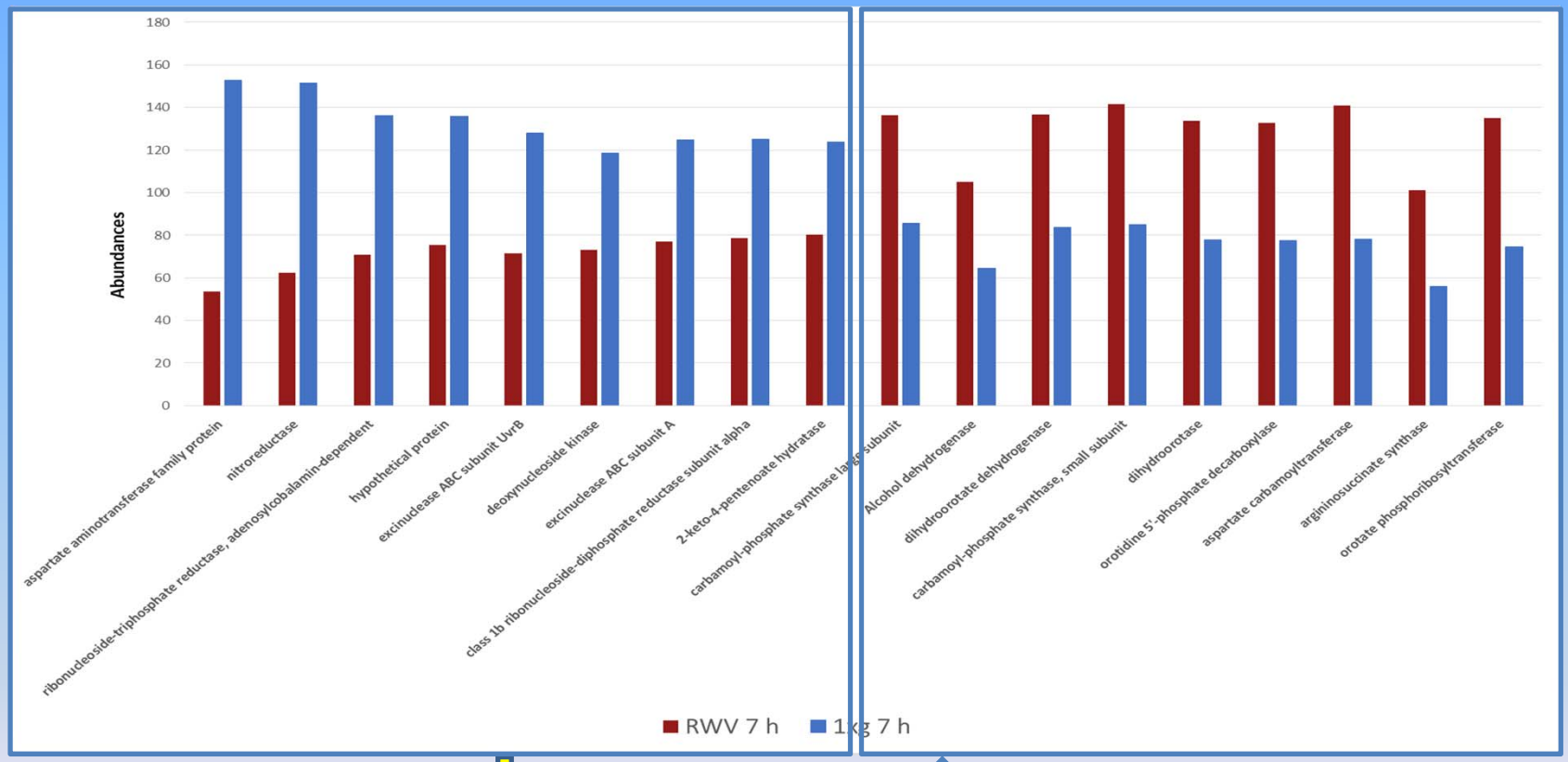
- Vitamin B12 metabolism
- DNA repair
- Arginine biosynthesis
- Oxidoreductase activity
- Alkaline shock protein

PROTEOMIC ANALYSIS

RESULTS

Differentially expressed proteins after 7 h under RWV

- 9 underexpressed
- 9 overexpressed



KEGG analysis

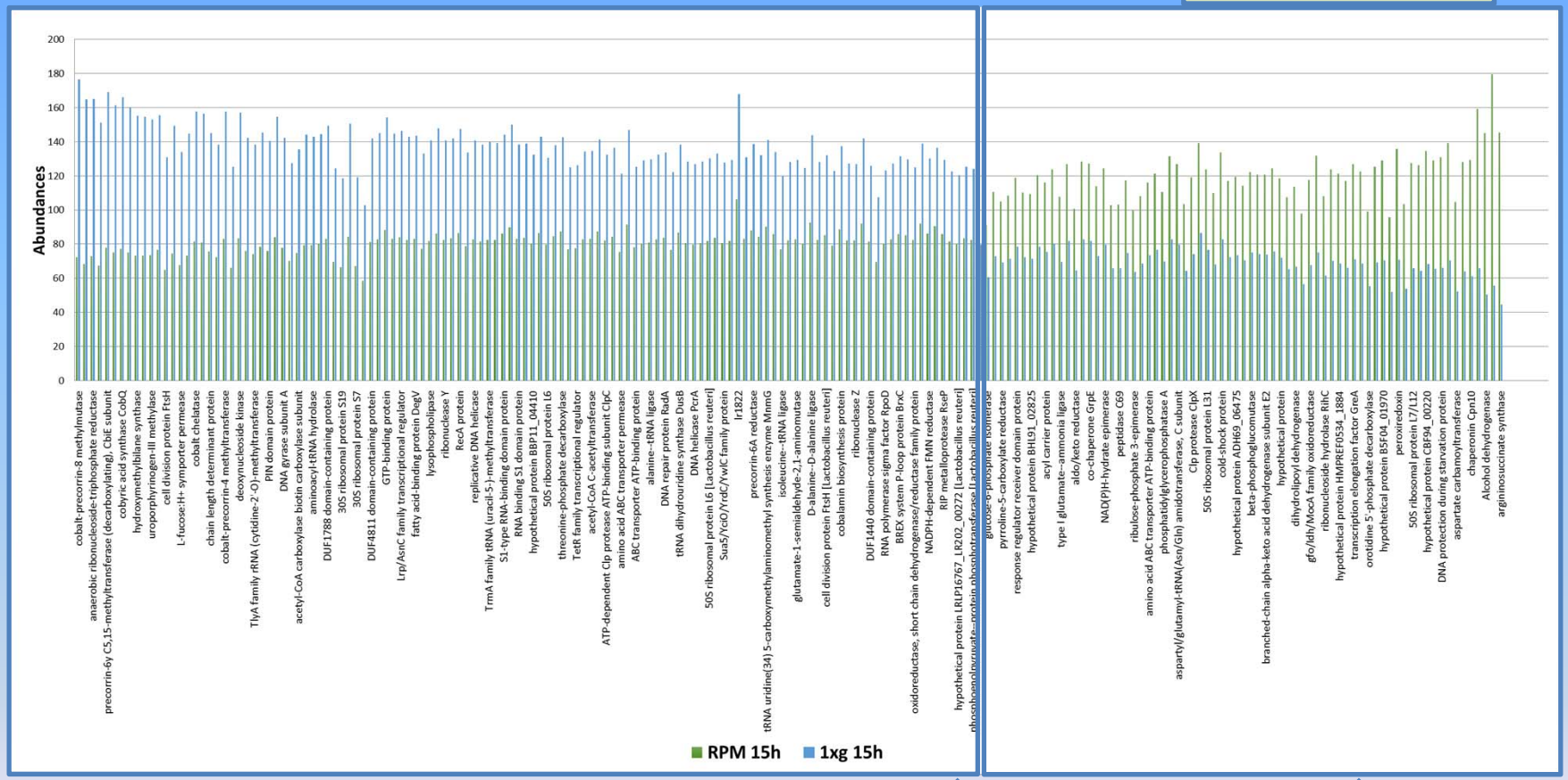
- DNA repair
- Pyrimidine metabolism
- Oxidoreductase activity
- Arginine biosynthesis

PROTEOMIC ANALYSIS

RESULTS

Differentially expressed proteins after 15 h in RPM

- 128 underexpressed
- 71 overexpressed



KEGG analysis

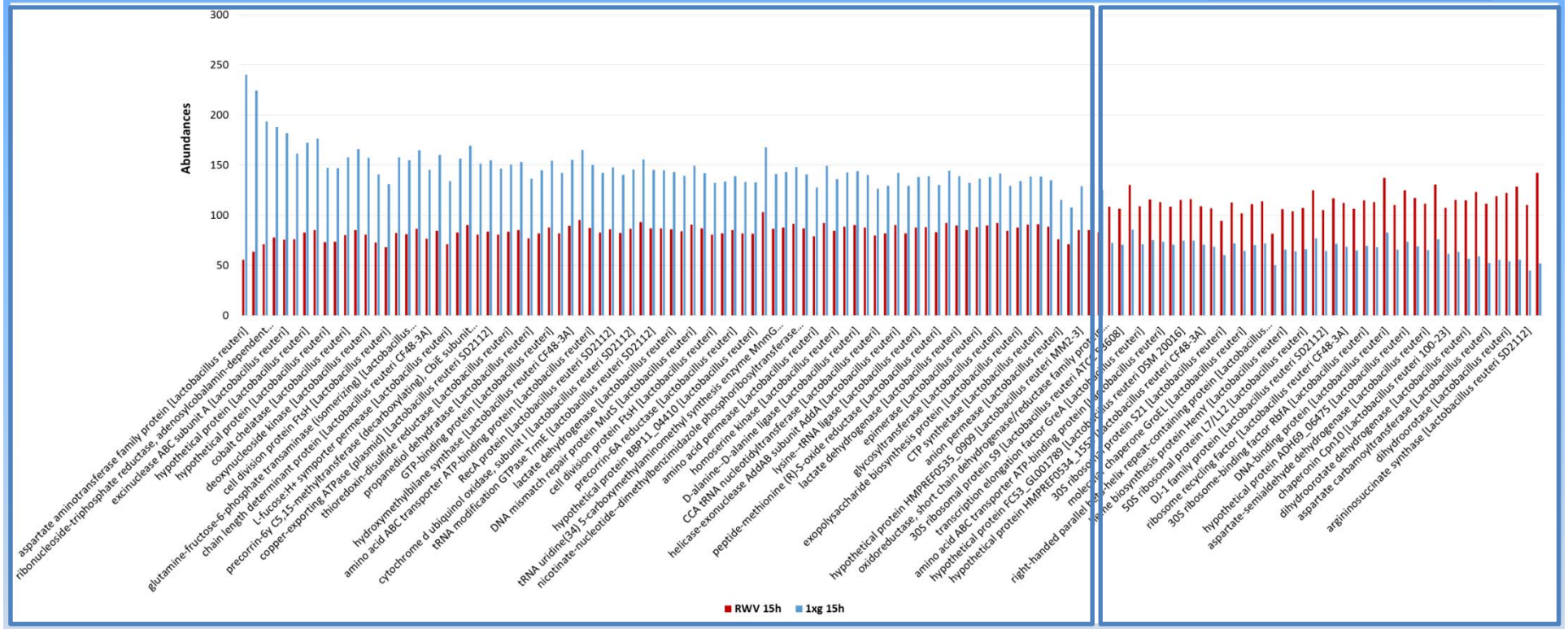
- DNA repair
- Vitamin B12 metabolism
- Stress protein
- Pyrimidine metabolism
- Arginine biosynthesis
- Oxidoreductase activity
- Alkaline shock protein

PROTEOMIC ANALYSIS

RESULTS

Differentially expressed proteins after 15 h under RWV

- 84 underexpressed
- 43 overexpressed



KEGG analysis

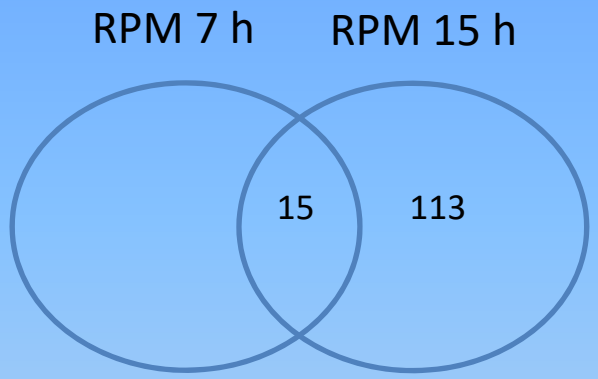
- DNA repair
- Vitamin B12 metabolism
- Stress protein
- Pyrimidine metabolism
- Arginine biosynthesis
- Oxidoreductase activity
- Alkaline shock protein

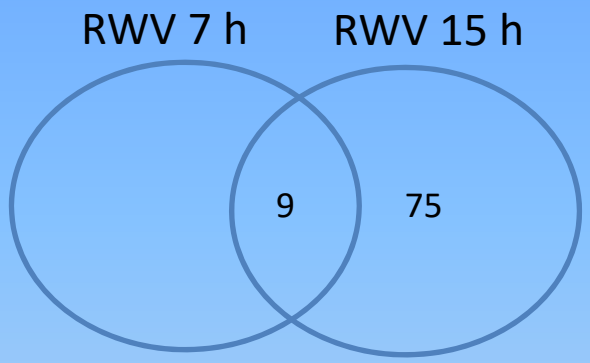
PROTEOMIC ANALYSIS

RESULTS

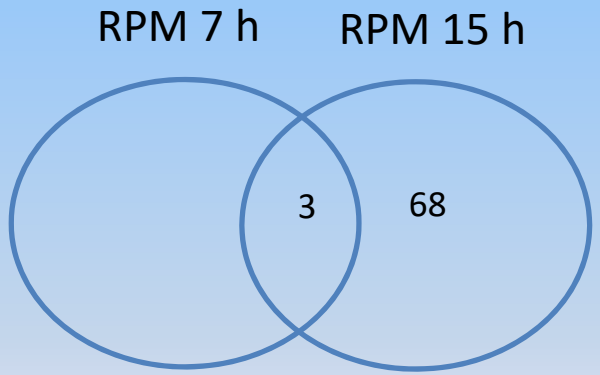
Venn Diagrams between differentially expressed proteins at 7 h and 15 h in RPM and RWV systems

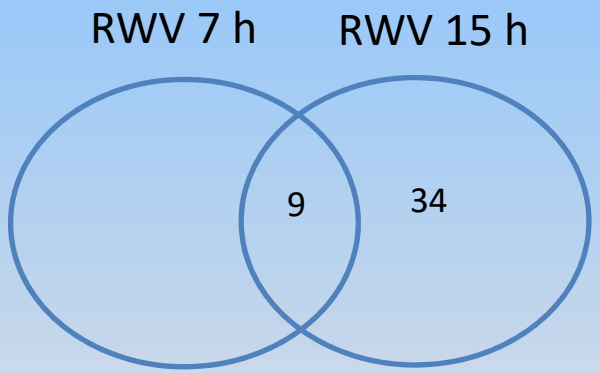
Underexpressed proteins





Overexpressed proteins





There is an overlap of identified proteins

Samples treated for 15 h to microgravity seem more interesting then 7 h samples

PROTEOMIC ANALYSIS

RESULTS

Underexpressed proteins

Protein	Accession number	Unique peptides	RPM 7 h/1xg 7 h abundance	RPM 15 h/1xg 15 h abundance	RWV 7 h/1xg 7 h abundance	RWV 15 h/1xg 15 h abundance
nitroreductase	1150049146	5	0.31	0.23	0.41	0.28
aspartate aminotransferase family protein	1198708410	9	0.32	0.203	0.35	0.23
ribonucleoside-triphosphate reductase, adenosylcobalamin-dependent	1189224164	18	0.51	0.30	0.52	0.36
excinuclease ABC subunit UvrB	1172352046	11	0.52	0.36	0.56	0.41
excinuclease ABC subunit A	1150049070	15	0.59	0.37	0.62	0.41
cobalt-precorrin-8 methylmutase	489765998	6	0.61	0.41	//	0.48
2-keto-4-pentenoate hydratase	1198461062	6	0.62	0.41	0.65	0.52
class 1b ribonucleoside-diphosphate reductase subunit alpha	1150816566	2	0.66	0.46	0.62	0.47
precorrin-6y C5,15-methyltransferase	227184664	7	0.64	0.46	//	0.53

Oxidative stress

Nucleotide-excision repair

Vitamin B12 metabolism

DNA de novo synthesis

PROTEOMIC ANALYSIS

RESULTS

Overexpressed proteins

Protein	Accession number	Unique peptides	RPM 7 h/1xg 7 h abundance	RPM 15 h/1xg 15 h abundance	RWV 7 h/1xg 7 h abundance	RWV 15 h/1xg 15 h abundance
Argininosuccinate synthase	336449577	16	2.55	3.27	1.80	2.47
Orotate phosphoribosyltransferase	1198462252	9	//	1.85	1.81	2.75
Asp23/Gls24 family envelope stress response protein	489766905	11	1.82	2.42	//	//
Alcohol dehydrogenase	970365880	2	2.38	2.88	1.62	1.62
Aspartate carbamoyltransferase	1231603022	13	//	2.00	1.80	2.13
Orotidine 5'-phosphate decarboxylase	1190868479	6	//	1.79	1.71	2.32
Dihydroorotase	1150052847	12	//	1.92	1.71	2.27
Carbamoyl-phosphate synthase, small subunit	324977945	3	//	//	1.667	2.096
Dihydroorotate dehydrogenase	112943111	8	//	1.732	1.634	2.031

Arginine biosynthesis

Alkaline shock protein

Oxidative stress

Pyrimidine biosynthesis

CONCLUSIONS

- The analysis of *L. reuteri* growth under simulated microgravity revealed the same growth rate compared to terrestrial gravity condition;
- The treated samples to simulated microgravity showed a more marked antimicrobial activity than the control one;
- The expression of some stress genes was significantly different under simulated microgravity and it suggests that other pathways may be compromised;
- Higher survival was shown after GI passage by cells grown under simulated microgravity;
- No differences were shown at morphological level;
- The proteomic analysis revealed patterns of decreased Vitamin B12 metabolism and decreased DNA biosynthesis and patterns of increased pyrimidine and arginine biosynthesis and increased some oxidative stress-related proteins.

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