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THE EFFECTS OF ISS-LIKE IONIZING RADIATION ON THE PROTEOME AND METABOLOME OF UREOLYTIC AND NITRIFYING BACTERIA

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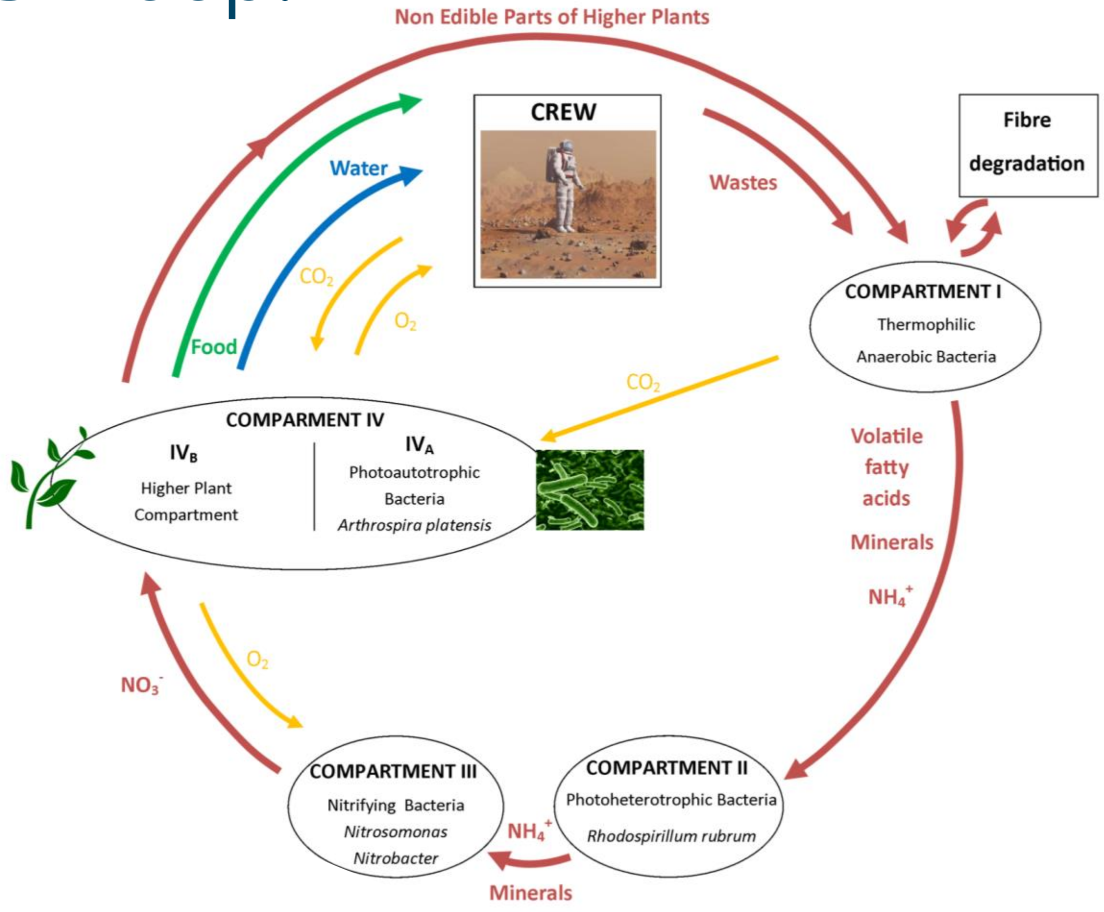
Where are we in MELiSSA loop?

- Urine nitrification by
    - (i) urease positive heterotrophs →hydrolysis of urea to ammonia and for aerobic oxidation of organic compounds in urine,
    - (ii) ammonia oxidizing bacteria →aerobic oxidation of ammonia to nitrite (nitritation),
    - (iii) nitrite oxidizing bacteria →aerobic oxidation of nitrite to nitrate (nitratation).
- URINIS project

→ providing a proof of principle that urine nitrification is possible under Space conditions in the ISS



$CO(NH_2)_2$ UREOLYSIS → NH_4^+ NITRITATION → NO_2^- NITRATATION → NO_3^-

Comamonas testosteroni I2

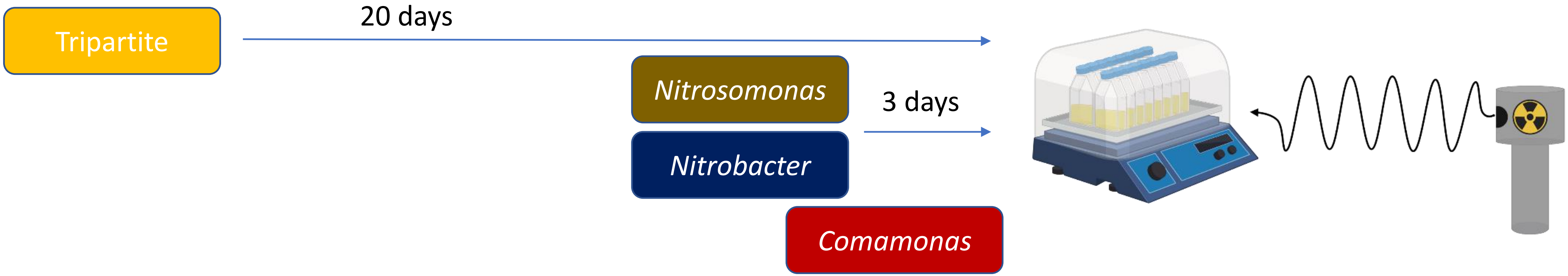
Nitrosomonas europaea ATCC 19178

Nitrobacter winogradskyi ATCC 25391

Why doing radiation and microgravity experiment?

- Ionizing radiation (IR) → damage to DNA, lipids, proteins, and many metabolites (Reisz et al., 2013)
- Microgravity → limited transportation of nutrients and removing waste products, diffusion-only transport, difficult gas-liquid exchanges
- In combination, microgravity may interfere with the operation of cellular repair processes of DNA damaged by radiation → stronger impact (Horneck et al., 2010)

Radiation experiment



Tripartite

20 days

Nitrosomonas

Nitrobacter

Comamonas

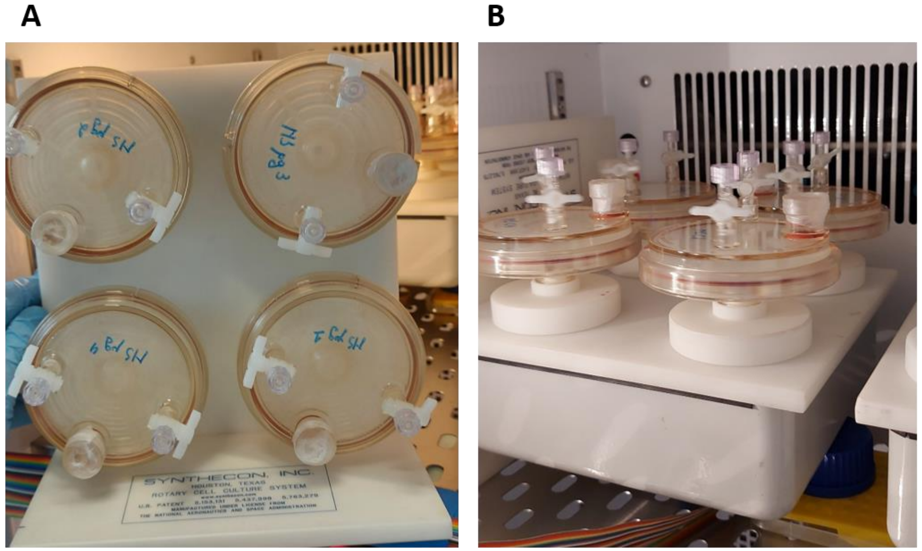
3 days

- Exposing to a ^{252}Cf neutron irradiation source in the Lab of Nuclear Calibrations (LNK) at SCK-CEN (Mol, Belgium) for 72 hours (4 replicates).
- Average dose rate of 5.09×10^{-1} mGy*h^{-1}

Overview of total absorbed dose and the corresponding days in space to be exposed to the total absorbed dose rate for every culture.

Strain	Total absorbed dose (mGy)	Equivalent days of space irradiation (d)
C. testosteroni	35.18	126
N. Europaea	38.17	136
N. winogradskyi	38.17	136
Tripartite culture	35.18	126

Microgravity experiment

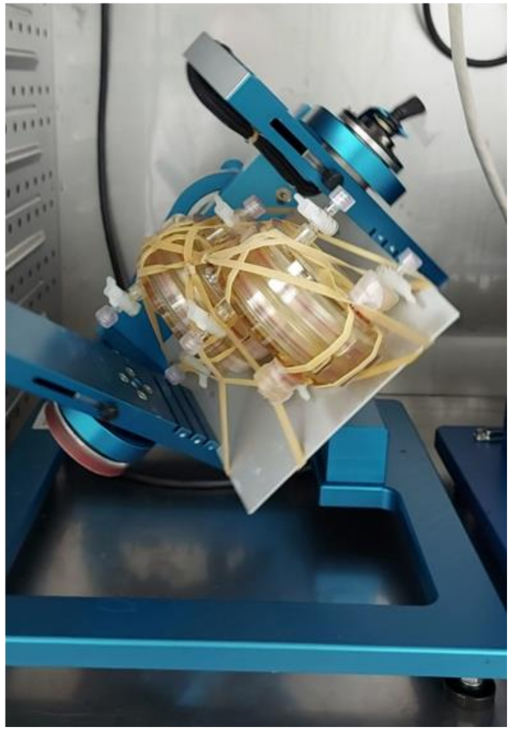


Cultures growing in (A) RWV and (B) normal gravity control conditions.

Simulated microgravity in a Rotating Wall Vessel (RWV)

- Inoculating *N. europaea* and *N. winogradskyi* cultures and injecting into Rotating Wall Vessels (RWV) (Synthecon)
- Removing air bubbles
- Growing in the dark at 30°C for 5 days.
- Rotating around the horizontal axis at 25 rpm

Microgravity experiment



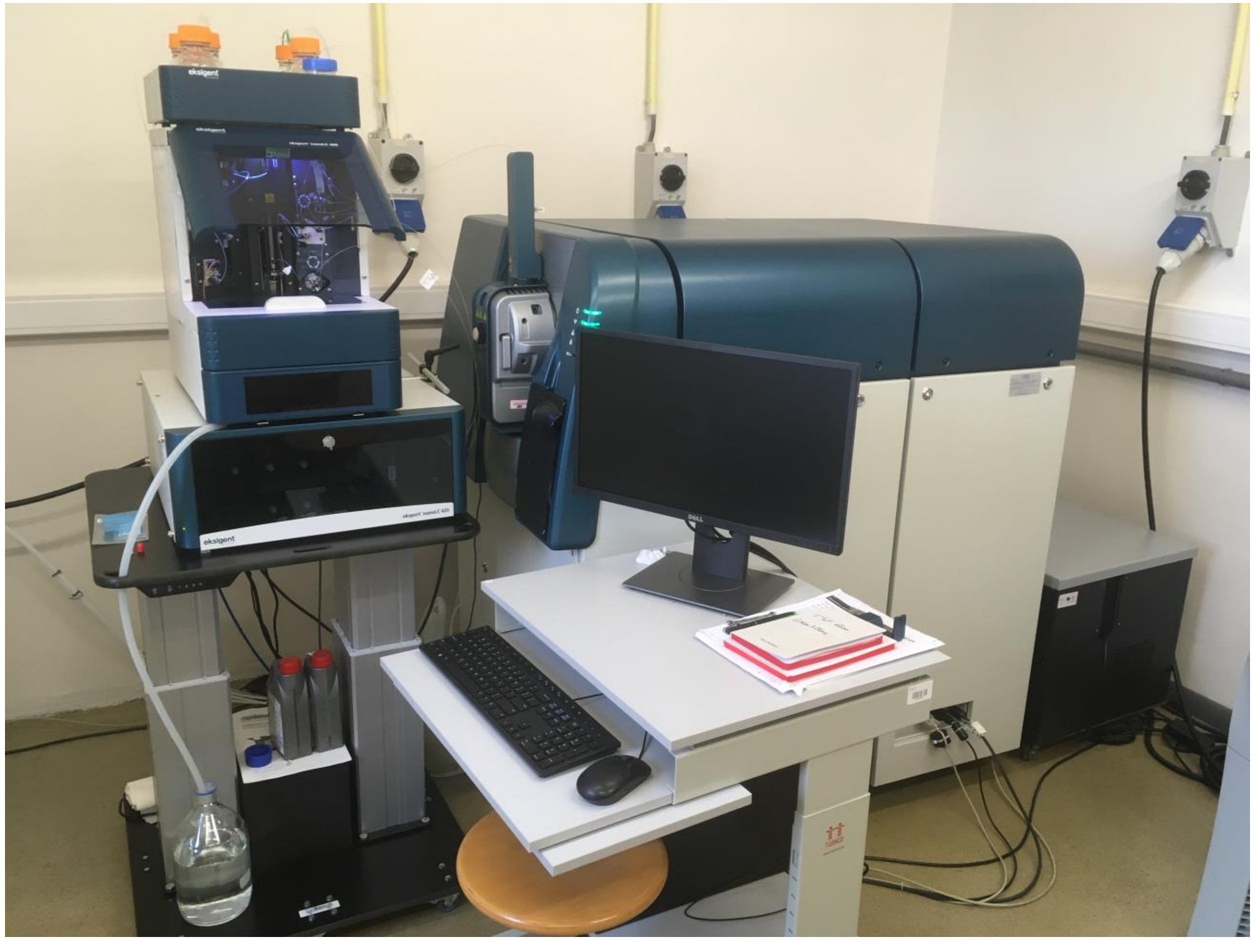
Simulated microgravity conditions on an RPM.

Simulated microgravity in a Random Positioning Machine (RPM)

- Inoculating N. europaea and N. winogradskyi cultures and injecting into RWVs.
- Mounting onto a Random Positioning Machine (RPM) (Airbus Defense and Space Netherlands)
- Growing in the dark at 30°C for 5 days
- Operated in real random mode with random direction and speed, angular velocity of 60 deg s-1.

Proteomic analysis

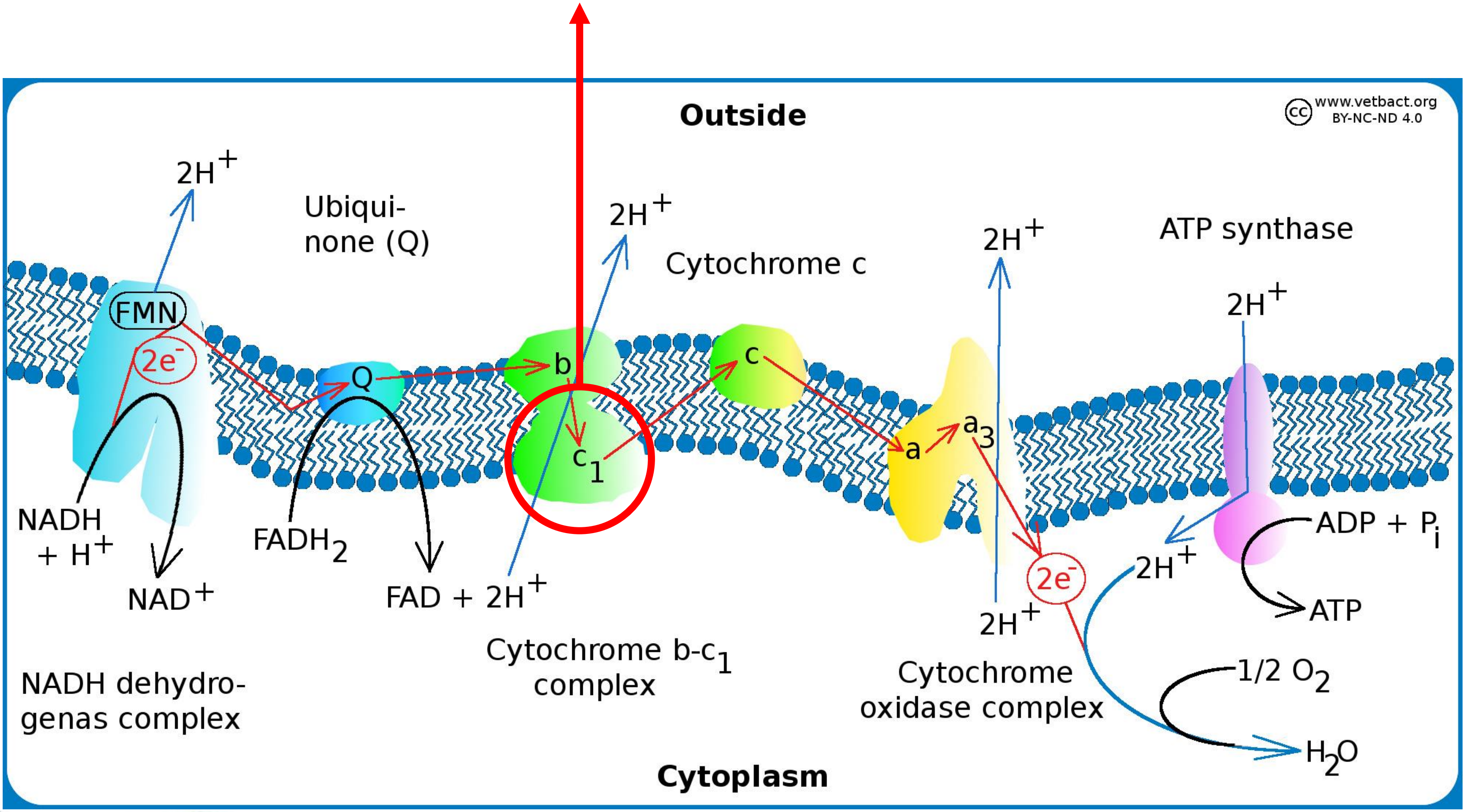
- AB SCIEX TripleTOF^{TM} 6600
- SWATH-based quantification
- Data processing by ProteinPilot (version 4.5, AB Sciex, USA) and Skyline software.
- Protein identification by at least 2 peptides
- *P* value <0.05 and fold change <0.66 or >1.5



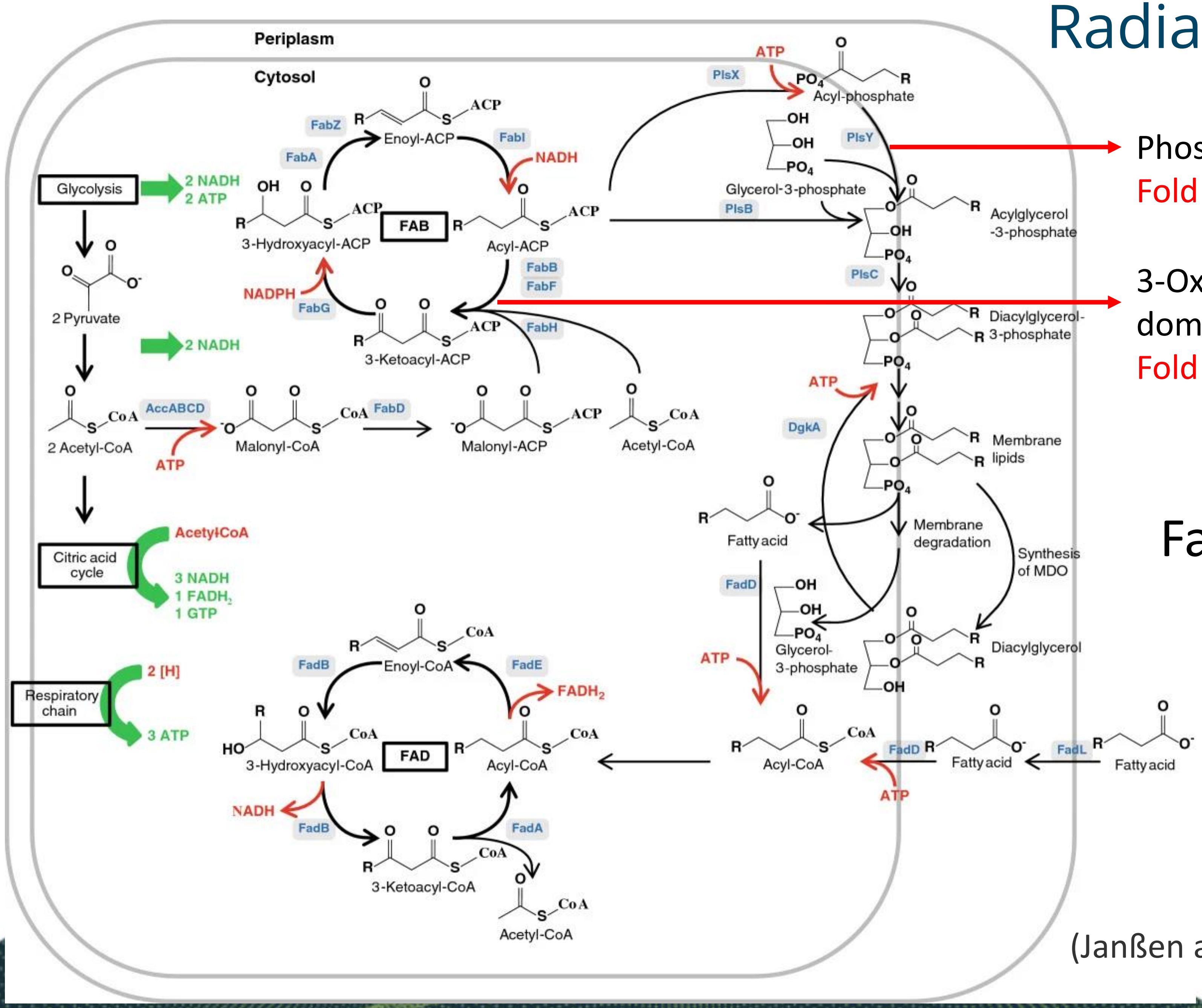
Radiation – *Comamonas testosteroni*

Down regulation of Cytochrome c1 (B7WUZ9) Fold change: 0.4862

Total protein identifications: 1809



Radiation – *C. testosteroni*



Phospholipid/glycerol acyltransferase (B7WSF1)
Fold change: 0.29

3-Oxoacyl-(Acyl-carrier-protein (ACP)) synthase III domain protein (B7WVC8)
Fold change: 0.5778

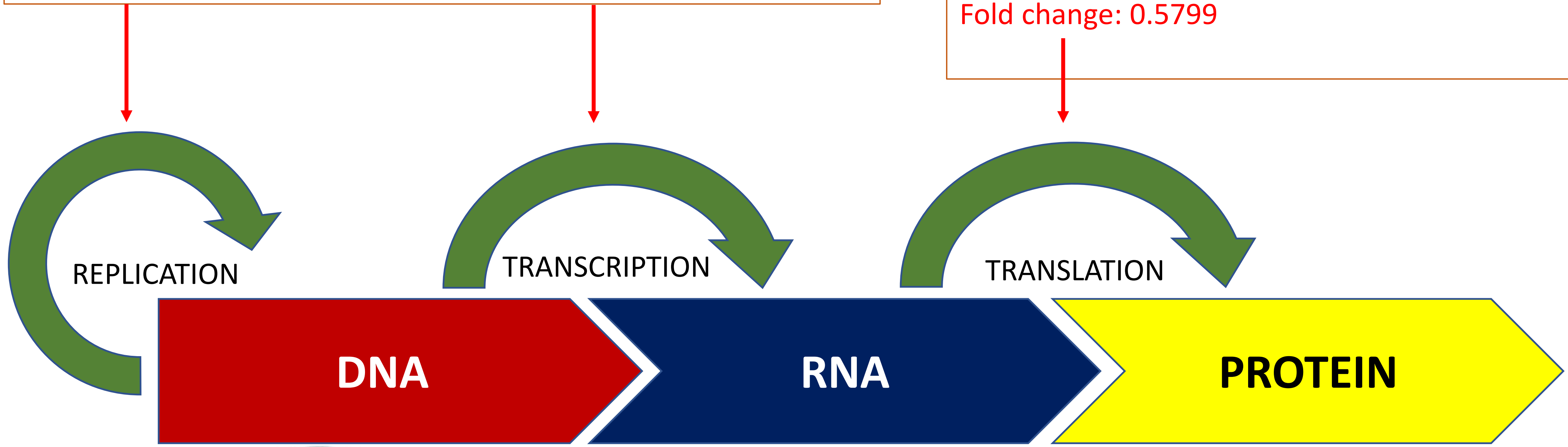
Fatty acid synthesis

(Janßen and Steinbüchel, 2014)

Radiation – *Comamonas testosteroni*

Ribonucleoside-diphosphate reductase subunit beta (B7WU75)
Fold change: 0.5932
Thymidylate synthase (B7X1J1) Fold change: 0.5979
Adenylosuccinate lyase (B7X279) Fold change: 0.6245

tRNA N6-adenosine threonylcarbamoyltransferase (B7WZK0)
Fold change: 0.5231
Isoleucine--tRNA ligase (B7WT40)
Fold change: 0.5799



Radiation – *Comamonas testosteroni*

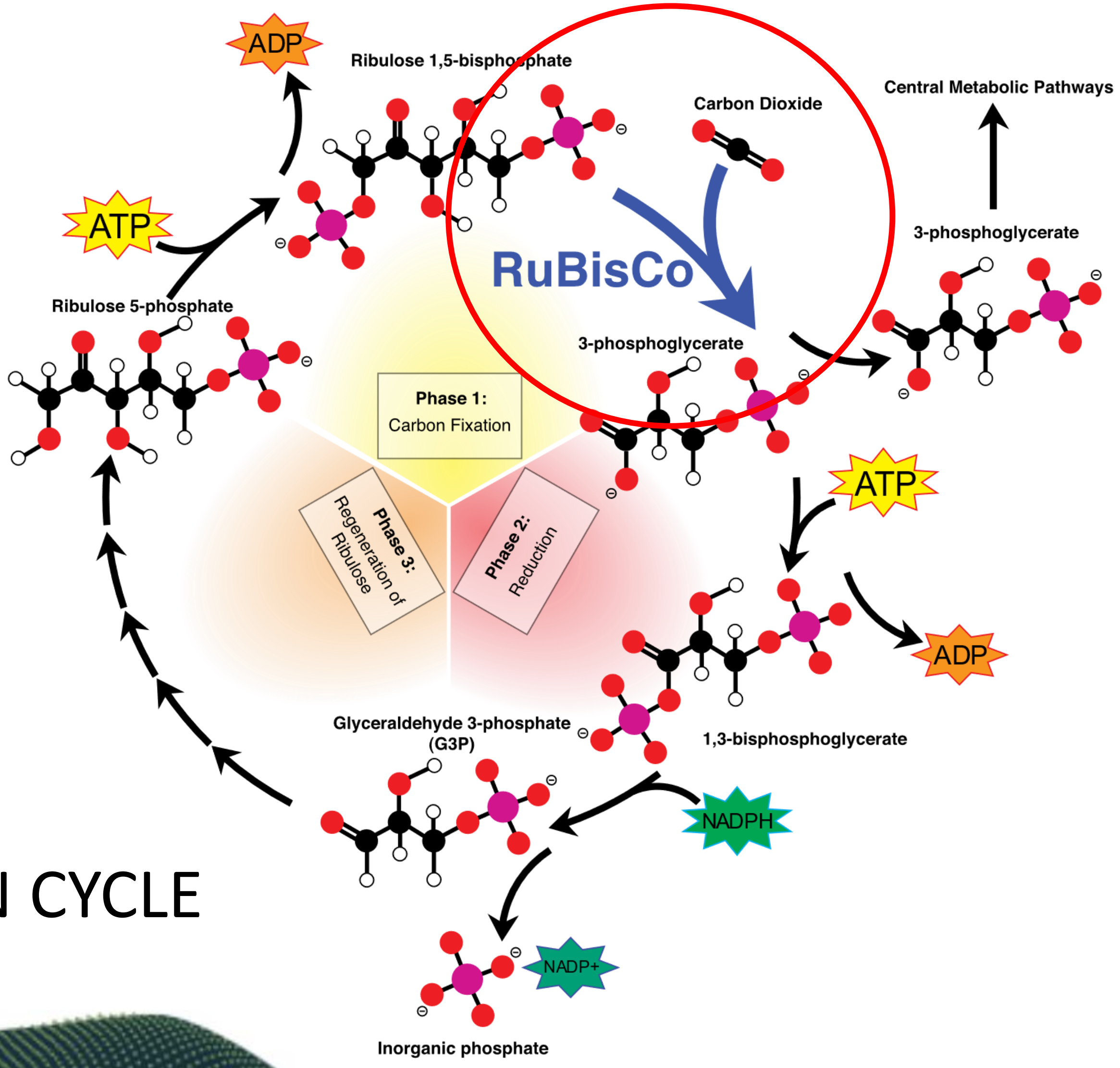
Protein Accession	Protein Description	Fold Change	Protein function
B7X3L5	Peptide-methionine (R)-S-oxide reductase	1.4854	Protein repair, response to oxidative stress
B7X5R4	Peptidyl-prolyl cis-trans isomerase	1.5541	Folding of newly synthesised proteins
B7WUW6	Sel1 domain protein repeat-containing protein	3.0583	Assembly of macromolecular complexes, activated under cellular stress, interactions between bacterial and eukaryotic host cells

Up regulation of proteins in response to stress or involved in protein folding and reparation

Radiation – *Nitrosomonas europaea*

Total protein identifications: 1216

Protein Accession	Protein Description	Fold Change
A0A837X6A4	Ribulose bisphosphate carboxylase large chain	1.379
Q93A92	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit CbbL (Fragment)	1.4681



CALVIN CYCLE

Radiation – *Nitrobacter winogradskyi*

Total protein identifications: 773

Protein Accession	Protein Description	Fold Change	Protein function
Q3SV94	Methyl-accepting chemotaxis sensory transducer with Pas/Pac sensor	1.2476	Chemotaxis signal transduction cascade, process environmental and intracellular sensory signals and alter the activity of the CheA histidine kinase.
Q3SS36	Lytic murein transglycosylase	1.2689	Non-hydrolytic cleavage of the peptidoglycan structures
Q3SNB6	Ferrous iron transport protein B	1.2594	Transfer of ferrous iron (Fe(II)) ions across the membrane

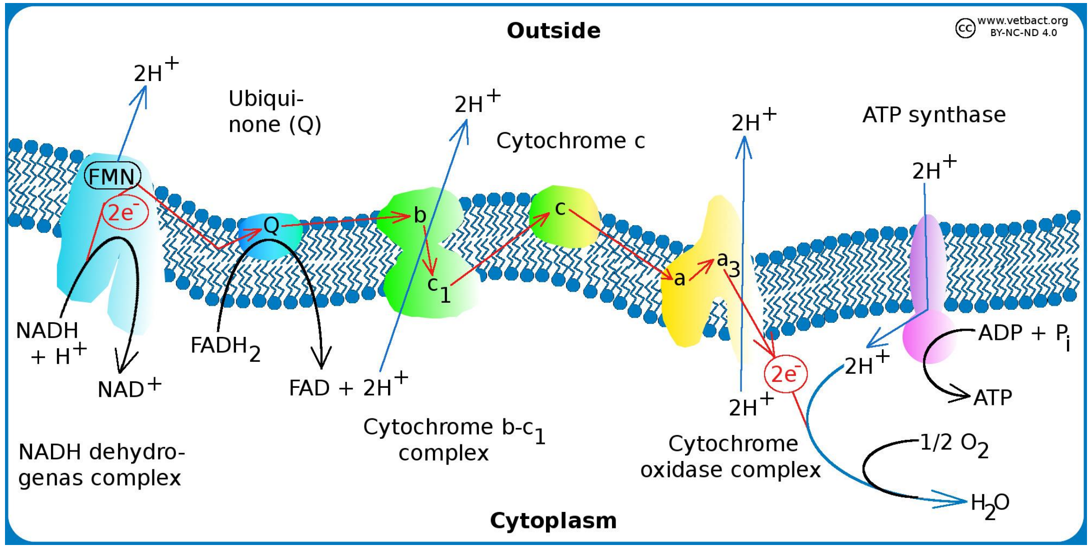
Very low fold changes

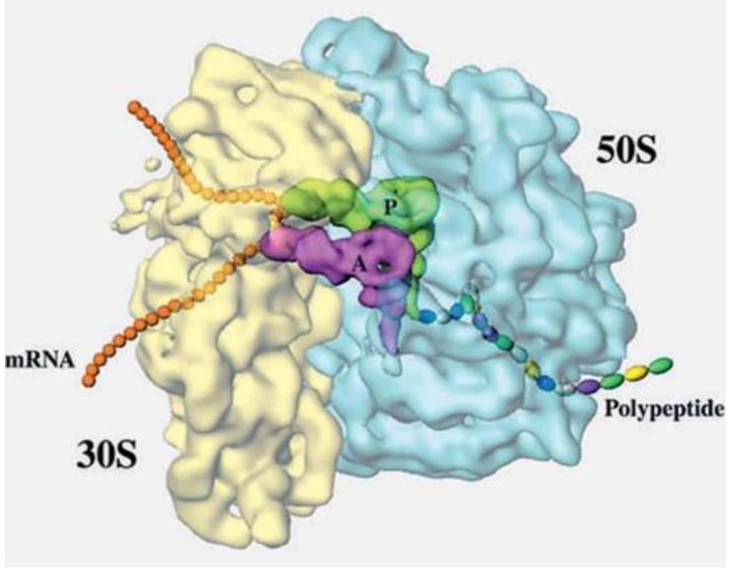
→Very limited effect detected

Radiation – *Tripartite* – *C. testosteroni*

Total protein identifications: 2553

Protein Accession	Protein Description	Fold Change	Protein function
B7WVX9	Cytochrome c class I	0.6066	Electron transport chain
B7X5W7	Deoxyuridine 5'-triphosphate nucleotidohydrolase	0.6121	Formation of dUMP, DNA synthesis
B7X381	Ribosomal subunit interface protein, putative	0.6098	Association of ribosomal subunits
B7WR64	50S ribosomal protein L17	0.6233	Maintain the subunit's structural integrity
B7WRZ1	30S ribosomal protein S6	0.6283	Small subunit's structural integrity
B7WZQ4	Ribosome-binding factor A	0.669	Maturation of the functional core of the 30S ribosomal subunit
B7WY92	50S ribosomal protein L7/L12	0.7054	Large subunit's structural integrity





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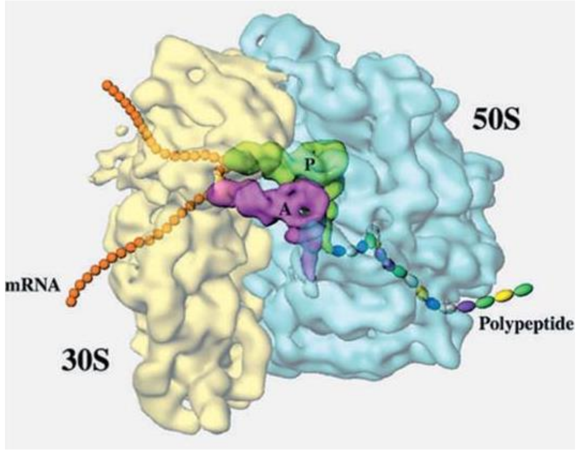
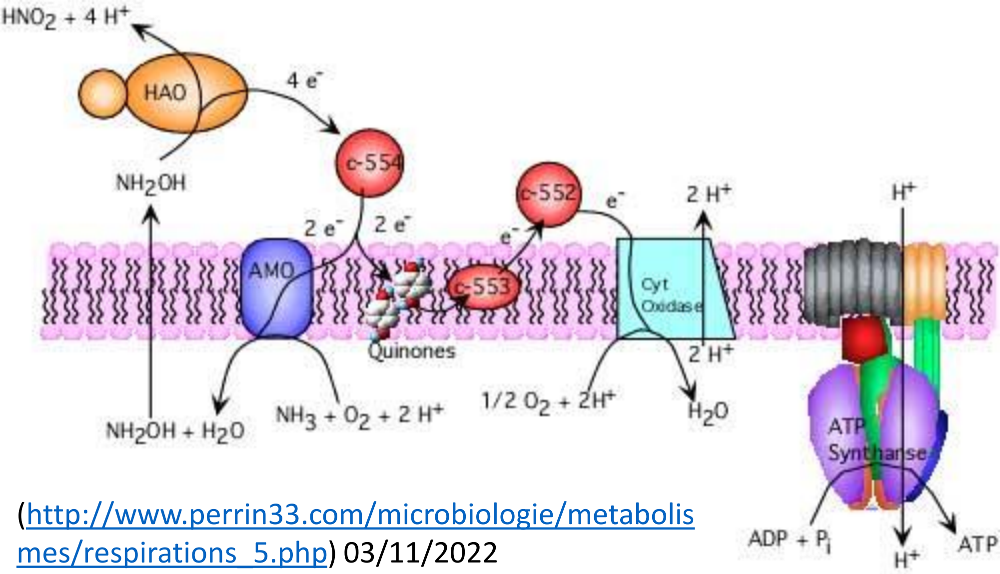
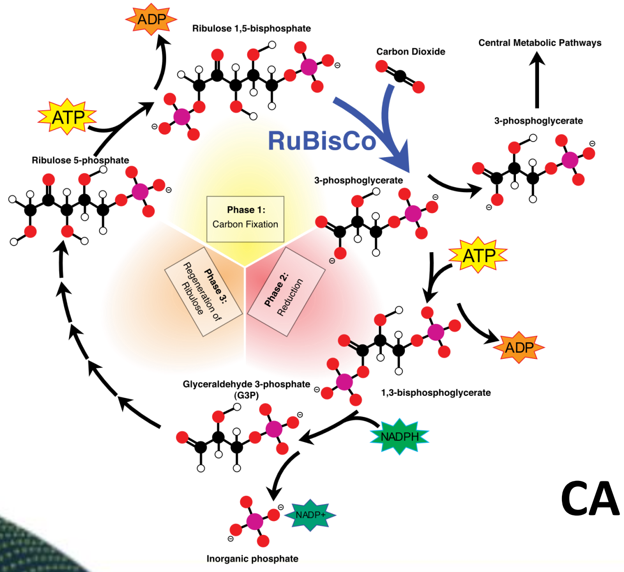
Radiation – *Tripartite – Nitrosomonas europaea*

Protein Accession	Protein Description	Fold Change	Protein function
A0A837WUL1	50S ribosomal protein L17	0.6626	Large subunit's structural integrity
A0A837X6A4	Ribulose bisphosphate carboxylase large chain	1.4912	Fixating CO2, Reductive pentose-phosphate cycle
Q82XP9	ATP synthase gamma chain	1.5831	ATP synthesis
Q82WC2	Cytochrome c-type biogenesis protein	1.8555	Electron transport chain





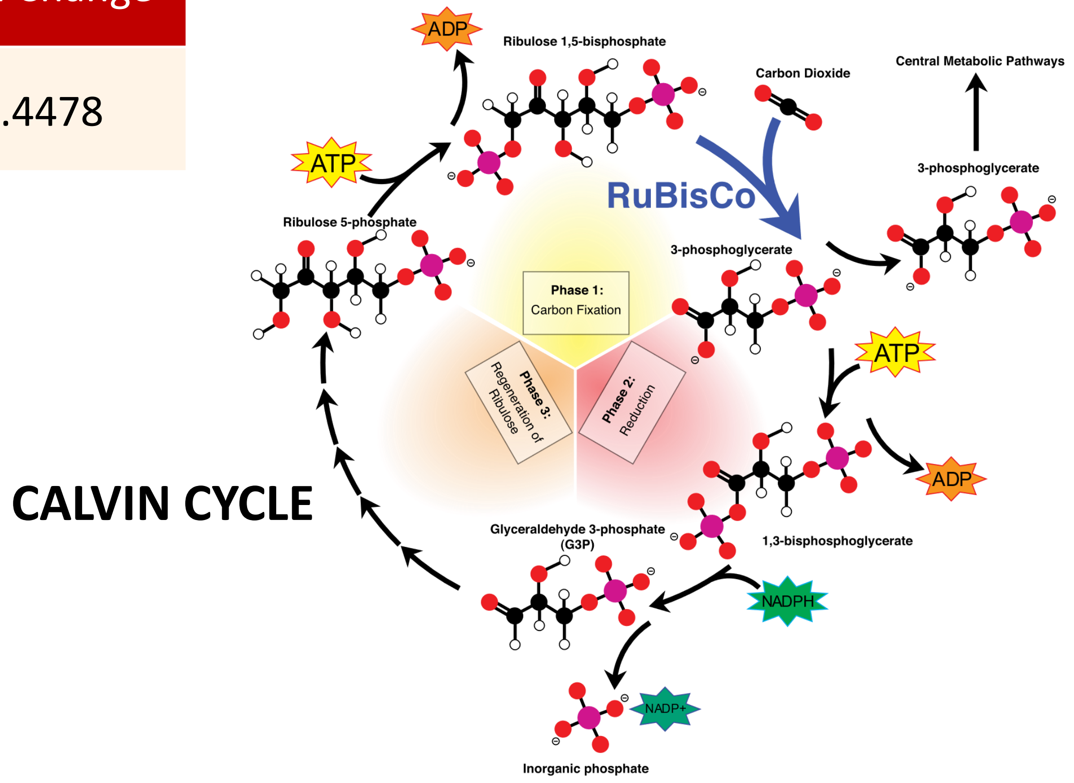
(http://www.perrin33.com/microbiologie/metabolismes/respirations_5.php) 03/11/2022



CALVIN CYCLE

Radiation – *Tripartite* – *Nitrobacter winogradskyi*

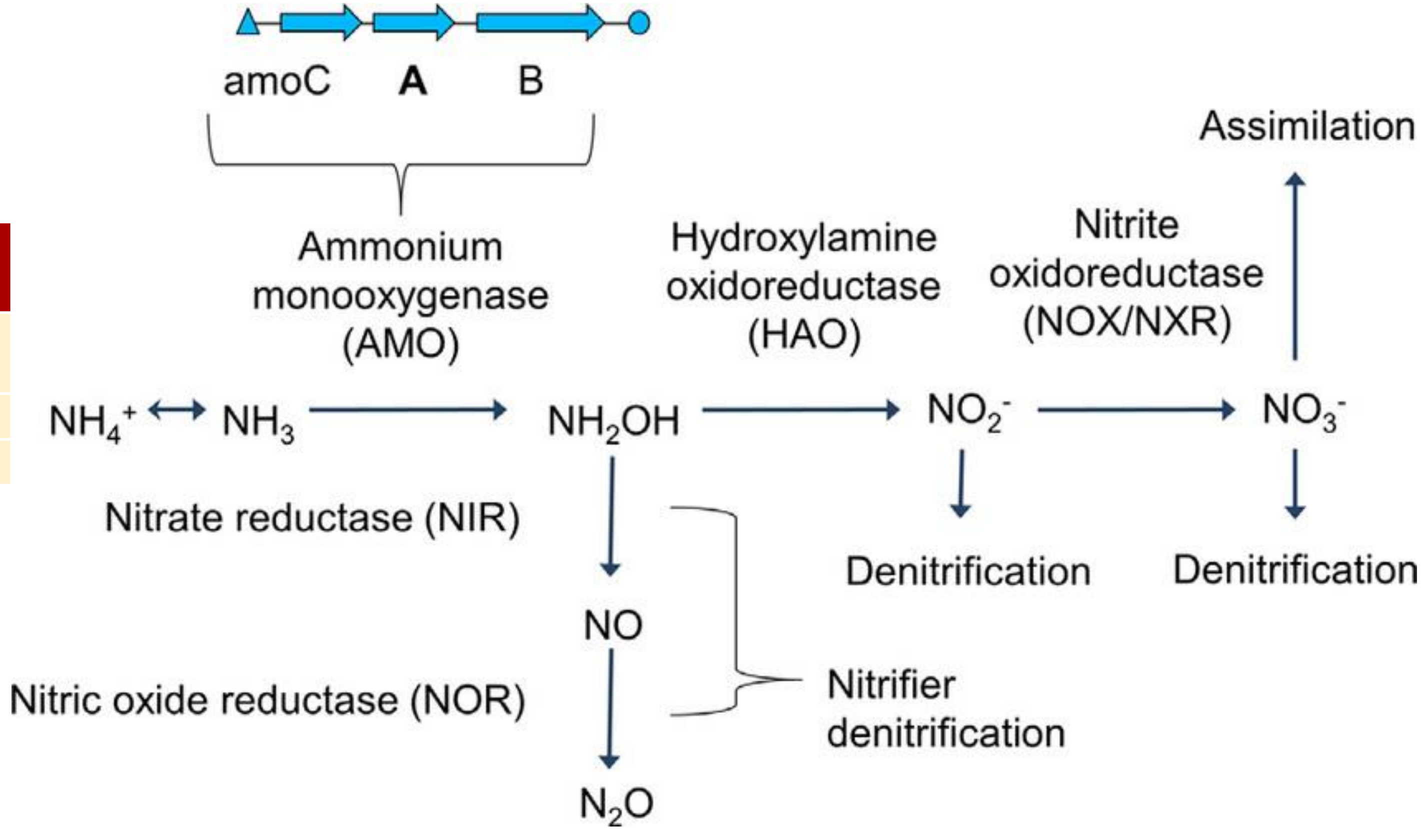
Protein Accession	Protein Description	Fold Change
Q9XD77	Ribulose bisphosphate carboxylase large chain	1.4478



Microgravity RWV – *Nitrosomonas europaea*

Total protein identifications: 1219

Protein Accession	Protein Description	Fold Change
H2VFV0	Ammonia monooxygenase 3 subunit C	0.4287
Q04507	Ammonia monooxygenase alpha subunit	0.63
Q04508	Ammonia monooxygenase beta subunit	0.644



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Ammonium monooxygenase (AMO)

Hydroxylamine oxidoreductase (HAO)

Nitrite oxidoreductase (NOX/NXR)

Assimilation

$NH_4^+ \leftrightarrow NH_3 \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^-$

Nitrate reductase (NIR)

Denitrification

Denitrification

NO

Nitric oxide reductase (NOR)

Nitrifier denitrification

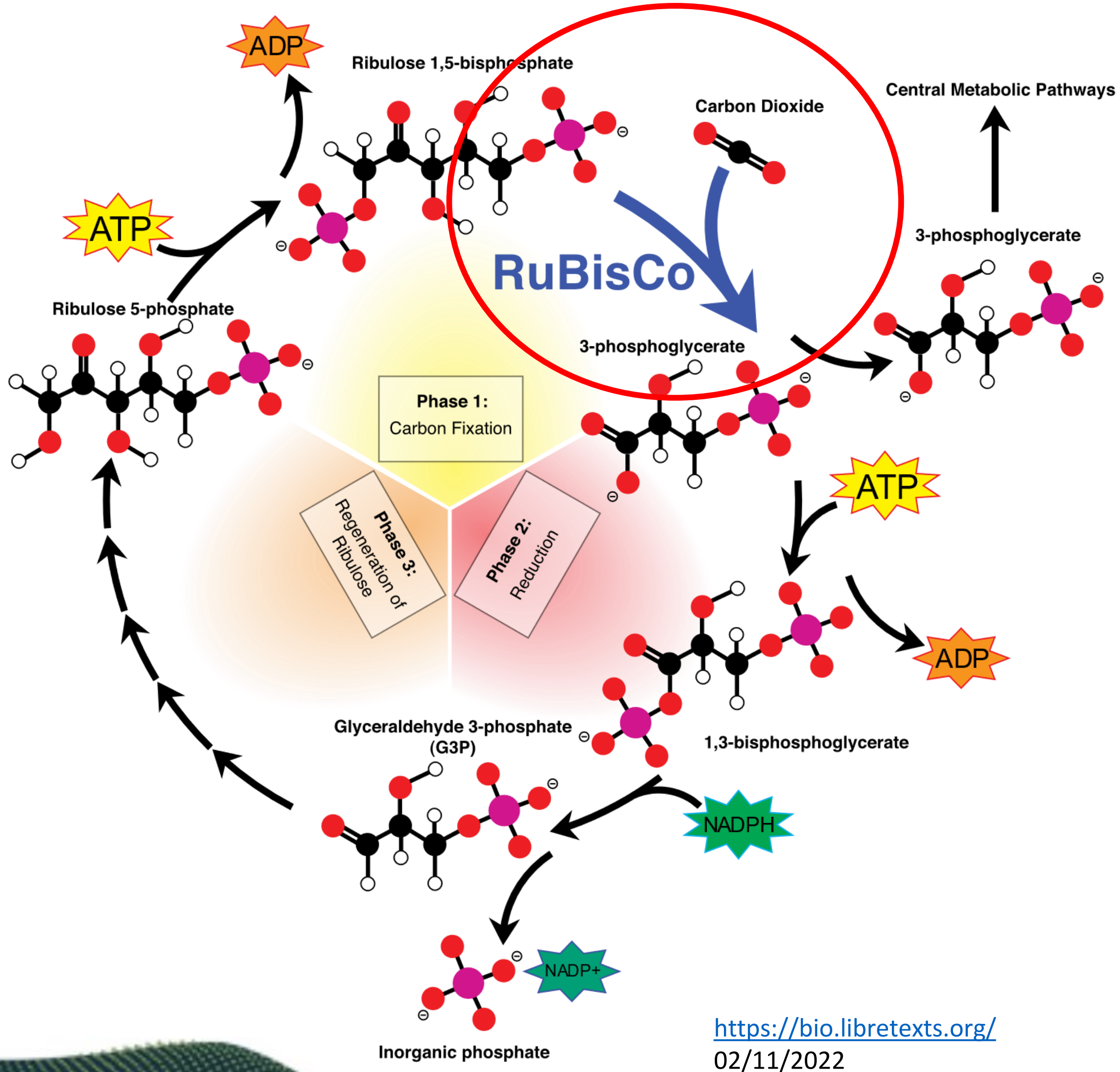
N_2O

(Levy-Booth et al., 2014)

Microgravity RWV – *Nitrosomonas europaea*

Protein Accession	Protein Description	Fold Change
A0A837X6A4	Ribulose bisphosphate carboxylase large chain	1.3768
A0A837X1A6	Ribulose bisphosphate carboxylase, small chain	1.3838
Q93A92	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit CbbL (Fragment)	1.4161

CALVIN CYCLE

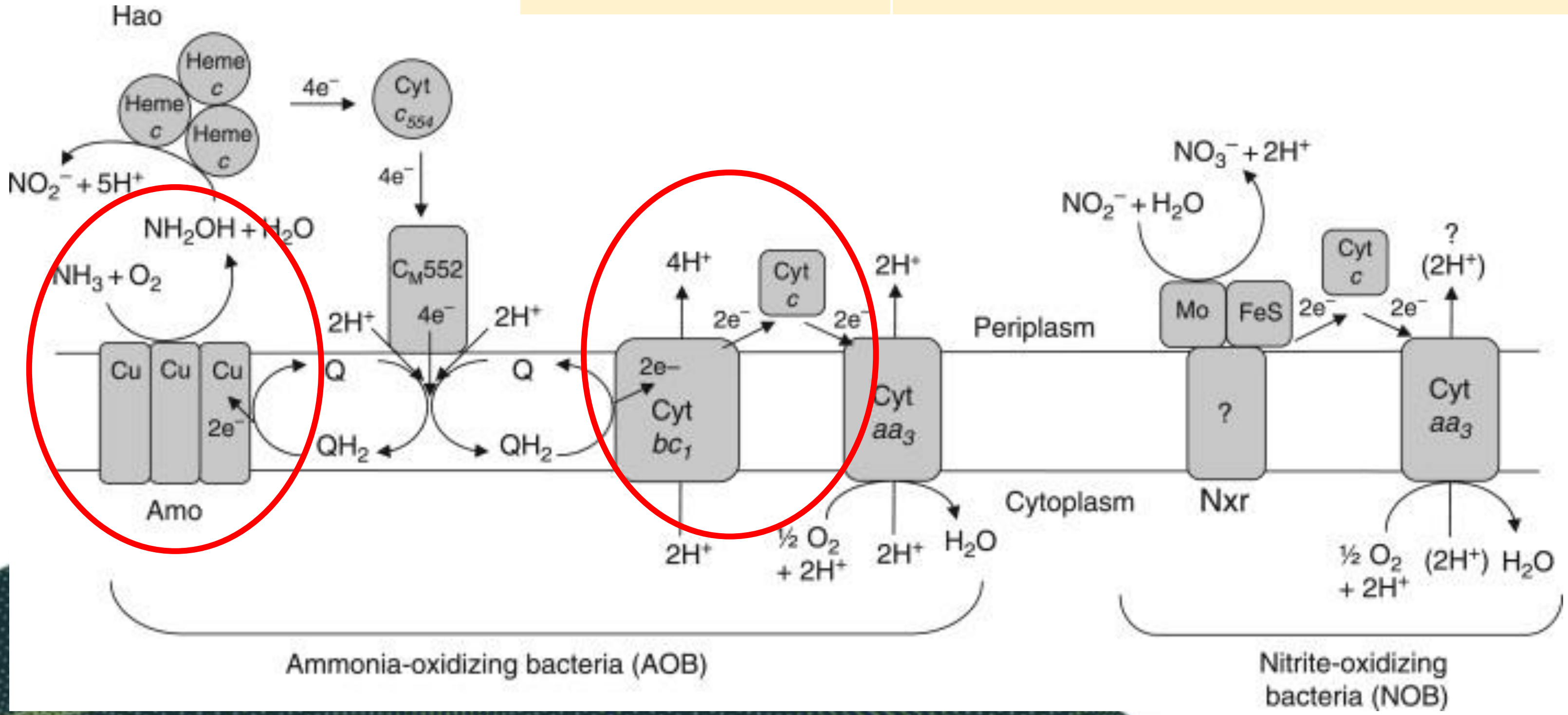


https://bio.libretexts.org/
02/11/2022

Microgravity RPM – *Nitrosomonas europaea*

Total protein identifications: 1216

Protein Accession	Protein Description	Fold Change
Q820N7	Cytochrome c, class IC:Cytochrome c, class I	0.2222
A0A837WZD6	Cytochrome-c oxidase chain II	0.5907
Q04507	Ammonia monooxygenase alpha subunit	0.628



(Cabello *et al.*, 2009)

Microgravity RWV– *Nitrobacter winogradskyi*

Total protein identifications: 1320

Protein Accession	Protein Description	Fold Change	Protein function
Q3SQ68	Propeptide, PepSY amd peptidase M4	0.4452	Nutrient production or pathogenicity
Q3SP87	Multicopper oxidase3	0.5689	Many different processes, including metal homeostasis, ascorbate metabolism, and phenolic substrate oxidation, also appear to play a supporting role in nitrite reduction

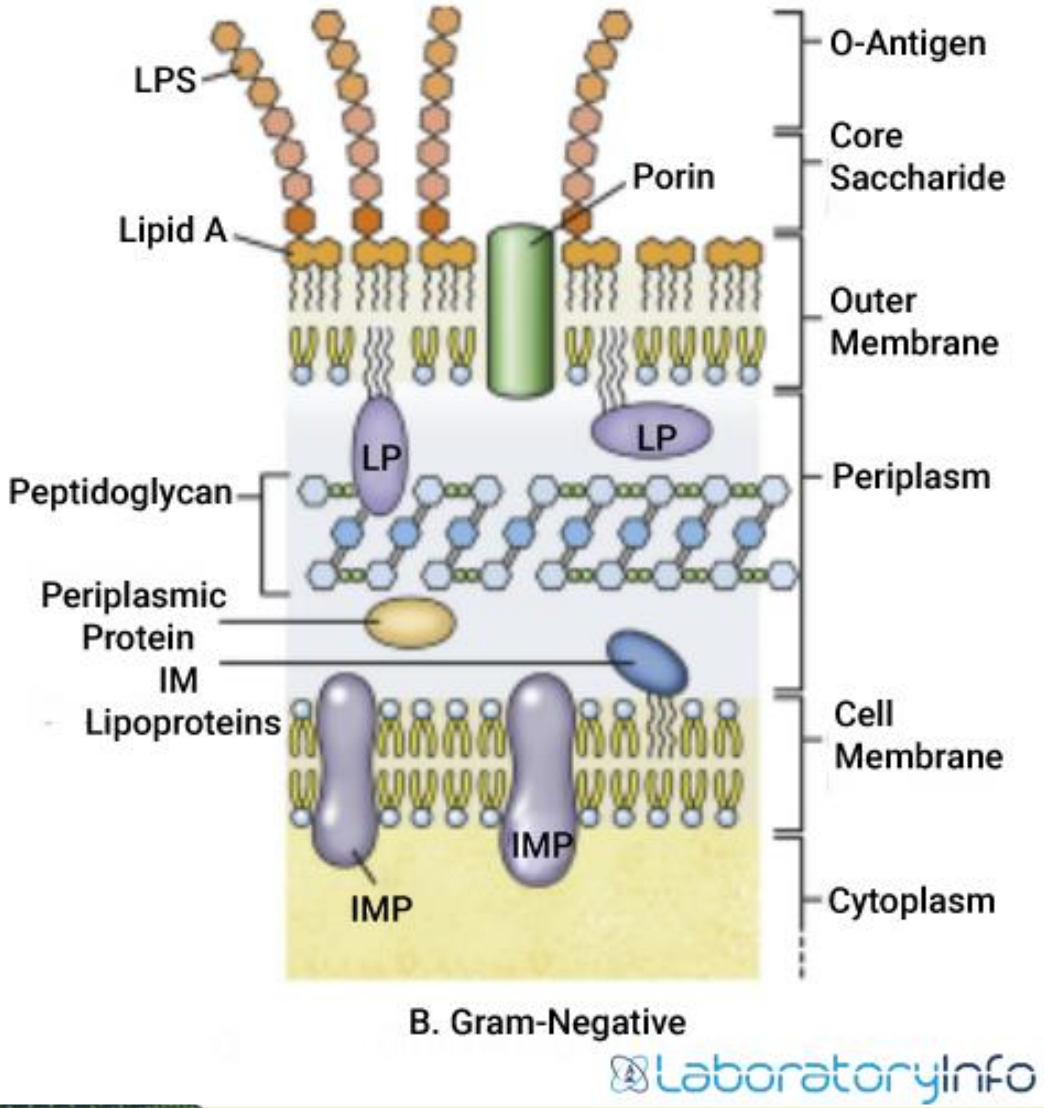
→ No clear changes

Microgravity RPM – *Nitrobacter winogradskyi*

Total protein identifications: 1320

Protein Accession	Protein Description	Fold Change
Q3SPQ0	ErfK/YbiS/YcfS/YnhG	0.4096
Q3SPT3	Cobyrinic acid a,c-diamide synthase	0.437
A0A4Y3WBQ0	SPOR domain-containing protein	0.5892
Q3SRA1	Lytic transglycosylase	0.6678
Q3SRZ5	Endolytic murein transglycosylase	0.6699

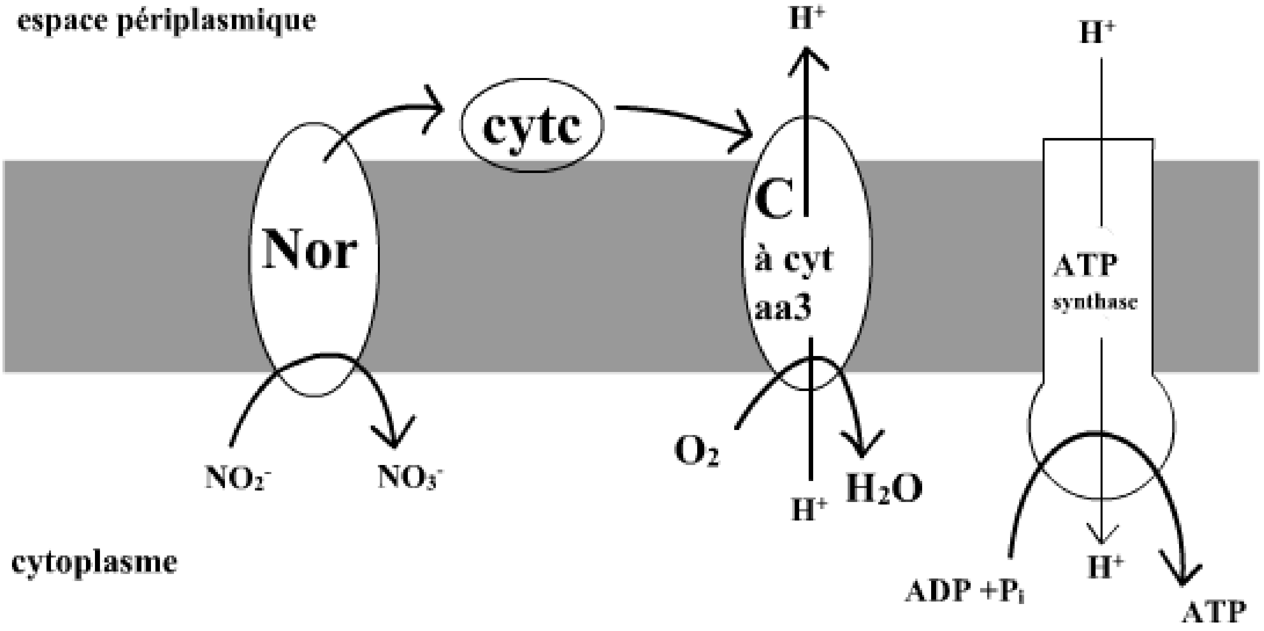
→ Down regulation of many proteins involved in the synthesis and modification of peptidoglycan wall



Microgravity RPM – *Nitrobacter winogradskyi*

Protein Accession	Protein Description	Fold Change
Q3SP88	Cytochrome c, class I	0.6035
Q3SWC9	Electron transport protein SCO1/SenC	0.608
Q3STD8	Cytochrome c-type biogenesis protein cycH / CcmH	0.6134
Q3SUR3	Cytochrome c, class I	0.6351
Q3SPC1	Ubiquinol-cytochrome c reductase iron-sulfur subunit	0.6482
Q3SQC0	Fe-S oxidoreductase	0.4864

→ May negatively affect the activity of nitrite oxidoreductase



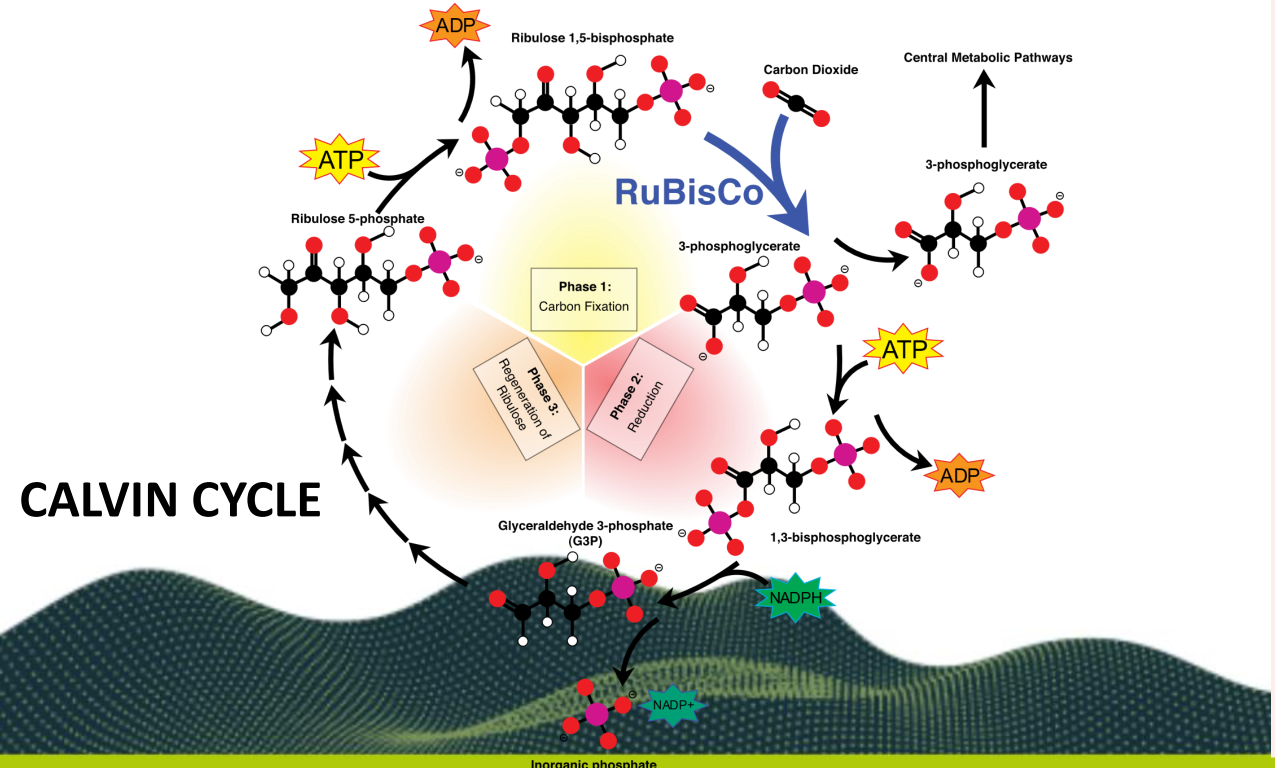
Electron transport chain in Nitrobacter

Nor: Nitrite oxidoreductase

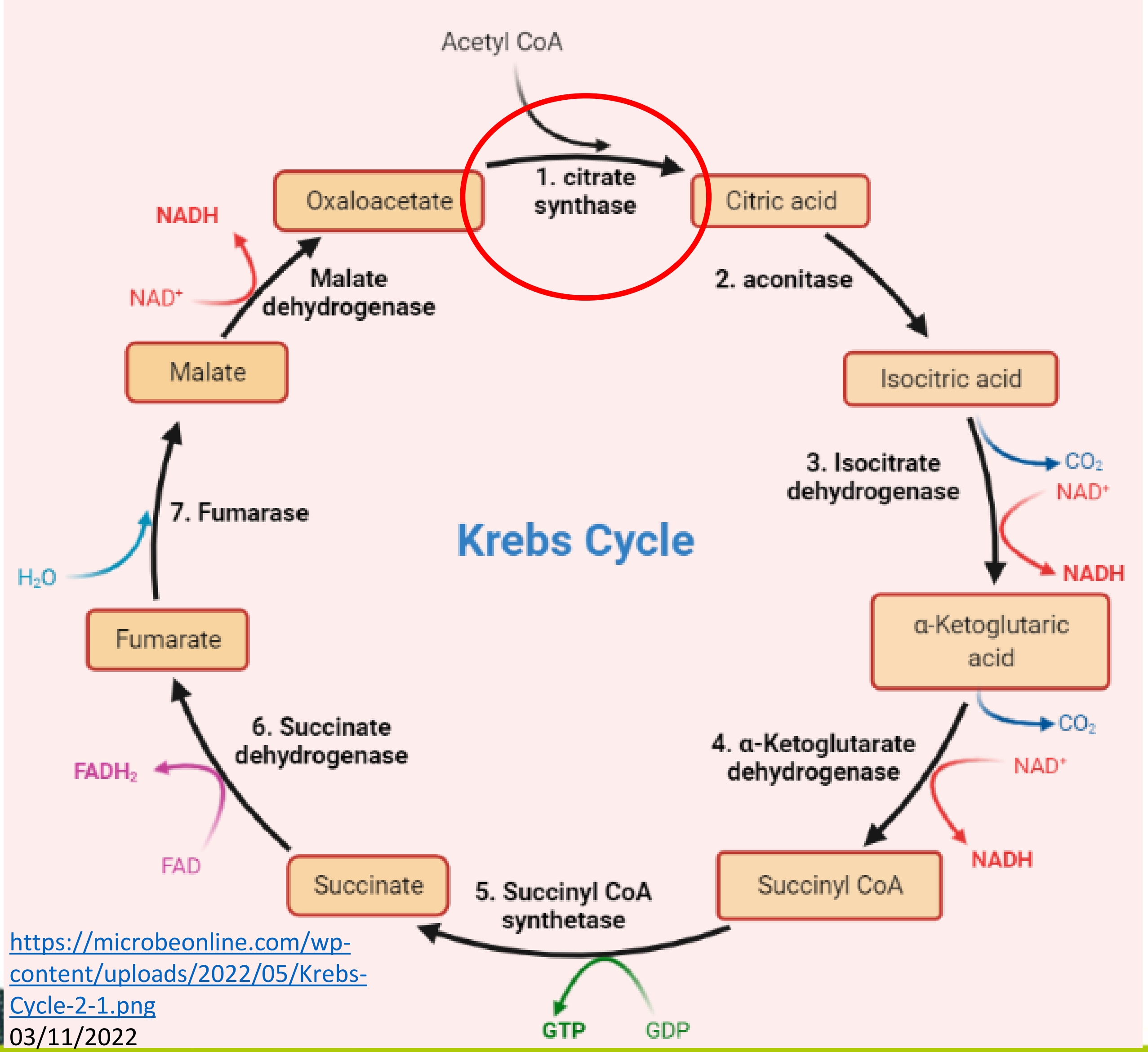
(http://www.perrin33.com/microbiologie/metabolismes/respirations_5.php) 03/11/2022

Microgravity RPM – *Nitrobacter winogradskyi*

Protein Accession	Protein Description	Fold Change
Q3SRI6	Citrate synthase	1.547
Q3SNG3	Ribulose 1,5-bisphosphate carboxylase small subunit	2.4564



CALVIN CYCLE



https://microbeonline.com/wp-content/uploads/2022/05/Krebs-Cycle-2-1.png
03/11/2022

Conclusions

- Not many differences observed in irradiated samples → possibly due to low dose
- Down regulation of ammonia monooxygenase in RPM → possible reduction of nitritation in *Nitrosomonas europaea* under microgravity condition
- Down regulation of cytochrome c under both radiation and microgravity simulation → negative impact on the electron transport chain
- Up regulation of ribulose 1,5-bisphosphate carboxylase (reductive pentose-phosphate cycle) observed in nitrifying bacteria under both radiation and microgravity simulating conditions

THANK YOU.

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Four-year position opened for a **post-doc** on:

Photosynthetic bacteria metabolism and PBR production

Please contact baptiste.leroy@umons.ac.be

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