MELISSA

MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

CREATING A CIRCULAR FUTURE

THE EFFECT OF ISS-LIKE IONIZING RADIATION AND MICROGRAVITY ON THE TRANSCRIPTOME OF N-CYCLE BACTERIA

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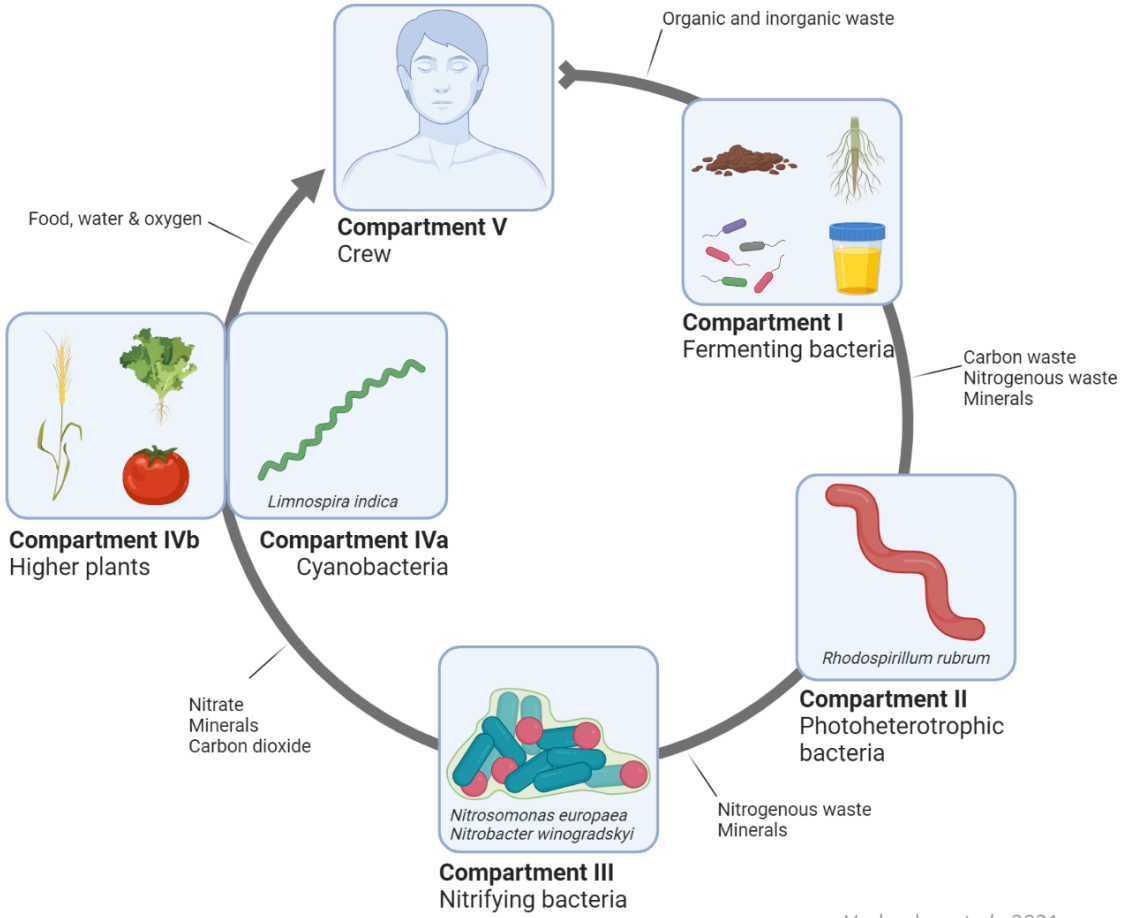
sck cen

Belgian Nuclear Research Centre

MELiSSA LOOP

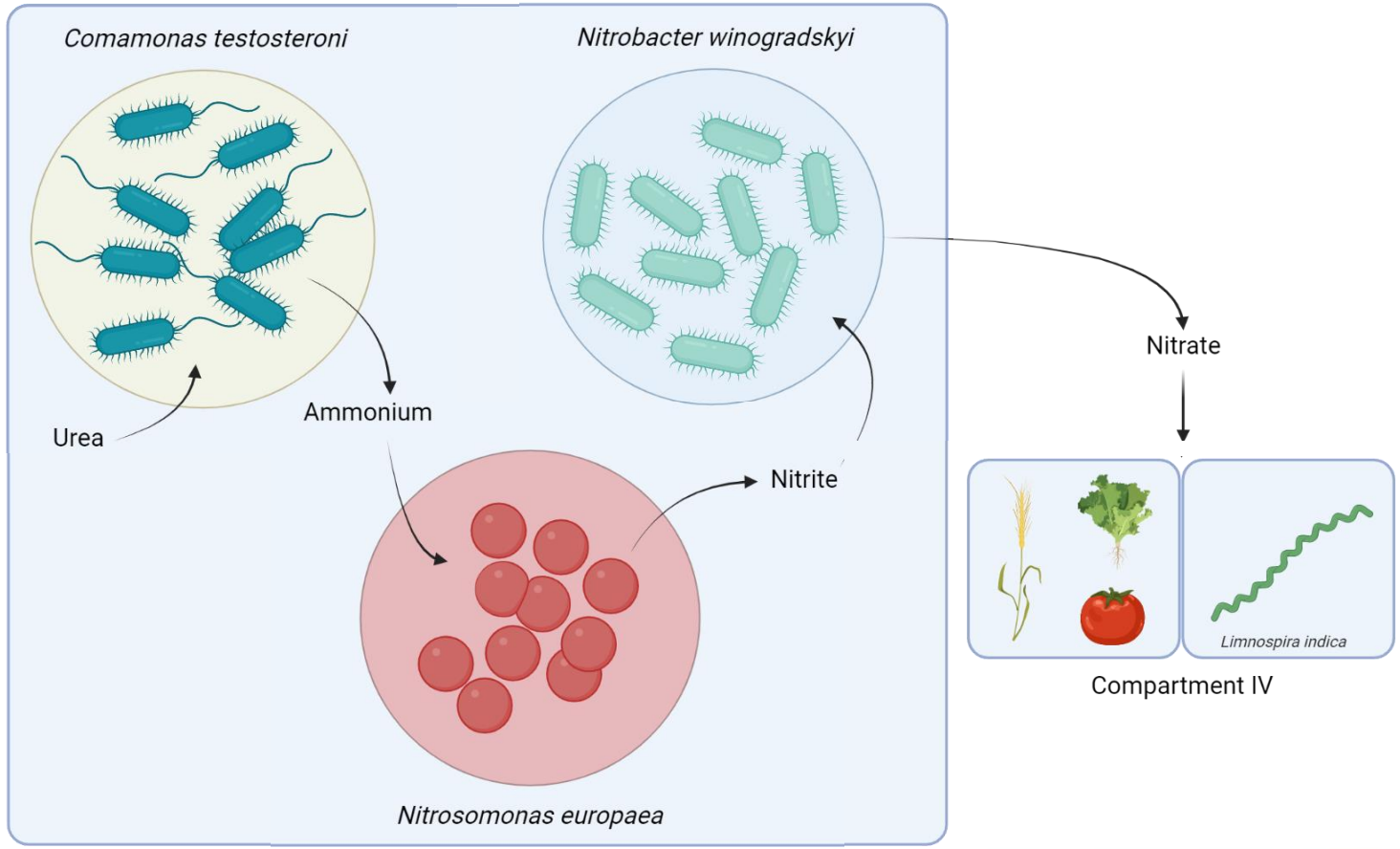
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

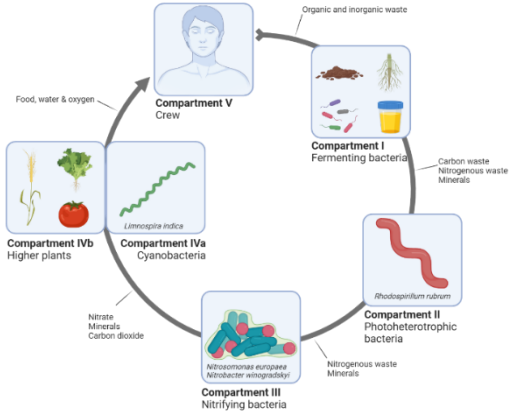


Verbeelen *et al.*, 2021

MELiSSA LOOP

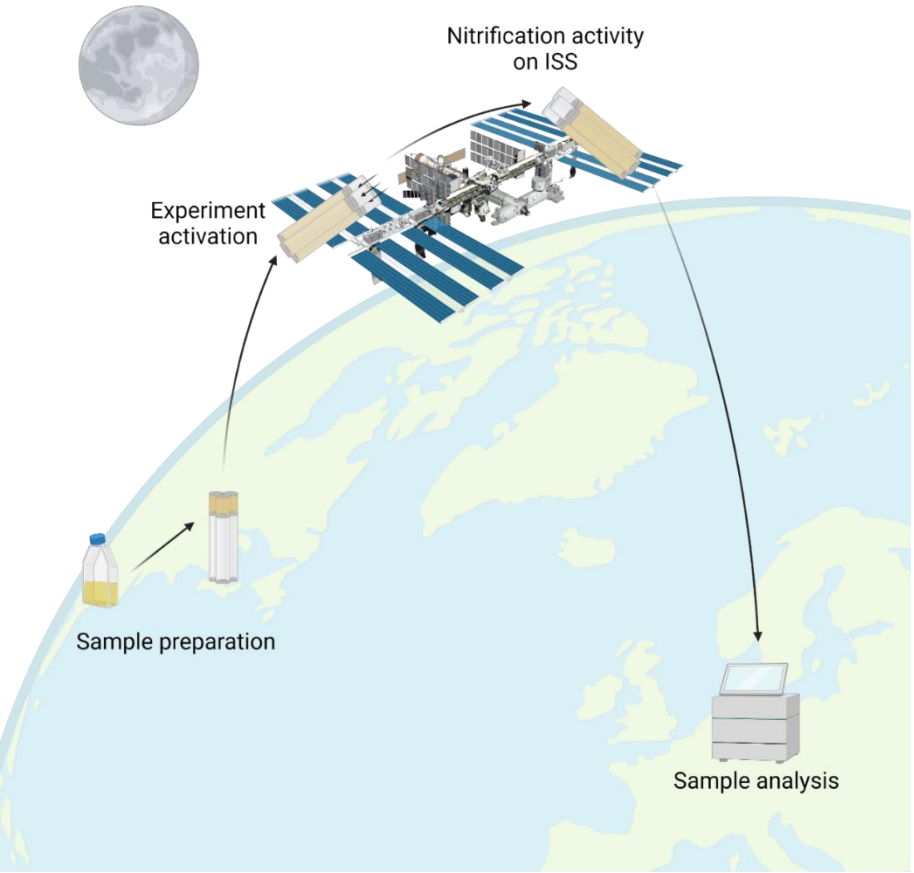
COMPARTMENT III: NITRIFICATION





- *Comamonas testosteroni*:
  - Urea → $NH_4^+ + CO_2$
- *Nitrosomonas europaea*:
  - $NH_4^+ \rightarrow NO_2^-$
- *Nitrobacter winogradskyi*
  - $NO_2^- \rightarrow NO_3^-$

URINE NITRIFICATION IN SPACE (URINIS)



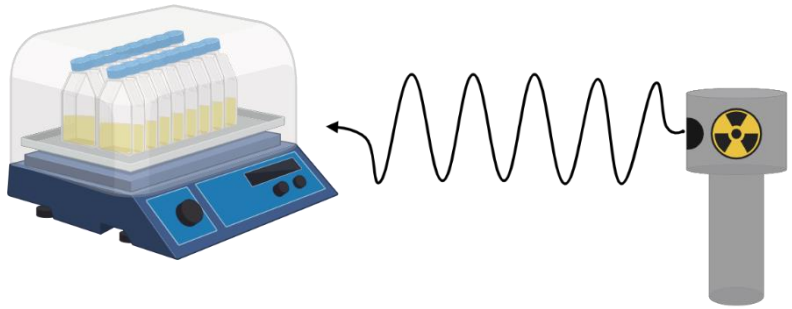
- Proof-of-concept study for nitrification on the ISS in Low Earth Orbit
  - Ionizing irradiation (280 µGy/day*)
  - Microgravity

*Berger *et al.*, 2016

- **Preparatory terrestrial experiments:**
  - Simulated ISS ionizing irradiation conditions
  - Simulated microgravity
  - Axenic cultures & tripartite community
  - Effect on ureolysis & nitrification
- **Whole transcriptome analysis**

CHRONIC LOW-DOSE IRRADIATION

CHRONIC LOW-DOSE IRRADIATION



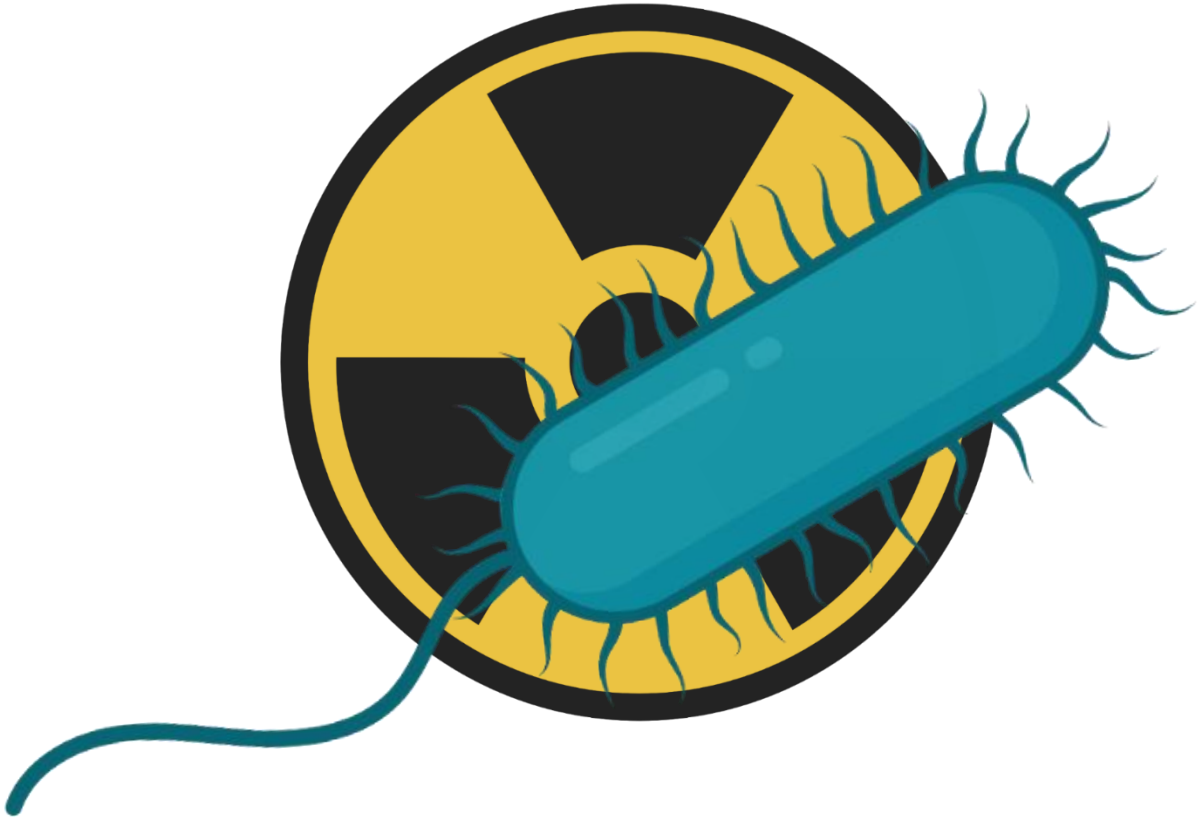
Cf-252 source

- **Simulation of ISS irradiation**
- **Accelerated life testing**:
  Total dose of a **4-month ISS stay** (36 mGy) over **3 days** of irradiation
- Cf-252 neutron source*: 509 µGy/h

*Akatov *et al.*, 2013

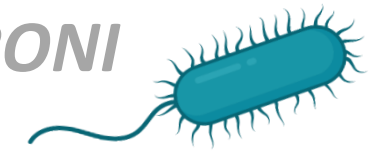
CHRONIC LOW-DOSE IRRADIATION

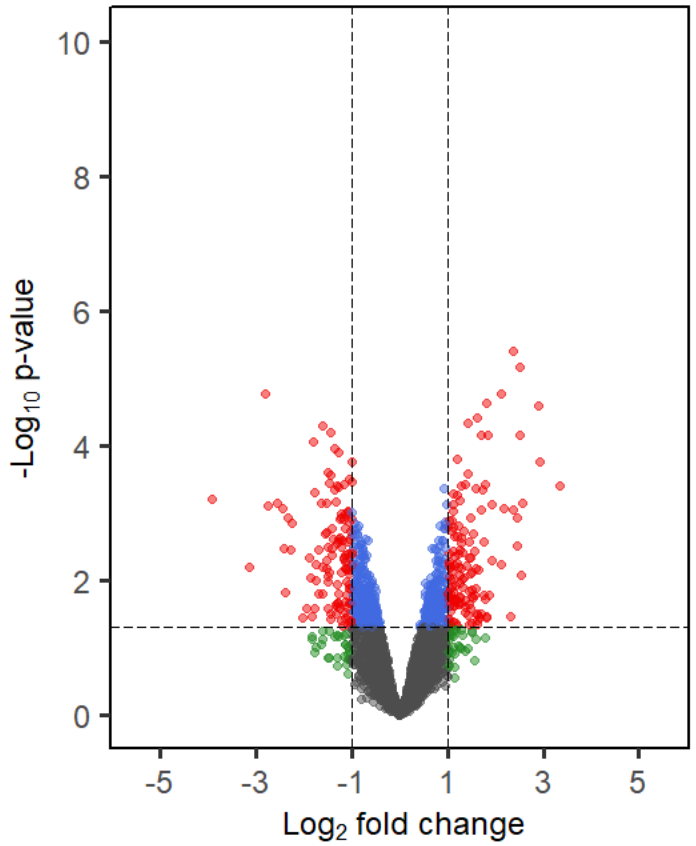
COMAMONAS TESTOSTERONI



CHRONIC LOW-DOSE IRRADIATION

COMAMONAS TESTOSTERONI



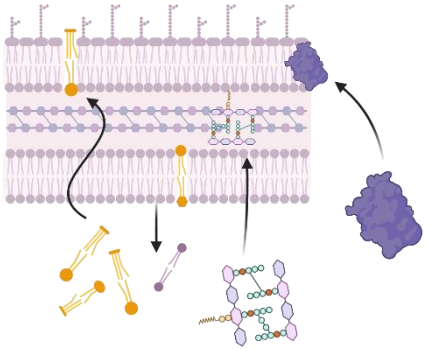


- Differentially expressed gene (DEG):
  - $\log_2$ fold change (FC) ≥ |1|
  - p-value < 0.05

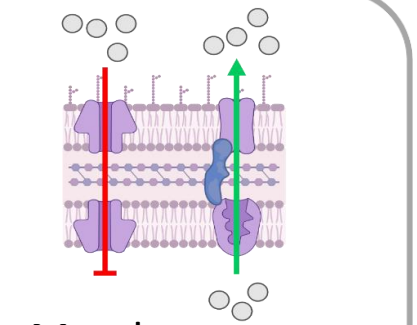
- DEG percentage: 4.28 % of total genome protein coding sequences (CDS) (= 5,771)
  - 56.28 % upregulated
  - 43.72 % downregulated

CHRONIC LOW-DOSE IRRADIATION

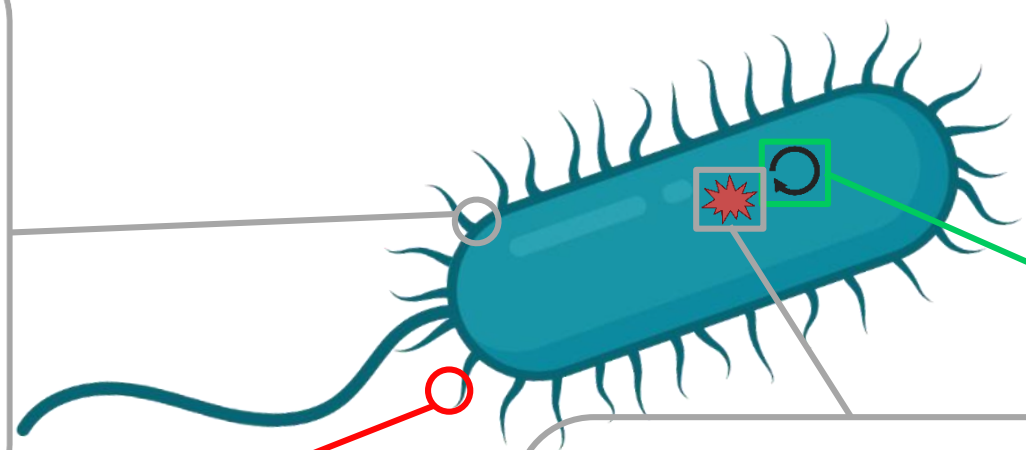
COMAMONAS TESTOSTERONI



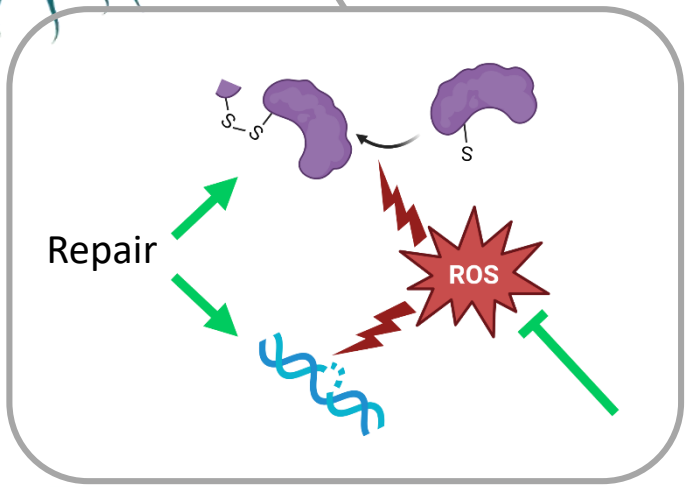
Cell envelope adaption

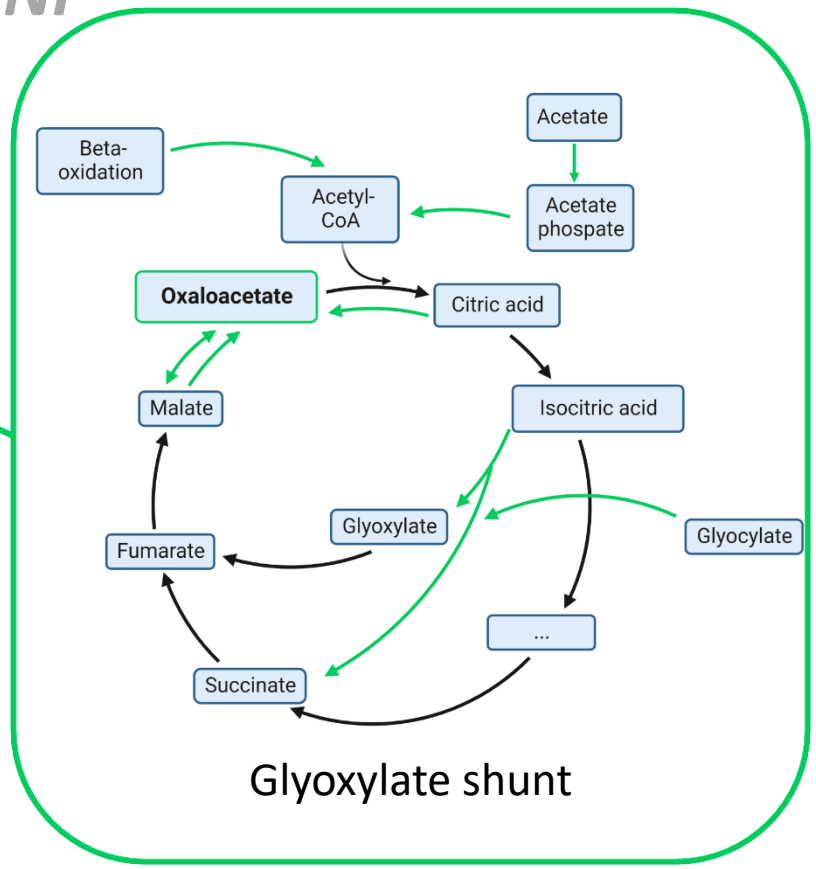


Metal transport



Pilus formation

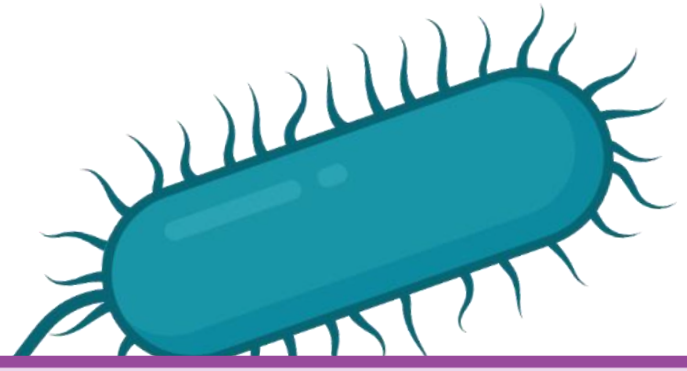




Glyoxylate shunt

CHRONIC LOW-DOSE IRRADIATION

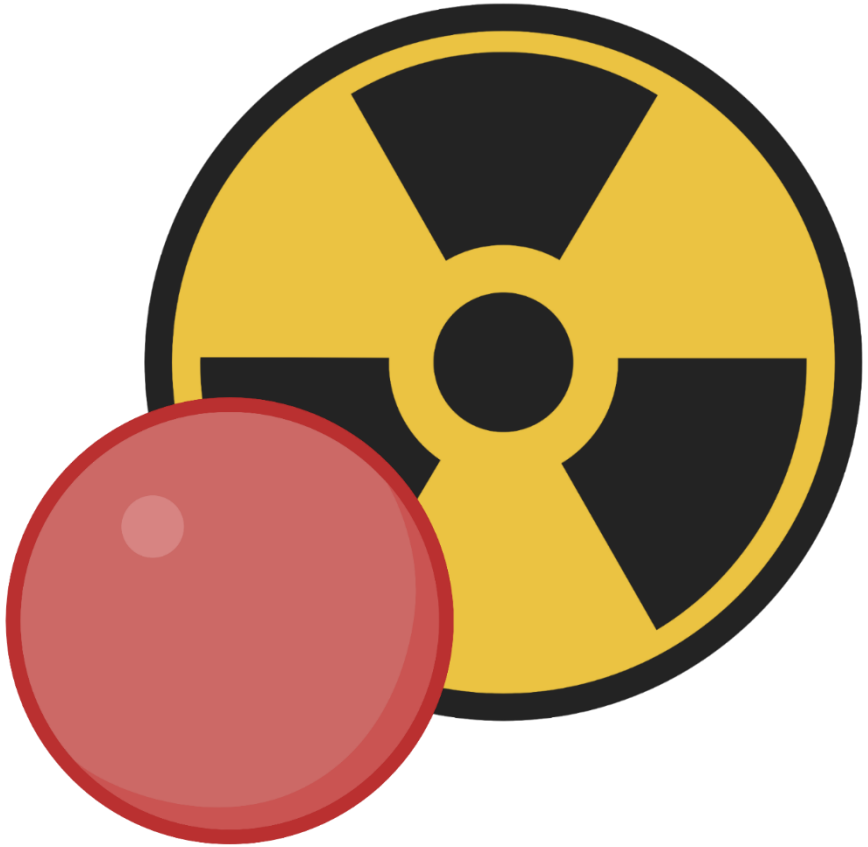
COMAMONAS TESTOSTERONI



No effect on ureolysis genes

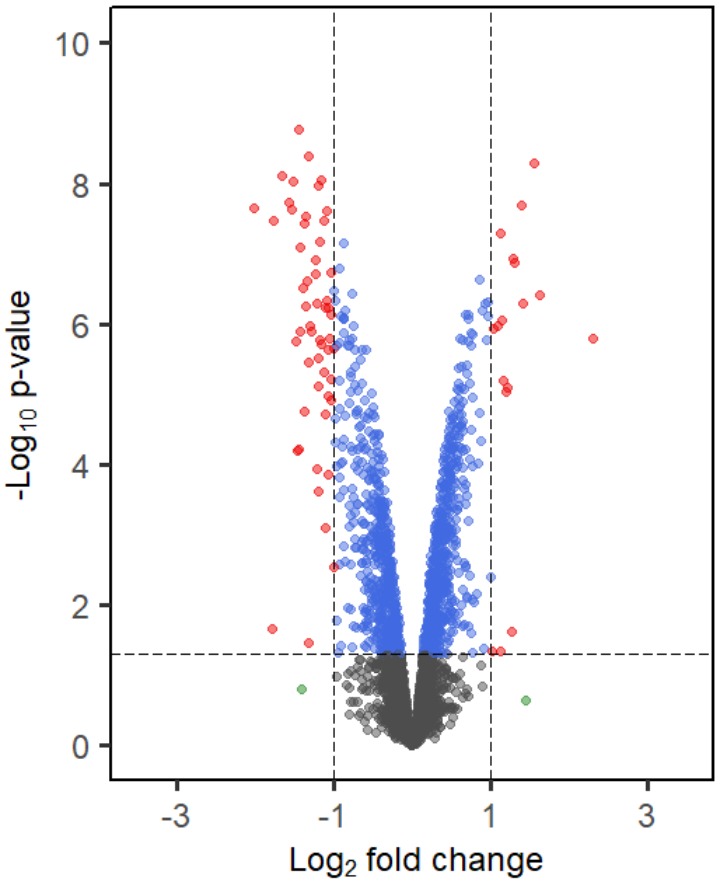
CHRONIC LOW-DOSE IRRADIATION

NITROSOMONAS EUROPAEA



CHRONIC LOW-DOSE IRRADIATION

NITROSOMONAS EUROPAEA



- DEG percentage: 2.55 % of total genome protein coding sequences (CDS) (= 2,783)
  - 23.94 % upregulated
  - 76.06 % downregulated

Not significant
log_2 FC > |1|
p-value < 0.05
DEG

CHRONIC LOW-DOSE IRRADIATION

NITROSOMONAS EUROPAEA

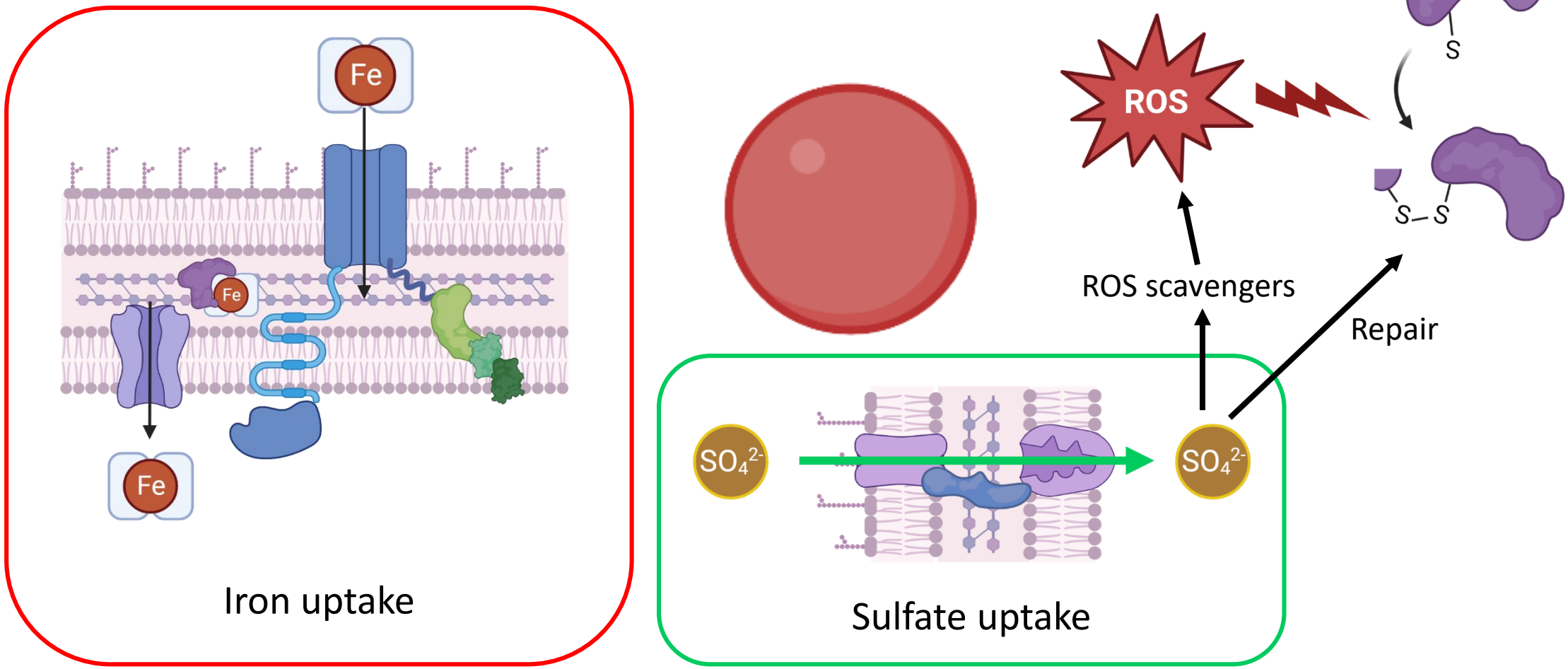
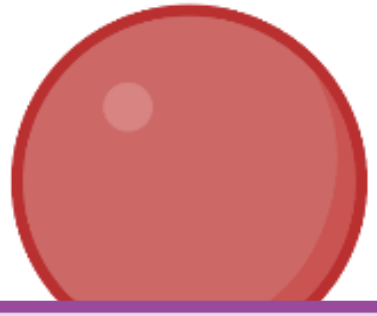


Figure adapted from Mey *et al.*, 2021

CHRONIC LOW-DOSE IRRADIATION

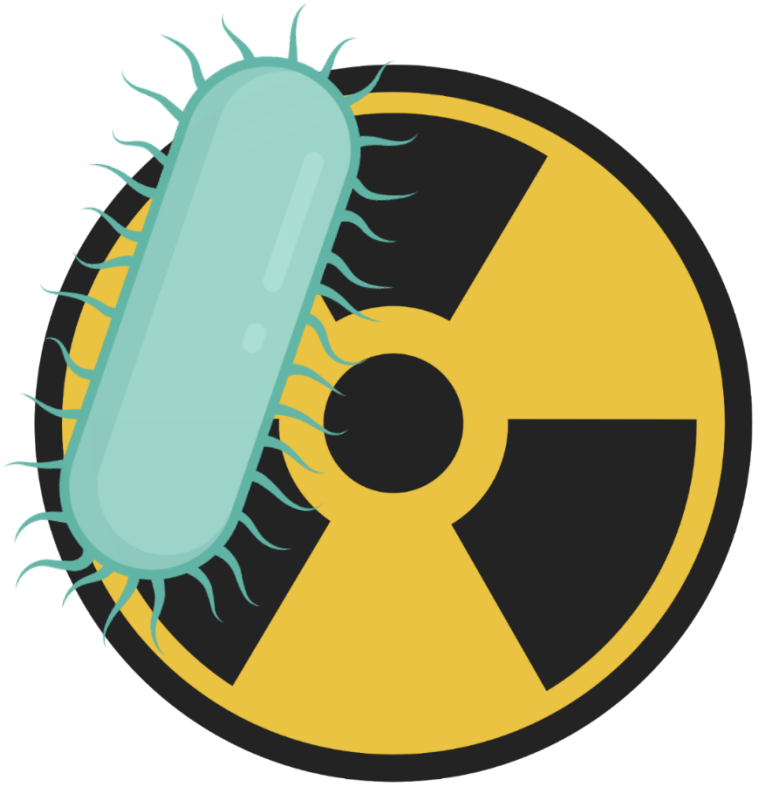
NITROSOMONAS EUROPAEA



No effect on nitrification genes

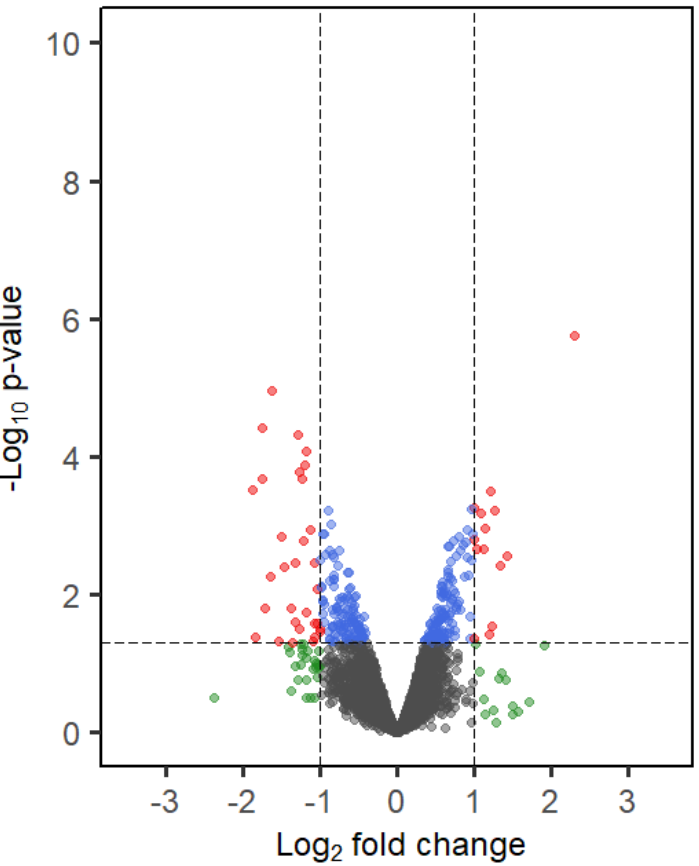
CHRONIC LOW-DOSE IRRADIATION

NITROBACTER WINOGRADSKYI



CHRONIC LOW-DOSE IRRADIATION

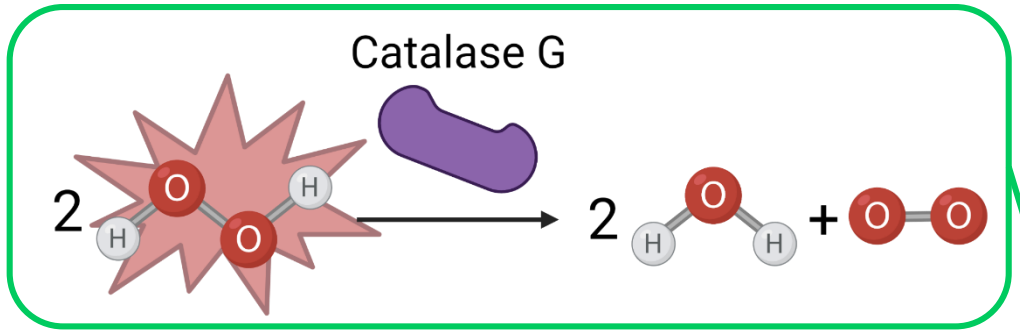
NITROBACTER WINOGRADSKYI

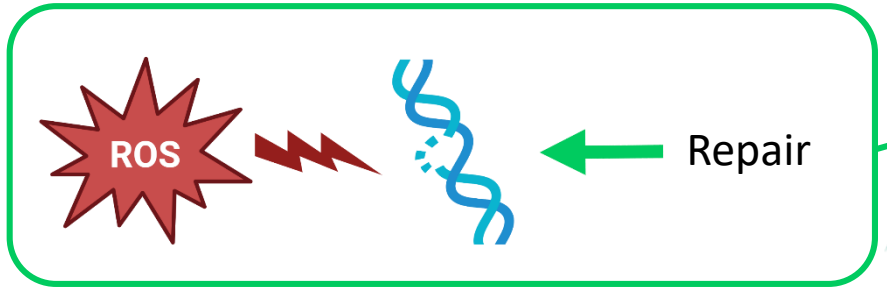


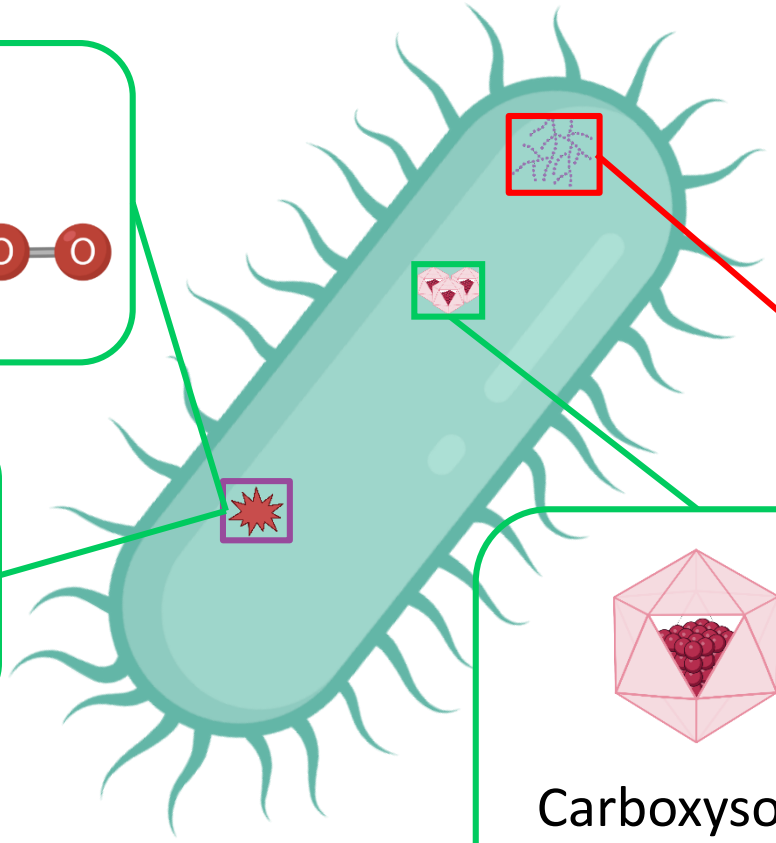
- DEG percentage: 1.23 % of total CDS (= 3,667)
  - 31.11 % upregulated
  - 68.89 % downregulated

CHRONIC LOW-DOSE IRRADIATION

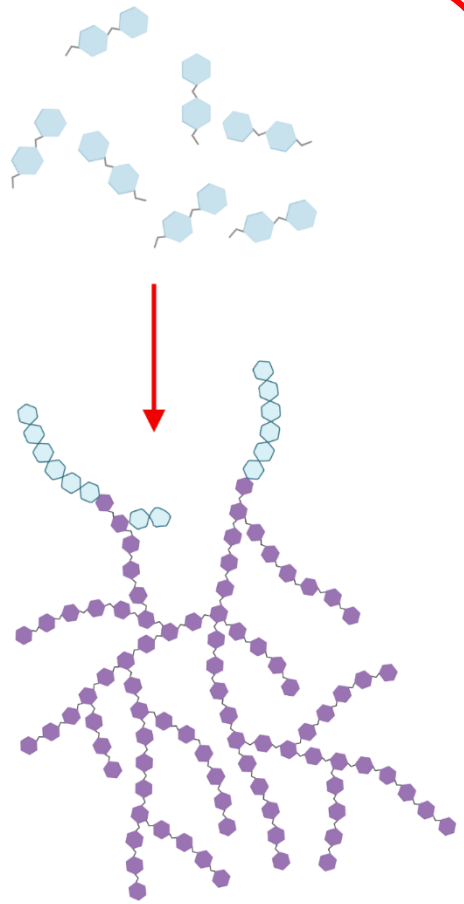
NITROBACTER WINOGRADSKYI







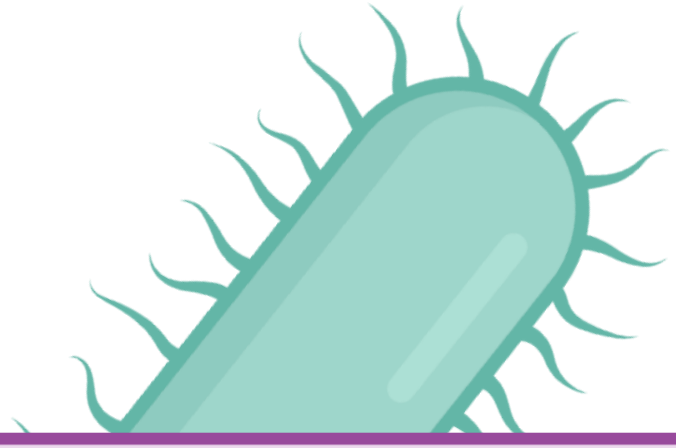
Carboxysome shell



Glycogen synthesis

CHRONIC LOW-DOSE IRRADIATION

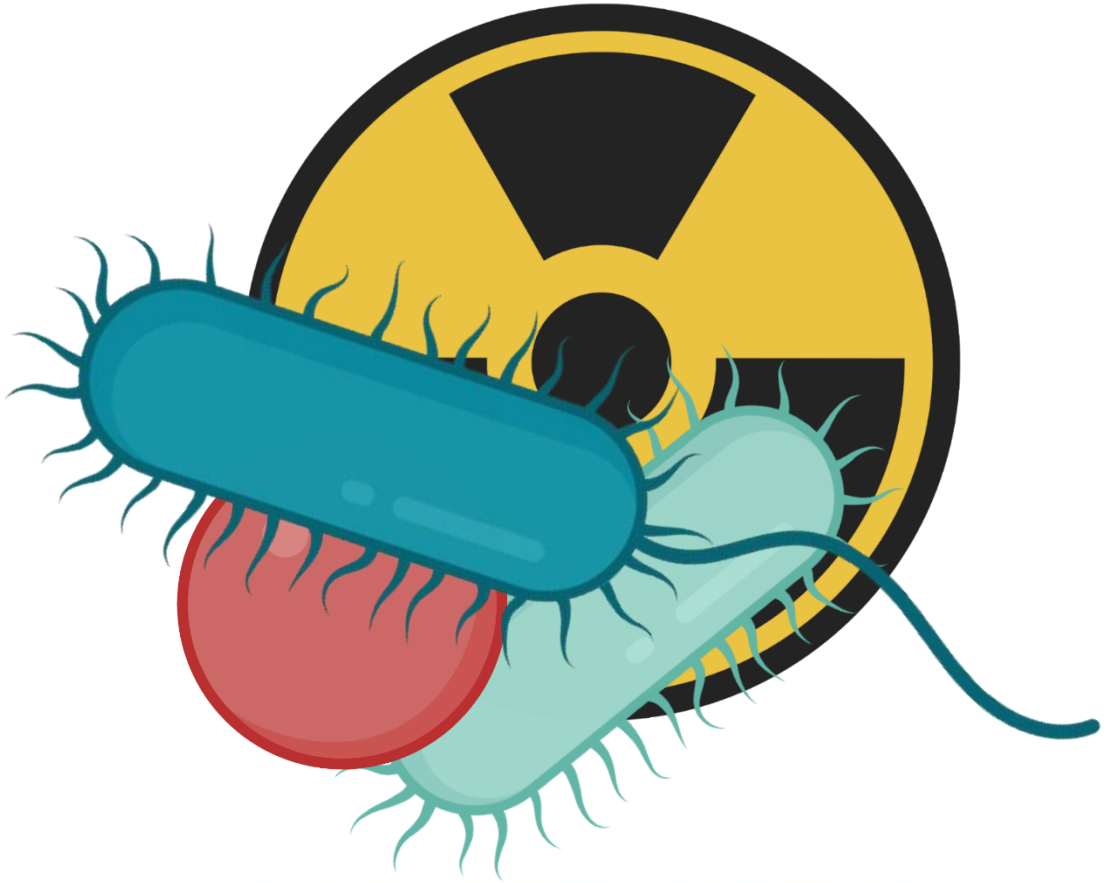
NITROBACTER WINOGRADSKYI



No effect on nitrification genes

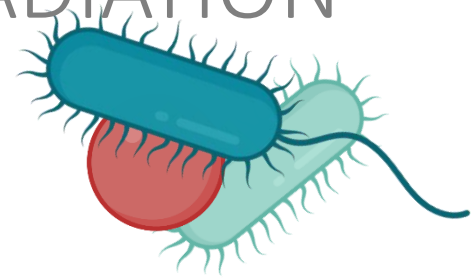
CHRONIC LOW-DOSE IRRADIATION

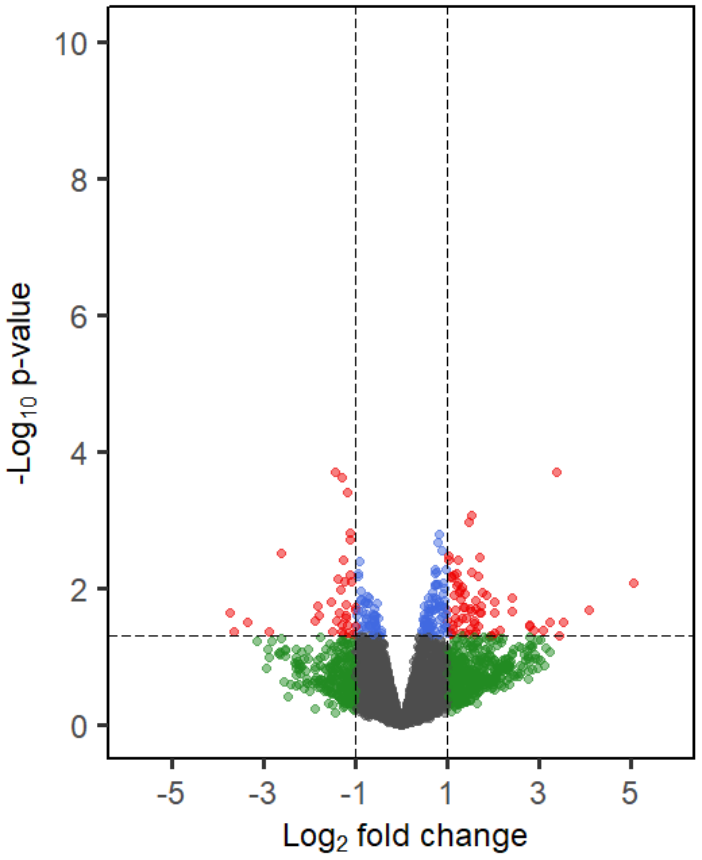
TRIPARTITE CULTURE



CHRONIC LOW-DOSE IRRADIATION

TRIPARTITE CULTURE



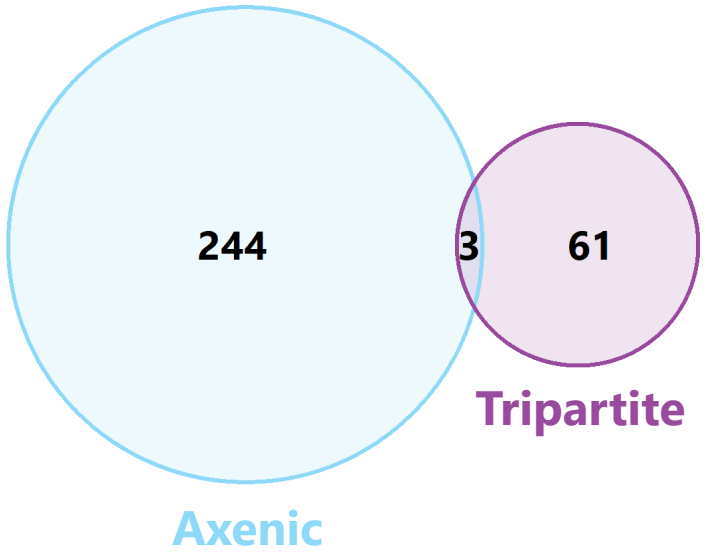


- DEG percentage: 0.85 % of total CDS (= 12,221)
  - 66.35 % upregulated
  - 33.65 % downregulated

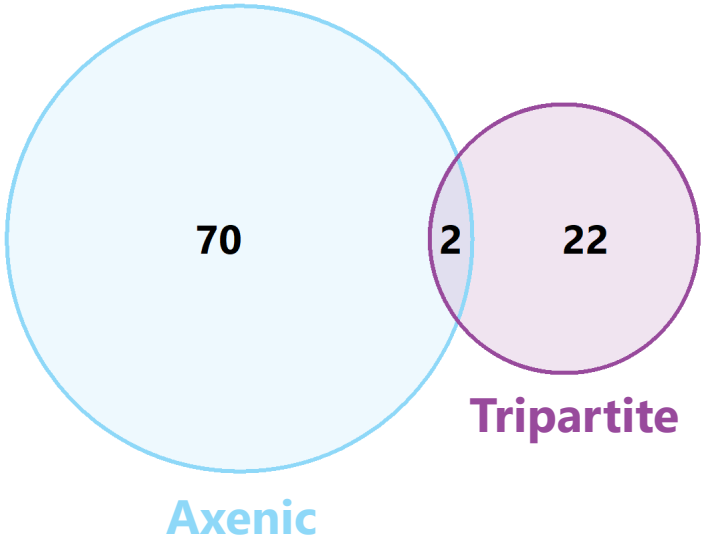
CHRONIC LOW-DOSE IRRADIATION

DEG OF AXENIC SPECIES VS. TRIPARTITE CULTURE SPECIES

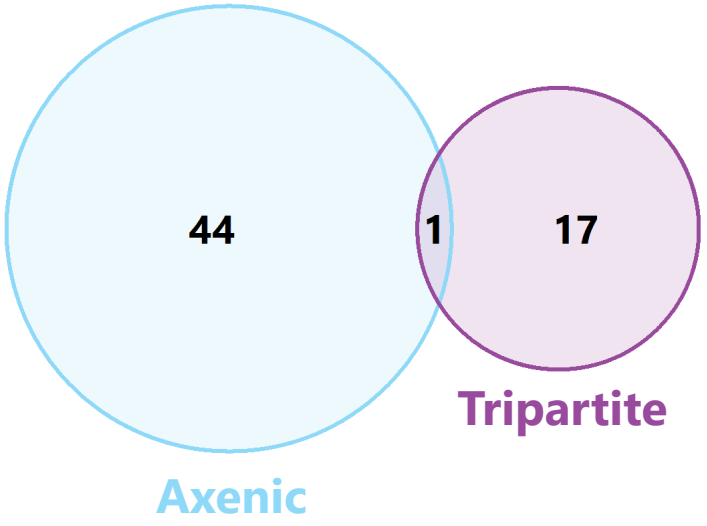
Comamonas testosteroni



Nitrosomonas europaea



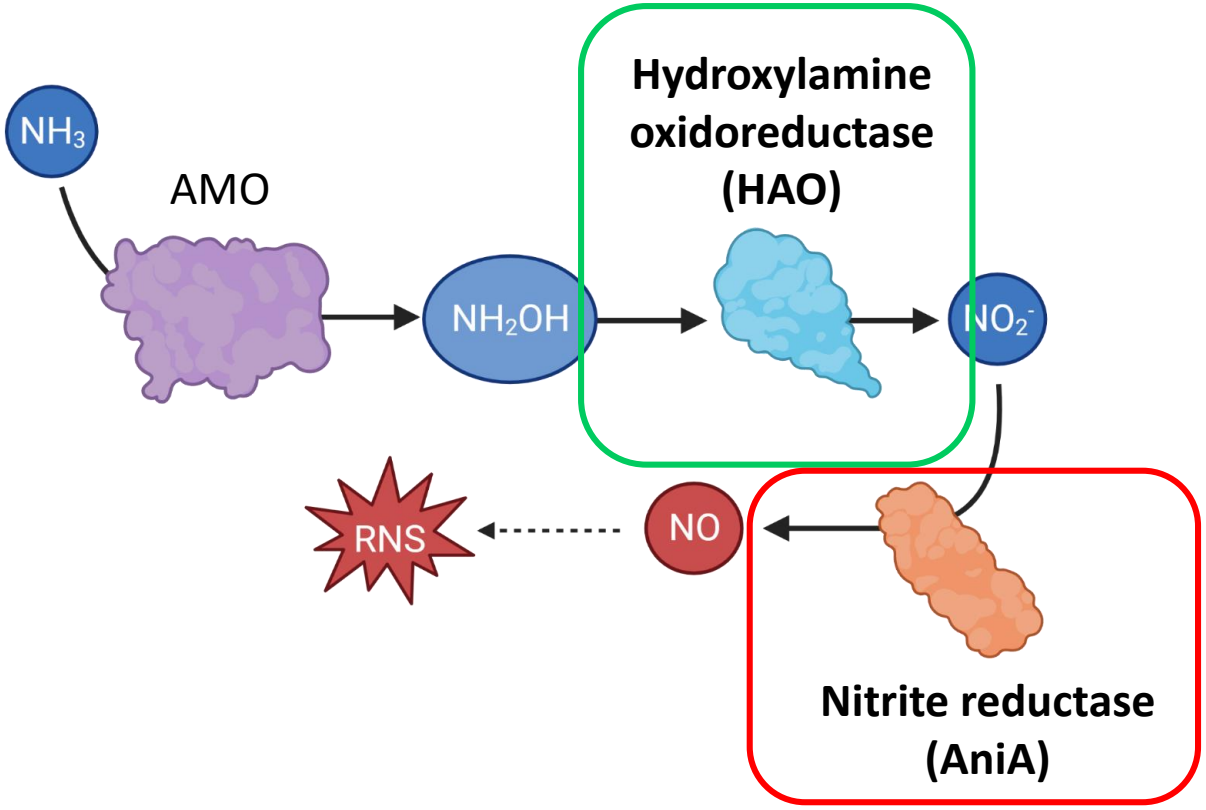
Nitrobacter winogradskyi



Similarities in stress response

CHRONIC LOW-DOSE IRRADIATION

TRIPARTITE CULTURE – *NITROSOMONAS EUROPAEA*



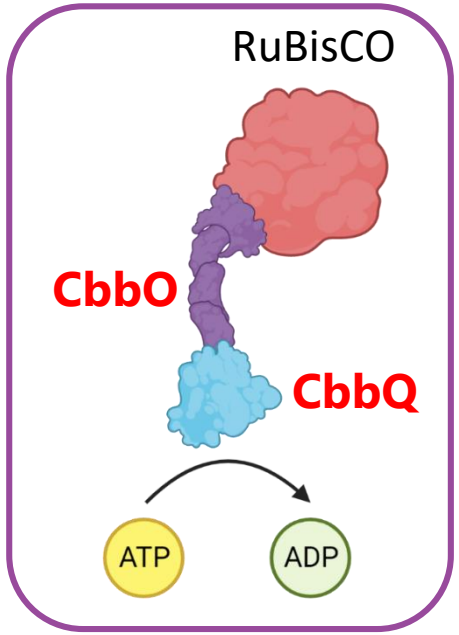
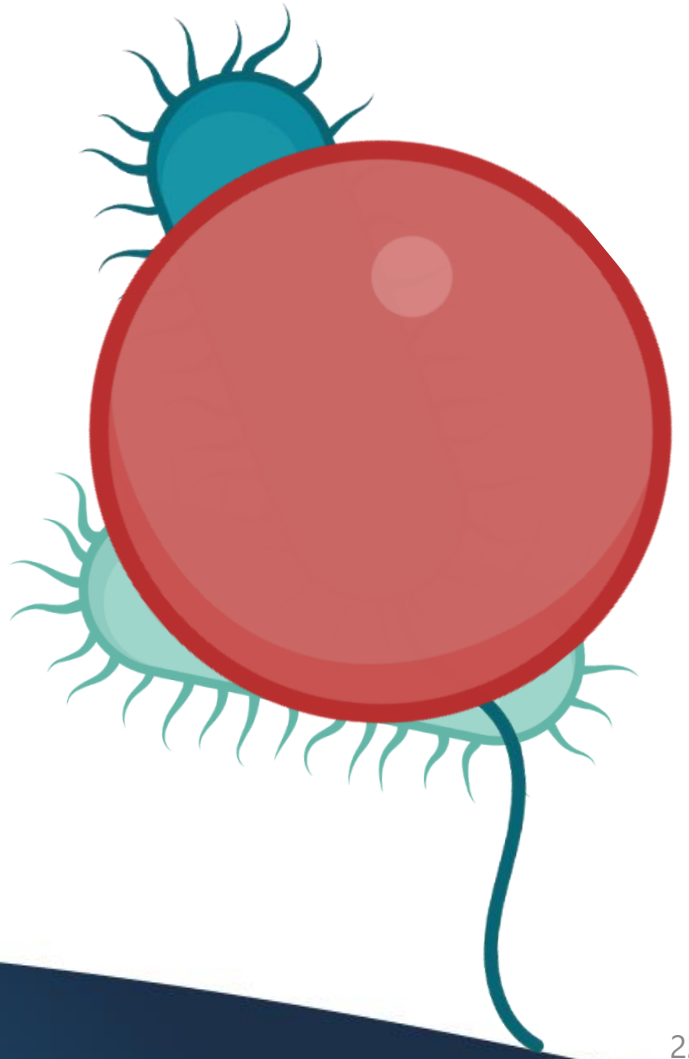
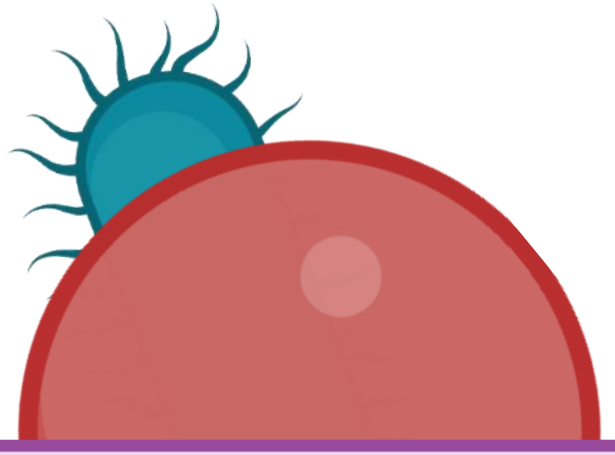


Figure adapted from Tsai *et al.*, 2015



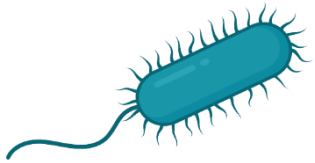
CHRONIC LOW-DOSE IRRADIATION

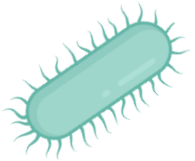
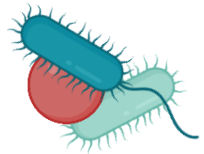
TRIPARTITE CULTURE – *NITROSOMONAS EUROPAEA*



Effect on nitrification genes

CHRONIC LOW-DOSE IRRADIATION



	Comamonas testosteroni	***Nitrosomonas europaea***	***Nitrobacter winogradskyi***	**Tripartite culture**
Oxidative stress response	- ROS scavengers - DNA repair - Cysteine biosynthesis - Glyoxylate shunt - Cell envelope adaption	- Sulfur import - Iron uptake	- ROS scavengers - Energy conservation	- ROS scavengers - Stress response proteins
Impact on ureolysis or nitrification genes	None	None	None	- *N. europaea hao3* - *N. europaea aniA* - *N. europaea* RuBisCO activators

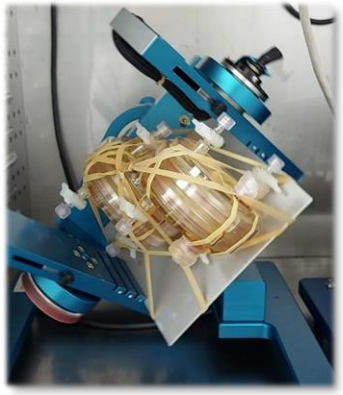
CONCLUSIONS

LOW-DOSE CHRONIC IRRADIATION

- Evidence of **subtle form of oxidative stress** in all **axenic strains** and **tripartite community**
- **Limited effect on nitrification** genes in low-dose chronic irradiation conditions in **tripartite community**

SIMULATED MICROGRAVITY

SIMULATED MICROGRAVITY



RANDOM POSITIONING MACHINE (RPM)

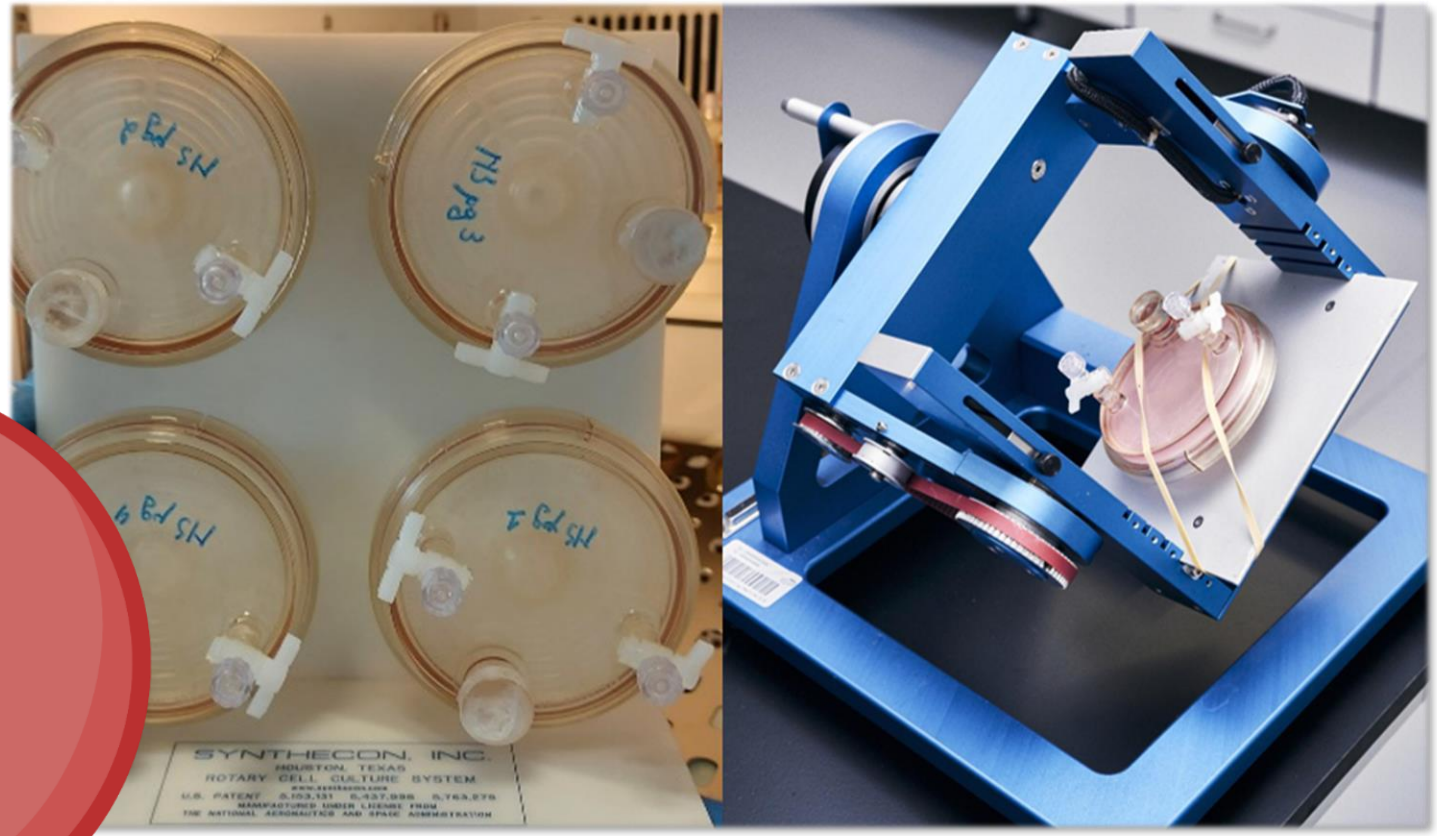
- Random 3D rotation
- Randomization of gravity vector
- Disruption of cell's gravity perception
- Low-shear modelled microgravity

ROTATING WALL VESSEL (RWV)

- 2D rotation
- Solid body rotation of liquid
- Continuous suspended orbid of cells
- Low-shear modelled microgravity

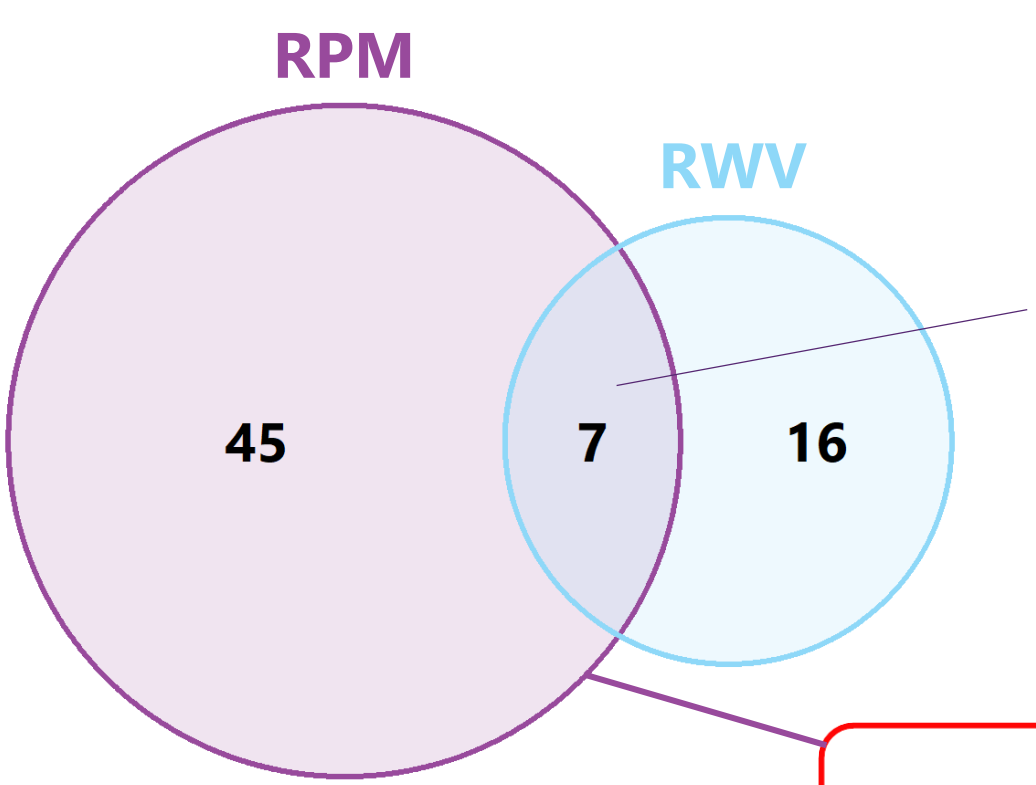
SIMULATED MICROGRAVITY

NITROSOMONAS EUROPAEA



SIMULATED MICROGRAVITY

NITROSOMONAS EUROPAEA



- RuBisCO
- Mercury transport
- Transposases & integrases
- Stress response

amoA2/amoB2
(ammonia monooxygenase)

SIMULATED MICROGRAVITY

NITROSOMONAS EUROPAEA

Localized nutritional deprivation

- Downregulation of central metabolism genes
  - Stress-induced mutagenesis
    - Upregulation of transposases and integrases
    - Observed during nutritional deprivation in other bacterial strains (Reviewed in Foster *et al.*, 2007)
  - Other stress response genes

RuBisCO

amoA2/amoB2 **(ammonia monooxygenase)**

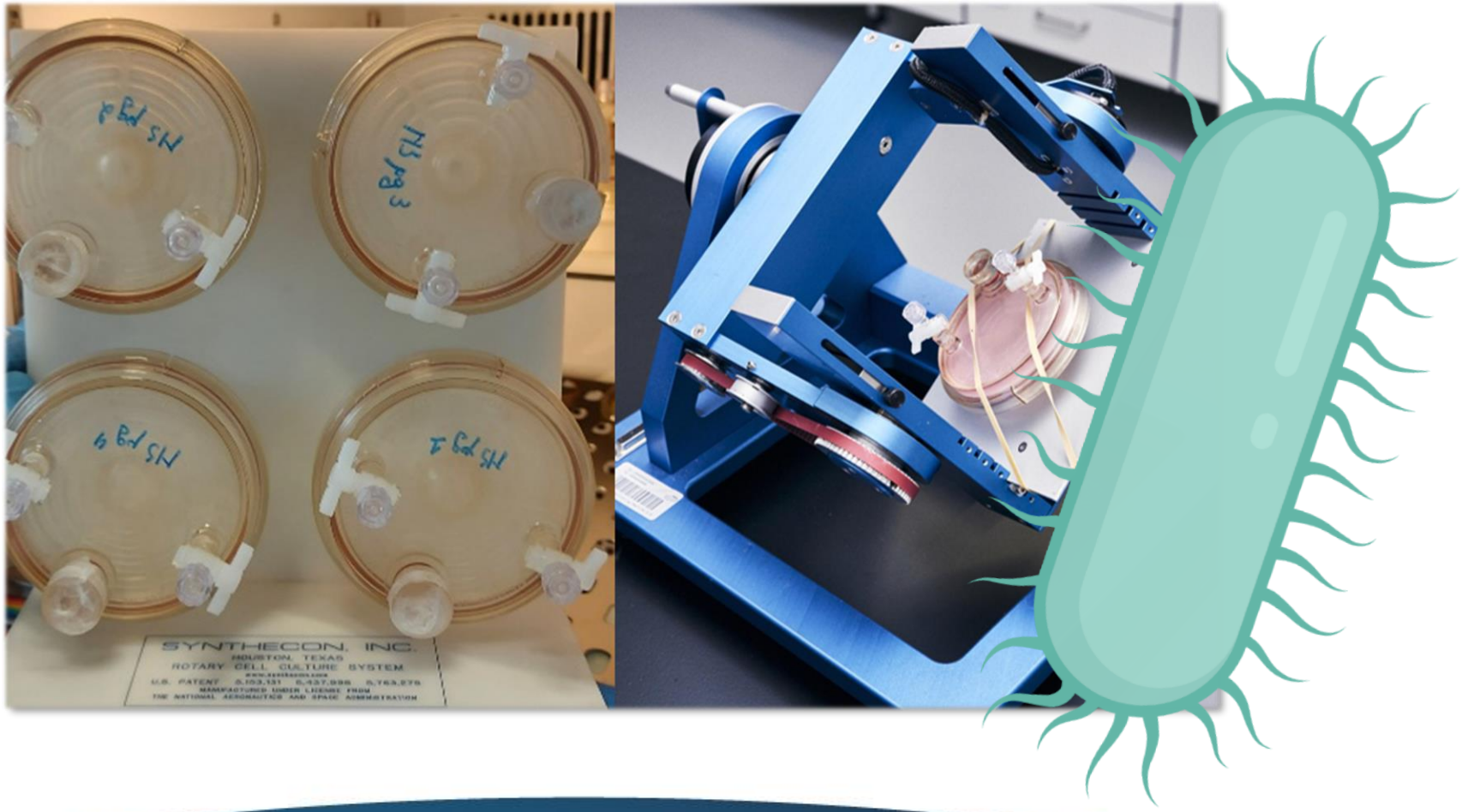
Transposases & integrases

Stress response

Mercury transport

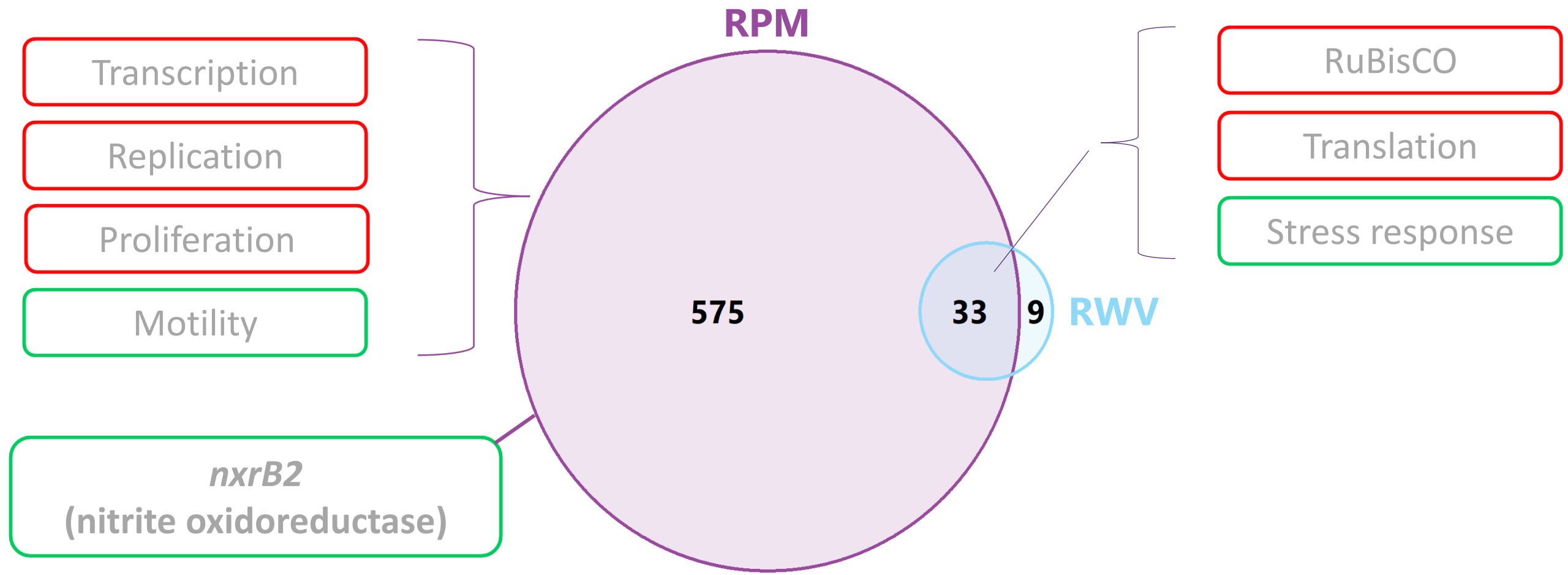
SIMULATED MICROGRAVITY

NITROBACTER WINOGRADSKYI

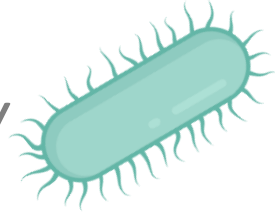


SIMULATED MICROGRAVITY

NITROBACTER WINOGRADSKYI – DIFFERENTIALLY EXPRESSED GENES



SIMULATED MICROGRAVITY



NITROBACTER WINOGRADSKYI

Localized nutritional deprivation

- Inhibition of cell growth and proliferation
- Central carbon metabolism inhibition

- Upregulation of *nxrB2* (nitrite oxidoreductase) → higher need for nitrite oxidation
- Inhibition of electron transport chain proteins
- Upregulation of early stage flagellum assembly proteins

nxrB2
(nitrite oxidoreductase)

RuBisCO

Translation

Transcription

Replication

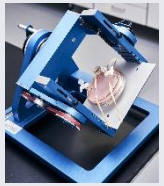
Proliferation

Motility

Stress response

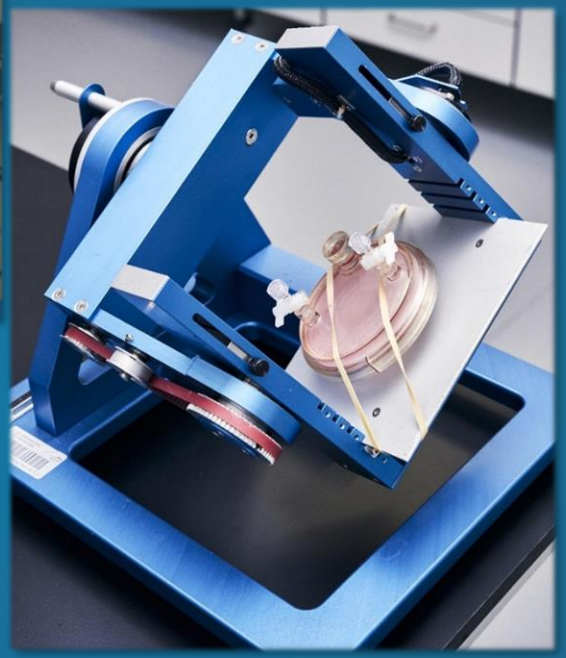
SIMULATED MICROGRAVITY

EFFECT ON NITRIFICATION

		Nitrosomonas europaea	*Nitrobacter winogradskyi*
	Random Positioning Machine (RPM)	- RuBisCO activation genes - *amoA2/amoB2* - Stress-induced mutagenesis	- RuBisCO operon - Cell growth, proliferation - Stress response genes - *nxrB2*
	Rotating Wall Vessel (RWV)	- RuBisCO activity - Stress-induced mutagenesis	- RuBisCO operon - Translation genes - Stress response genes
		Local nutritional deprivation	**Local nutritional deprivation**

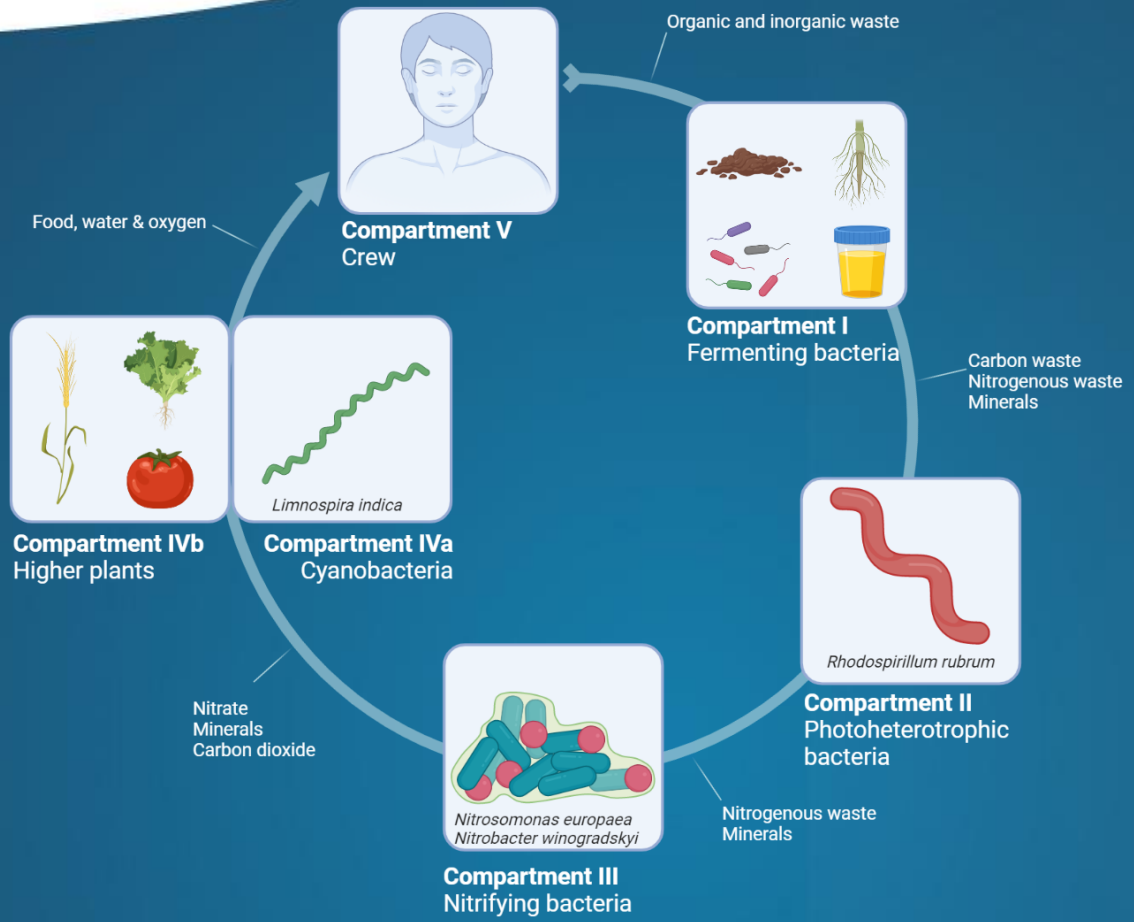
CONCLUSIONS

SIMULATED MICROGRAVITY



- Possible **nutritional deprivation** caused by elimination of fluid dynamics

- ***N. europaea* and *N. winogradskyi*:**
  - Downregulated central metabolism genes
  - Upregulated stress response genes
- Simulated microgravity has an **effect on nitrification**



CONCLUSIONS

CONSEQUENCES FOR MELISSA IN SPACE

1) Ionizing irradiation has no immediate consequence on ureolysis and nitrification activity

2) Simulated microgravity

- Local nutrient deprivation
- Current technology: Fixed-bed bioreactor
  - Effect on biofilm will be investigated in space experiment
  - Research towards improved mixing conditions

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Figures were created with BioRender.com

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MELiSSA
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

MELISSA

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THANK YOU.

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