MELISSA CONFRRENCE
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FULLY VIRTUAL

# Cellulose wastes management by microbial degradation

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# INTRODUCTION

The flight from Earth to Mars take about 520 days.
The crew possibly be consist of 6 cosmonauts.

Each of these are necessary daily of:

- ✓ oxygen (1 kg of liquid),
- ✓ water (1-2 litres),
- ✓ food (2-3 kg).

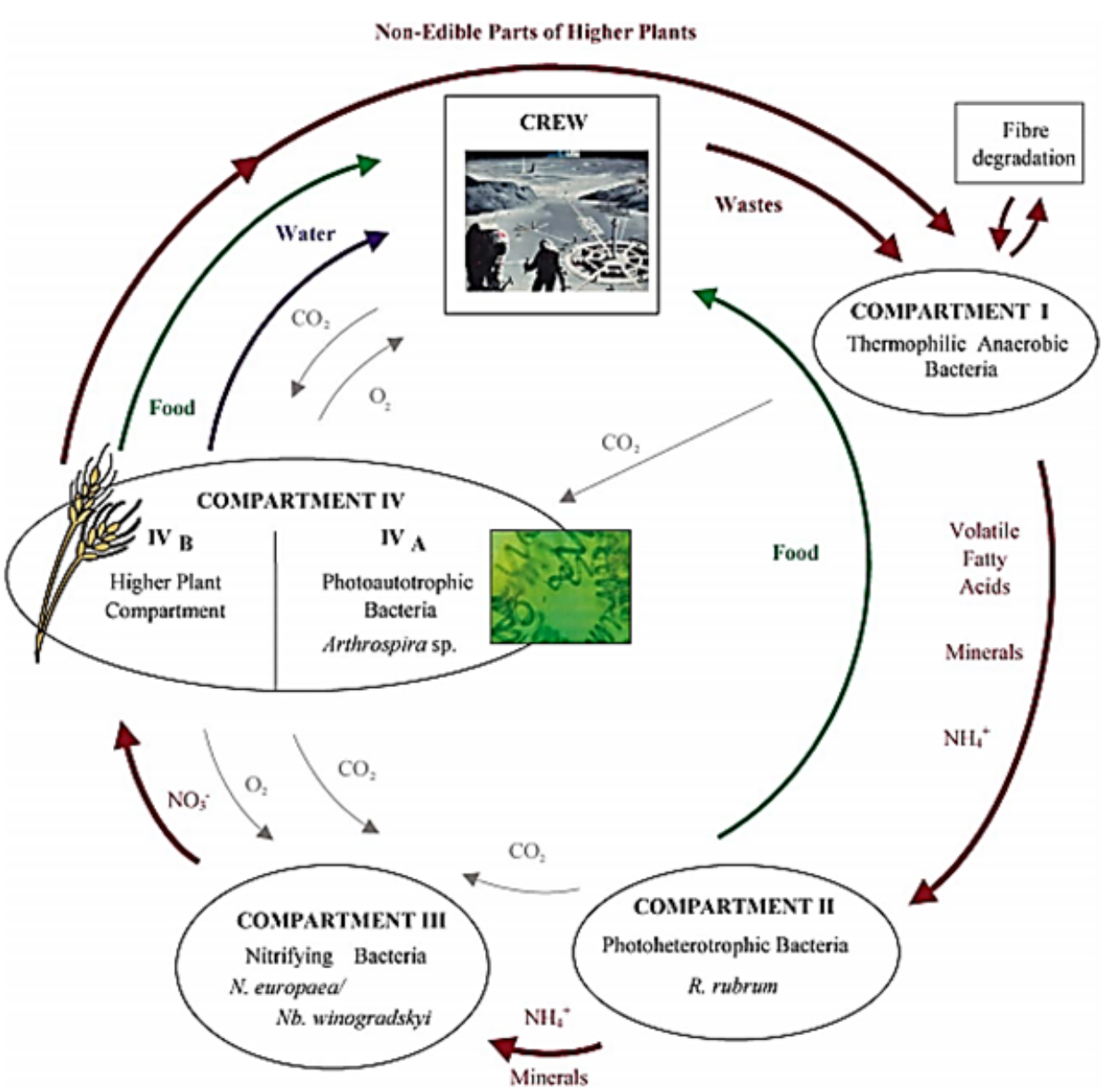
The total weight is about 5 kg/day or 30 kg/day for the entire crew.

For the whole period the cosmonauts will require oxygen, water and food from 15 to 16 t.

In addition, they will need hygienic materials.

All this suggests that the accumulation on board the spaceship to a large amount of organic waste. Waste in space stations of the Earth orbit missions are take out with transportation vessels, which burn in the Earth's atmosphere for long-duration missions such as the Moon orbit missions, Mars orbit missions - the waste is not allowed to throw away in space and have to be utilized. This extremely important task has not yet been determined, and also the creation of a closed ecological systems too. To successfully resolve this problem, they can be used in Lunar and Martian bases, and also to create such bases on Earth, for example, in the Arctic and in the deserts.

# Concept of the MELiSSA loop




Lasseur C., Brunet J., de Weever H., Dixon M., Dussap G., Godia F., Leys N., Mergeay M., Van der Straeten D., MELiSSA: The European Project of Closed Life Support System, Gravitational and Space Biology, 2010, 23, 2, 3-12.

# OBJECTIVES

*Aims of our study were to achieve:*

1. Maximum cellulose biodegradation in laboratory terrestrial and microgravity conditions
2. Useful laboratory model with the potential for implementation in waste management and environmental protection.

# Isolation of cellulolytic bacterial consortia and strains from different natural habitats

1. Methanogenic BR

2. Partly destroyed wood

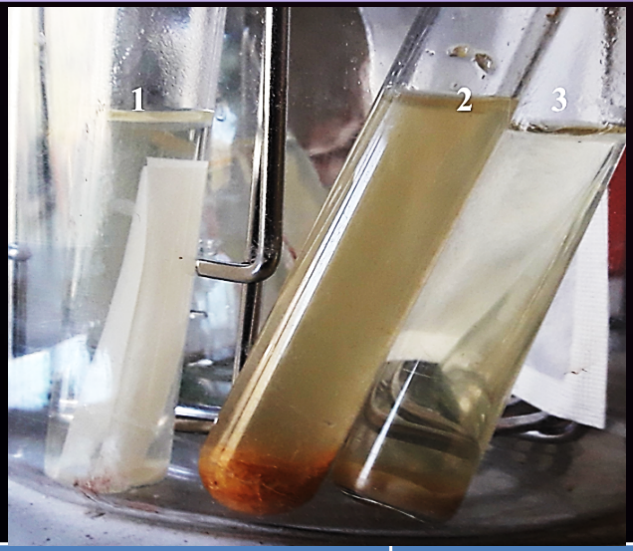
3. Goat feces

# Methods and materials

***Composition of the bacterial nutrient media :***
For *Ruminiclostridium cellulolyticum* (CM3) 520 DSMZ and for *Hungateiclostridium thermocellum* 122 DSMZ - cellobiosis was replaced by pre-cut Whatman filter paper or sterile medical gauze in 10 mg/ml.
Carboxymethylcellulose (CMC) agar; peptone cellulose solution (PCS) with 1% pretreated rye straw and Watman filter paper; soya-casein agar and Mueller Hinton agar.

***Growth conditions:***
**Aerobic cultivation** of bacteria in a thermostat at 37 °C.
**Microaerophilic cultivation** of bacteria **-** microaerophilic conditions created by candle jar in a thermostat at 37 °C.
**Anaerobic cultivation** of bacteria **-** anaerobic conditions in jars by gas-generating GasPakTM EZ bags for anaerobic container system (Becton Dickinson, 260678).

# Determining the degree of filter paper degradation by anaerobic mesophilic bacteria




***Legend:***

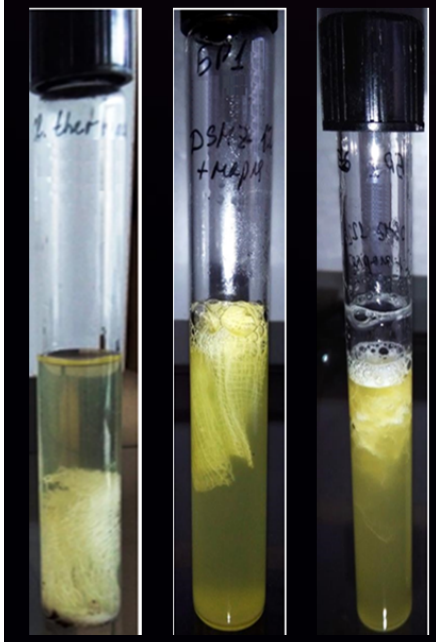
**1 – control (sterile nutrient medium loaded with filter paper**
**2 – glass tube, with visibly degraded cellulose inoculated with an anaerobic microbial population from BR2.**
**3 – glass tube, with visibly degraded cellulose inoculated with an anaerobic microbial population from BR1.**

| Parameters | Intervals for residual cellulose determination (days) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 7 | 14 | 17 | 22 | 56 |
| Amount of residual cellulose (mg/10 ml ± SD) | 90.2 ± 1.1 | 54.3 ± 0.6 | 35.2 ± 0.9 | 28.2 ± 1.1 | 23.2 ± 2.6 | 21.7 ± 3.9 |

# Comparative study of medical gauze biodegradation between the mesophilic and thermophilic bacteria

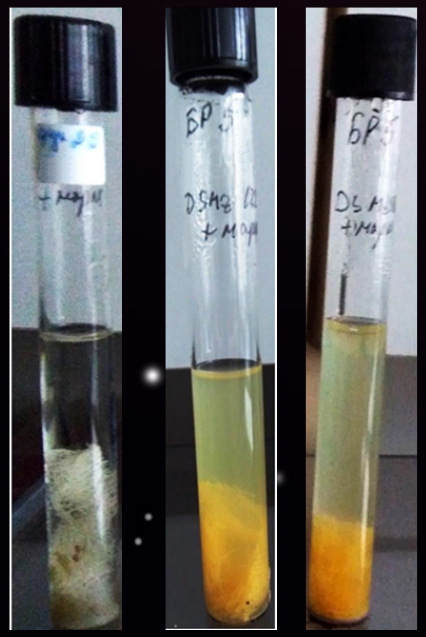
**Mesophilic bacteria**

Day 1 | Day 14 | Day 20



**Thermophilic bacteria**

Day 1 | Day 14 | Day 20



The analysis showed that on the 14th day at 55 °C the cellulose biodegradation proceeds faster than the tubes cultured at 37 °C. A change in the color of the medical gauze was observed. After vortexing of the glass tubes for 15 s the decomposed medical gauze was not visualized.

The formation of gas bubbles on the surface of the glass tubes was also observed.

| Temperature | Day 1 | Day 14 | Day 20 |
|---|---|---|---|
| 37 ºC | 95.9 mg/10 ml | 41.48 mg/10 ml | 41.10 mg/10 ml |
| 55 ºC | 89.7 mg/10 ml | 64.8 mg/10 ml | 63.5 mg/10 ml |

# Aerobic bacterial biodegradation of various cellulose-rich substrates

| Days | Substrate | Partially decomposed wood | Goat feces | Ruminal contents of calf |
|---|---|---|---|---|
| Day 6 | Toilet paper | √√√ | × | × |
| | Wet wipes | × | × | × |
| | Filter paper | × | × | × |
| Day 9 | Toilet paper | √√√ | √ | × |
| | Wet wipes | × | × | × |
| | Filter paper | √ | × | × |
| Day 27 | Toilet paper | √√√ | √ | × |
| | Wet wipes | × | × | × |
| | Filter paper | √ | × | × |

***Legend:*** √√√ - full decomposed; √ - partly decomposed and × - undegradable

# Microaerophilic bacterial biodegradation of various cellulose-rich substrates

| Days | Substrate | Partially decomposed wood | Goat feces | Ruminal content of calf |
|---|---|---|---|---|
| Day 6 | Toilet paper | √√√ | √ | × |
| | Wet wipes | × | × | × |
| | Filter paper | × | × | × |
| Day 19 | Toilet paper | √√√ | √ | × |
| | Wet wipes | × | × | × |
| | Filter paper | √√√ | × | × |
| Day 32 | Toilet paper | √√√ | √ | × |
| | Wet wipes | × | × | × |
| | Filter paper | √√√ | × | × |
| Day 36 | Toilet paper | √√√ | √ | × |
| | Wet wipes | × | × | × |
| | Filter paper | √√√ | × | × |

***Legend:***

√√√ - full decomposed;

√ - partly decomposed;

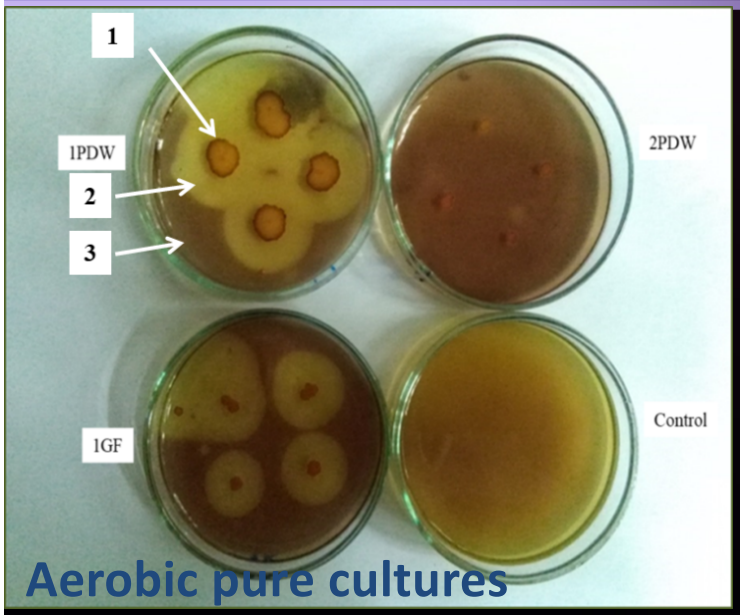
× - undegradable

# Anaerobic bacterial biodegradation of various cellulose-rich substrates

| Days | Substrate | Partially decomposed wood | Goat feces |
|---|---|---|---|
| **Day** 15 | Toilet paper | √ | √ |
| | Wet wipes | × | × |
| | Filter paper | × | × |
| **Day** 17 | Toilet paper | √ | √√√ |
| | Wet wipes | × | × |
| | Filter paper | × | × |

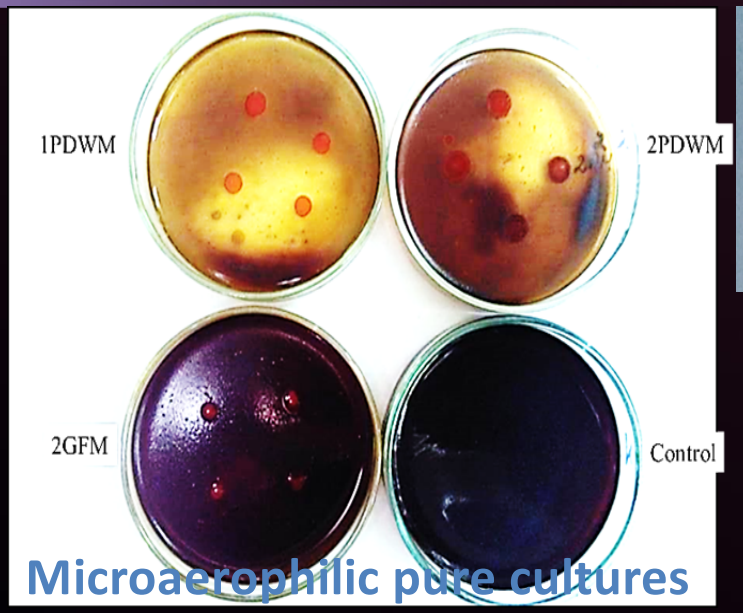
***Legend:*** √√√ - full decomposed; √ - partly decomposed and × - undegradable

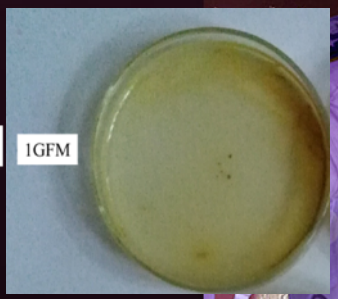
# Screening for cellulolytic activity of isolated single colonies from bacterial populations




## Aerobic pure cultures

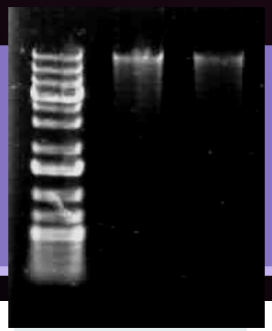
1 PDW – colony 1 isolated from partly destroyed wood, positive result
2 PDW – colony 2 isolated from partly destroyed wood, negative result
1 GF – colony 1 isolated from goat faces, positive result
Control – without bacterial strain




## Microaerophilic pure cultures

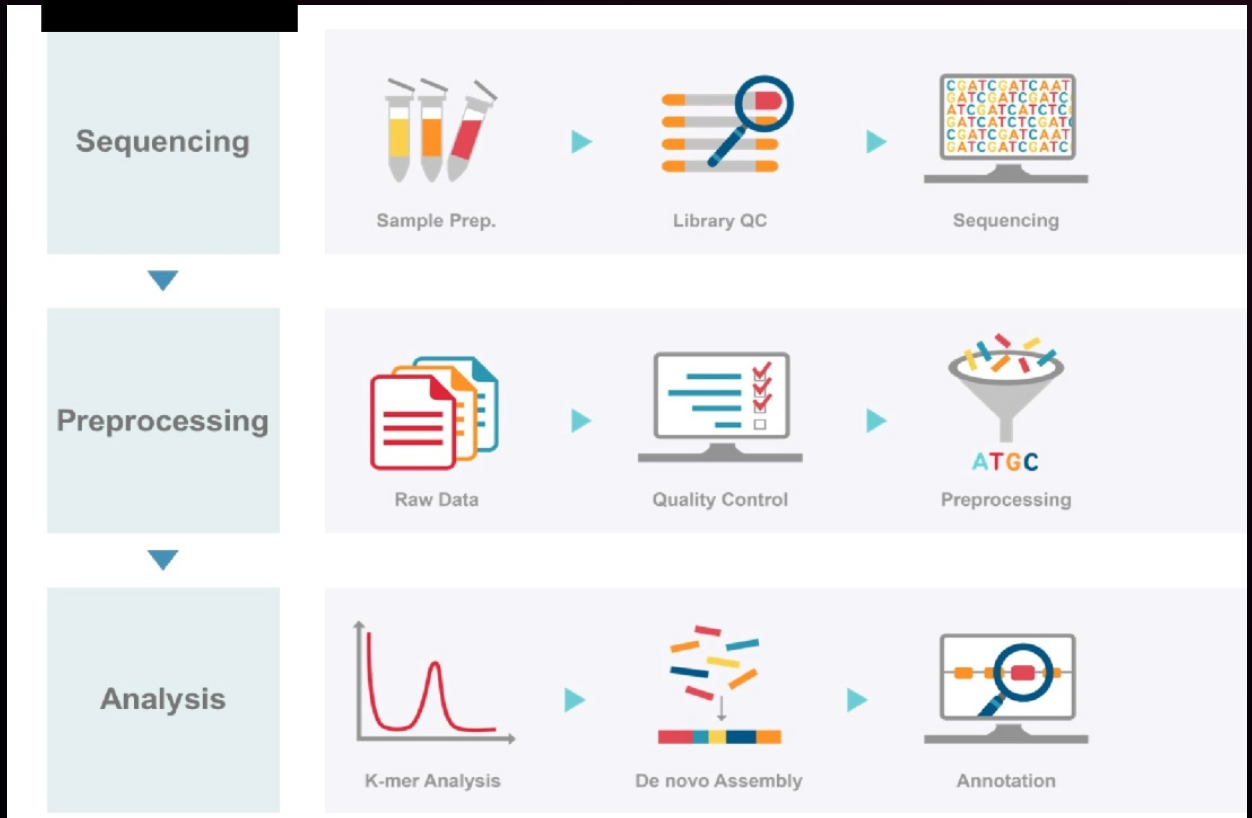
1 PDWM – colony 1 isolated from partly destroyed wood at microaerophilic conditions, positive result; 2 PDWM – colony 2 isolated from partly destroyed wood at microaerophilic conditions, positive result; 1 GFM – colony 1 isolated from goat feces, at microaerophilic conditions, positive result; 2 GFM – colony 2 isolated from goat feces at microaerophilic conditions, negative result; Control – without bacterial strain.



## Isolation of total DNA

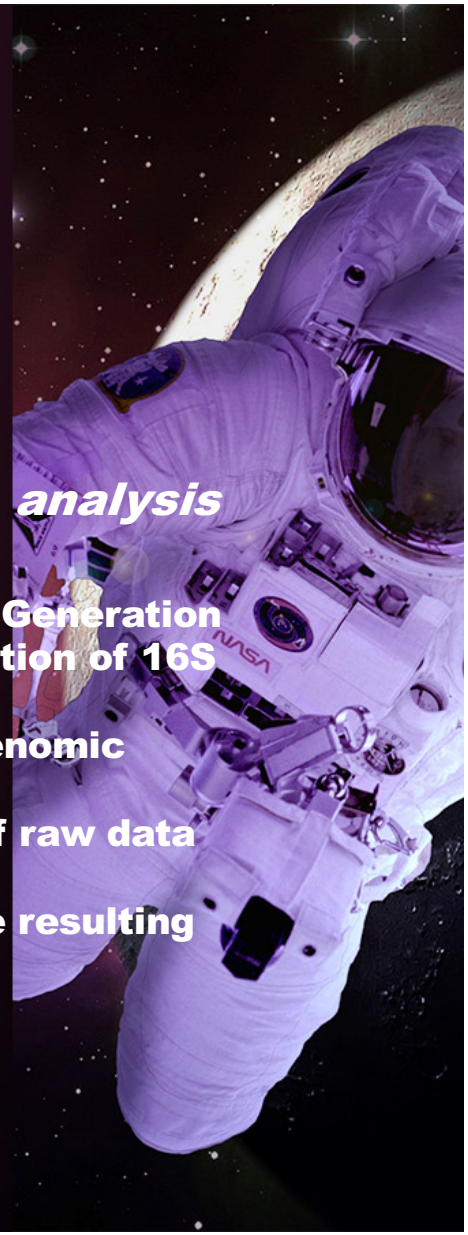
DNA - 1µg

λ260/λ280 = 1.71

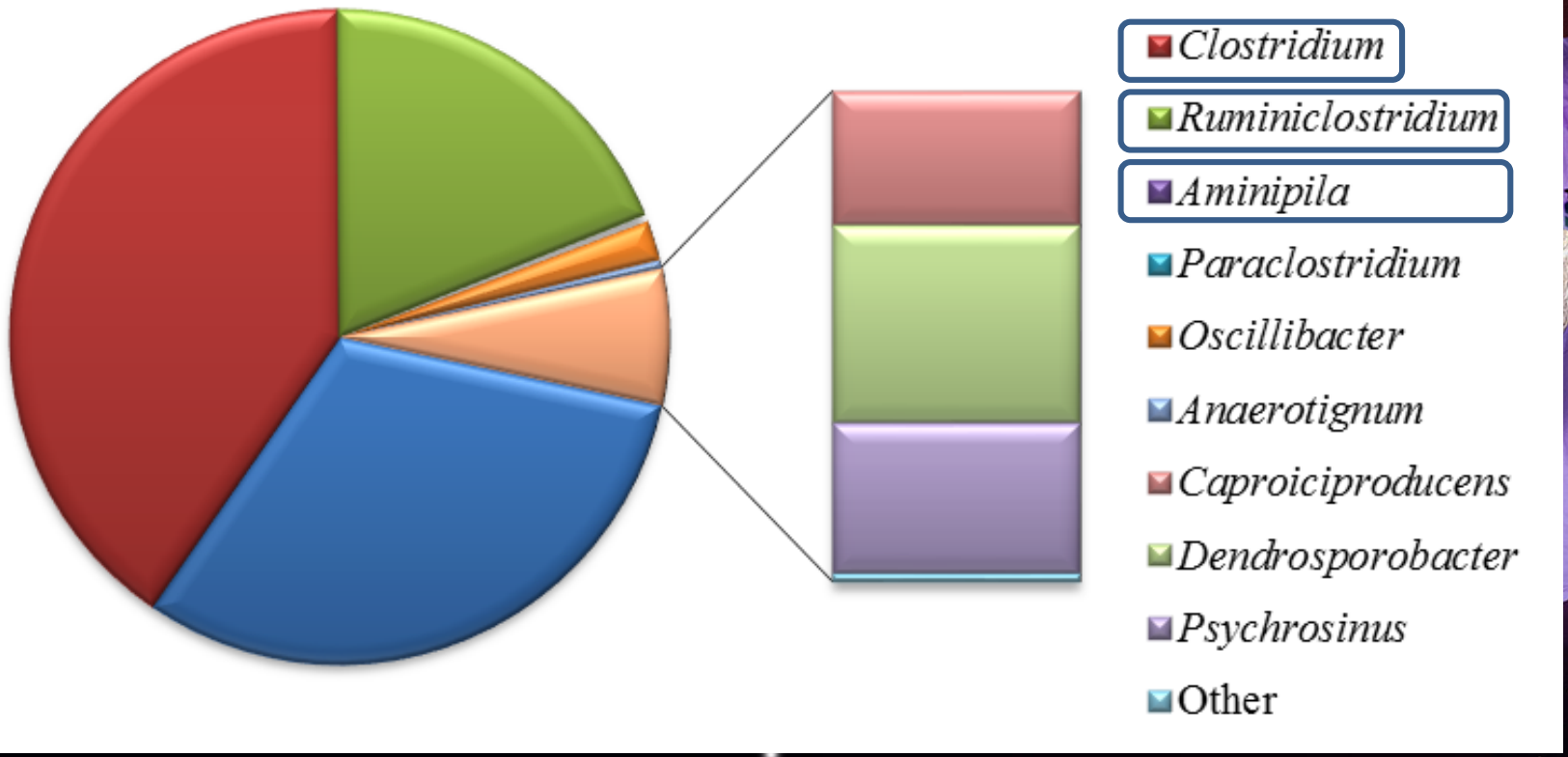



## *Stages of DNA analysis*

1. Genomic Library Generation by PCR Amplification of 16S rDNA
2. Sequencing of genomic library
3. Quality control of raw data
4. Assembling
5. Annotation of the resulting sequences



# Genus composition of mesophilic bacterial population isolated from bioreactor by metagenomic analysis

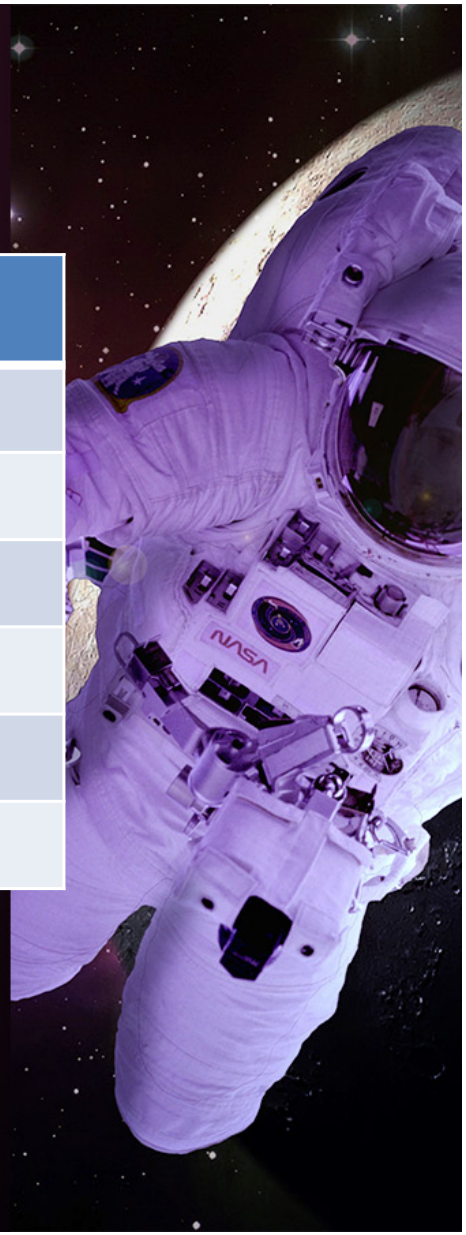



# 16S rDNA identification of isolated single colonies

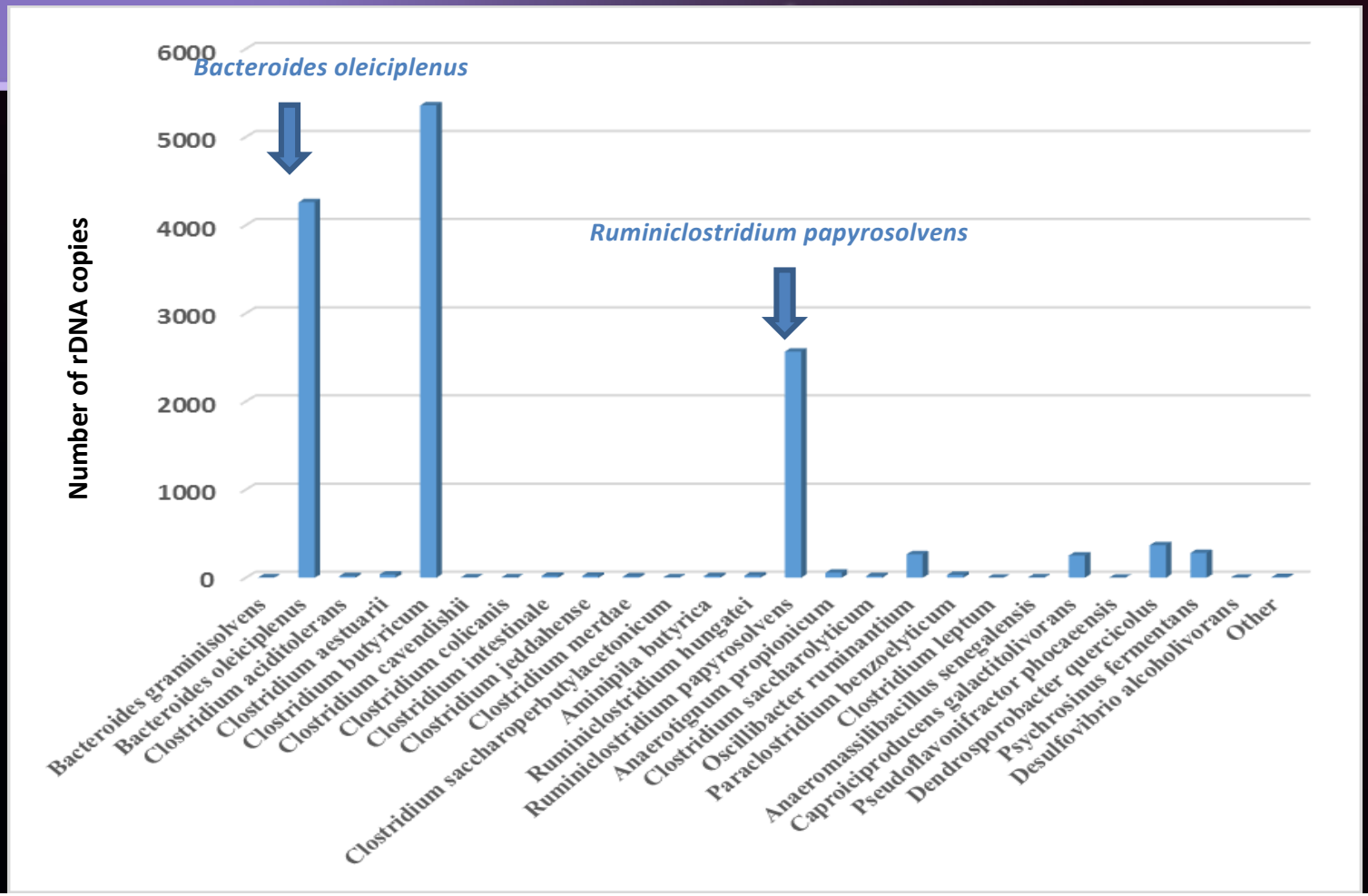
| Isolate | Species | Homology (%) |
|---|---|---|
| 1PDWM | *Brevibacillus laterosporus* | 99.46 |
| 1PDW | *Bacillus cereus* | 100 |
| 1GF | *Pseudomonas stutzeri* | 99.90 |
| 1GFM | *Bacillus thermoamylovorans* | 99.10 |
| 2PDW | *Lysinibacillus macrolides* | 100 |
| 2PDWM | *Bacillus velezensis* | 100 |

***Legend:***

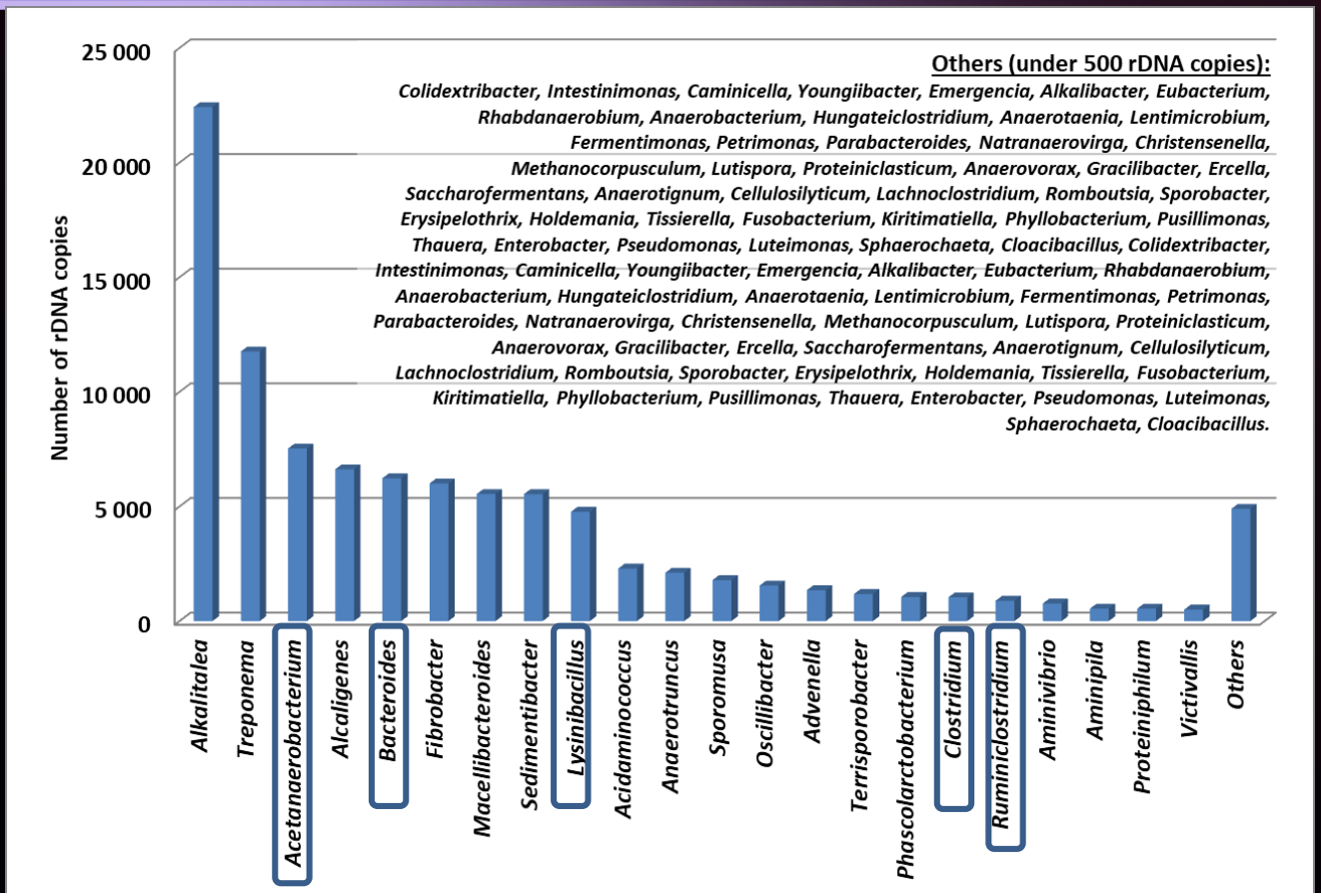
1PDWM – colony 1 isolated from partly destroyed wood, microaerophiles
1PDW – colony 1 isolated from partly destroyed wood, aerophiles
1GF – colony 1 isolated from goat faces, aerophiles
1GFM – colony 1 isolated from goat faces, microaerophiles
2PDW–colony 2, isolated from partly destroyed wood, aerophiles
2PDWM – colony 2, isolated from partly destroyed wood, microaerophiles



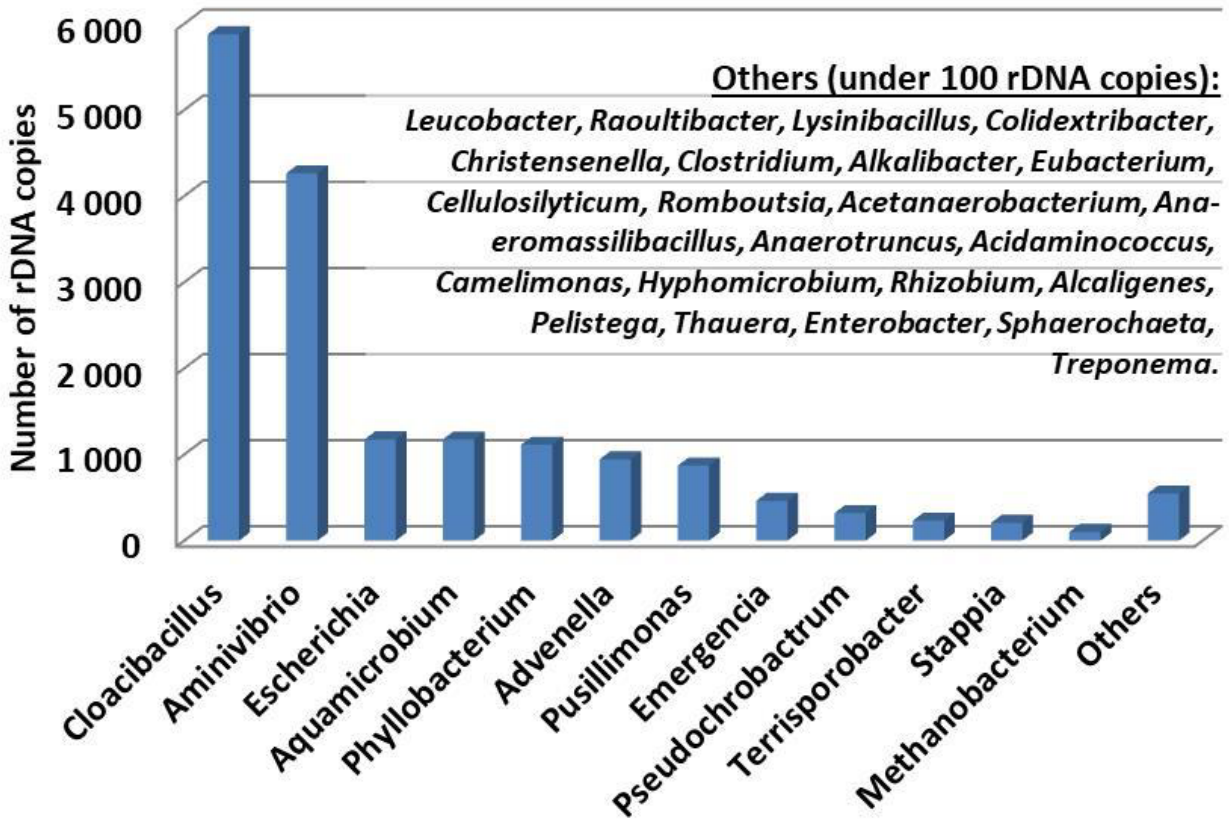
# Species composition of mesophilic bacterial population isolated from bioreactor by metagenomic analysis




# Genus composition of aerobic bacterial population isolated from goat faeces by metagenomic analysis

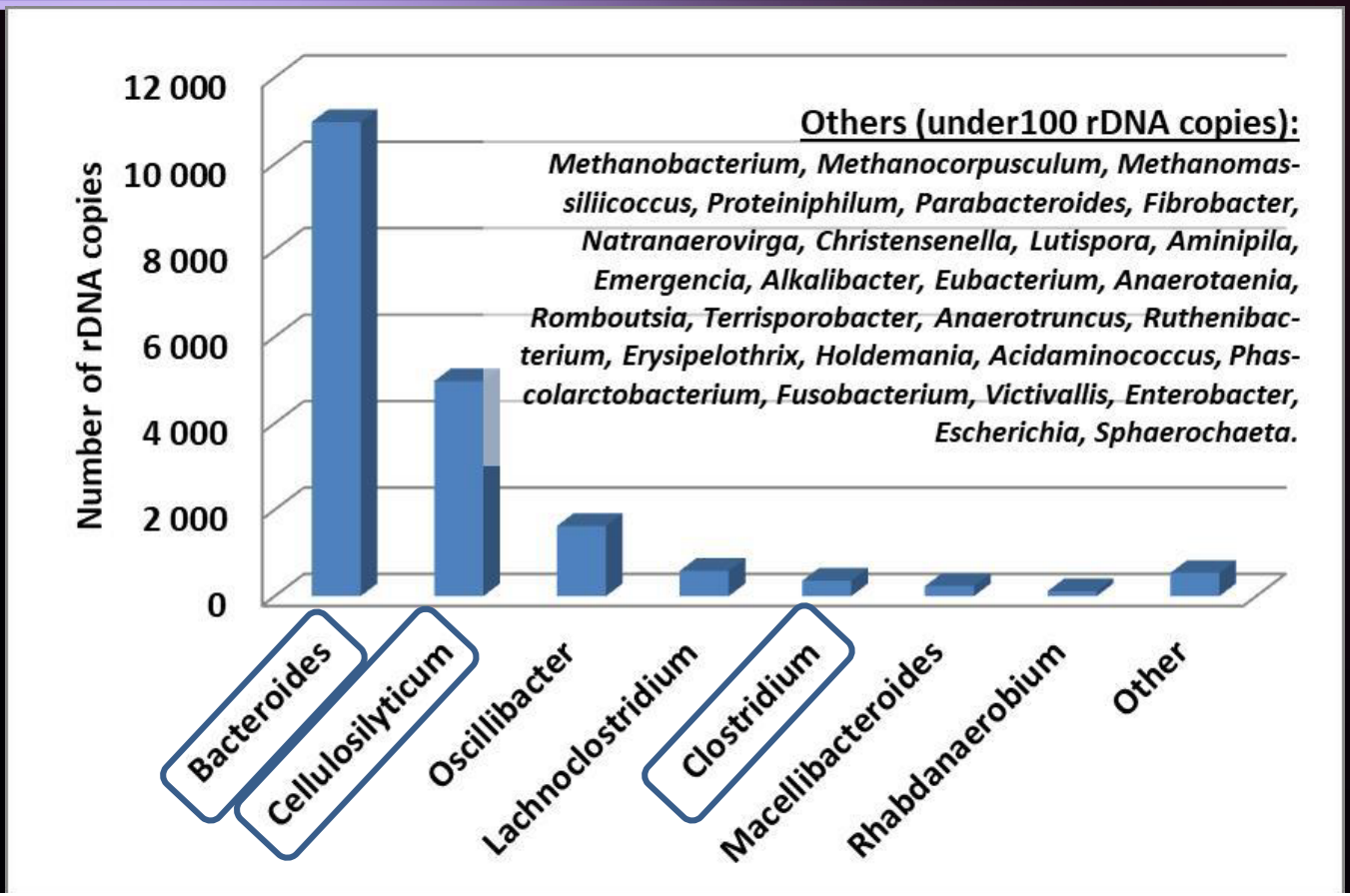



# Genus composition of microaerophilic bacterial population isolated from goat faeces by metagenomic analysis



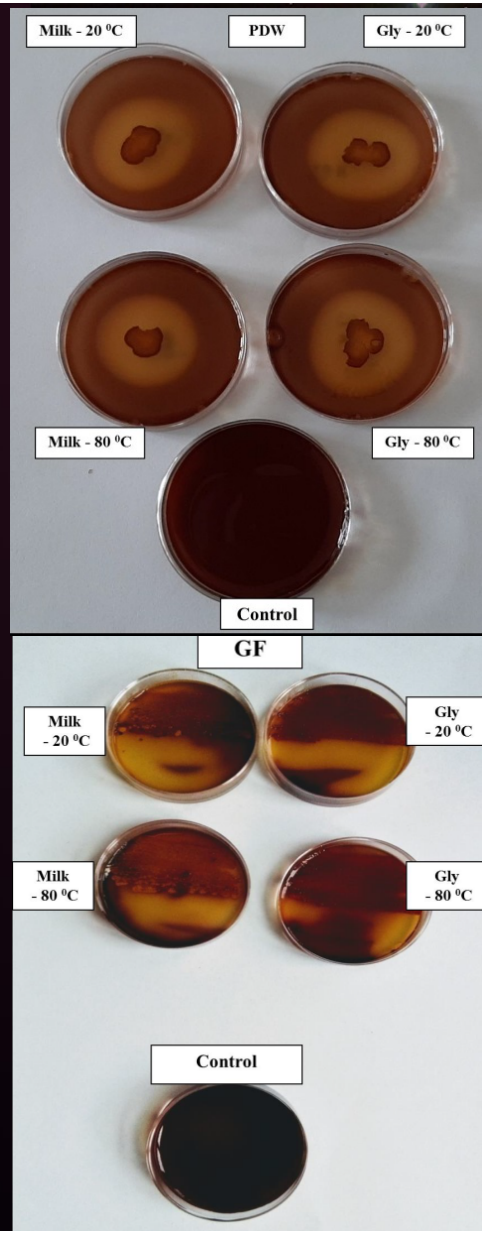

**The microaerophilic population isolated from goat feces is characterized with many pathogenic genera such as Escherichia. Therefore, this sample is not of interest for further experimental work related with long-therm space missions.**

# Genus composition of anaerobic bacterial population isolated from goat faeces by metagenomic analysis



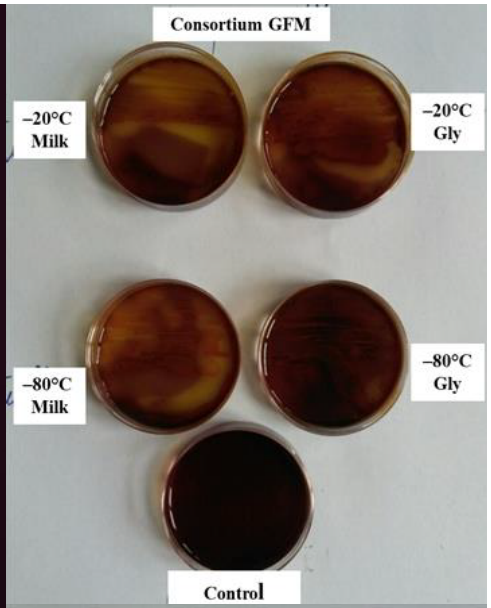

# Storage of aerobic microbial population and individual strains with cellulolytic activity (Day 30 and Day 90)

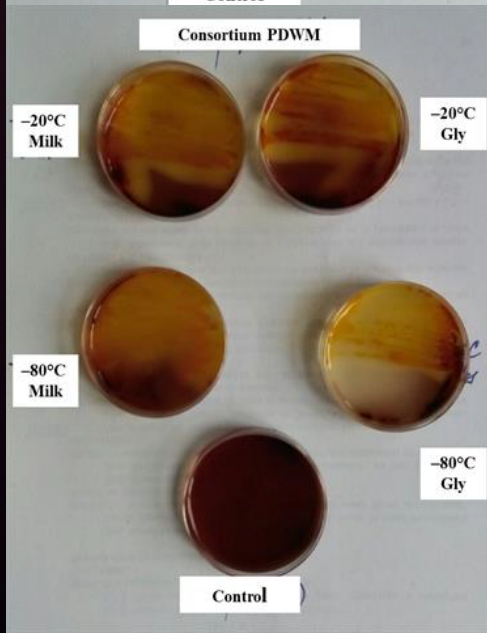
| Days | Aerobic cryocultures | Temperature / cryoprotectant | | | |
|---|---|---|---|---|---|
| | | –20°C milk | –20°C glycerol | -80°C milk | -80°C glycerol |
| Day 30 | Consortium GF | √√√ | √√√ | √√√ | √√√ |
| | Consortium PDW | √√√ | √√√ | √√√ | √√√ |
| | 1GF | √√√ | √√√ | √√√ | √√√ |
| | 1PDW | √√√ | √√√ | √√√ | √√√ |
| | 2PDW | √√√ | √√√ | √√√ | √√√ |
| Day 90 | Consortium GF | √√√ | √√√ | √√√ | √√√ |
| | Consortium PDW | √√√ | √√√ | √√√ | √√√ |
| | 1GF | √√√ | √√√ | √√√ | √√√ |
| | 1PDW | √√√ | √√√ | √√√ | √√√ |
| | 2PDW | √√√ | √√√ | √√√ | √√√ |



# Storage of microaerophilic microbial population and individual strains with cellulolytic activity (Day 30 and Day 90)

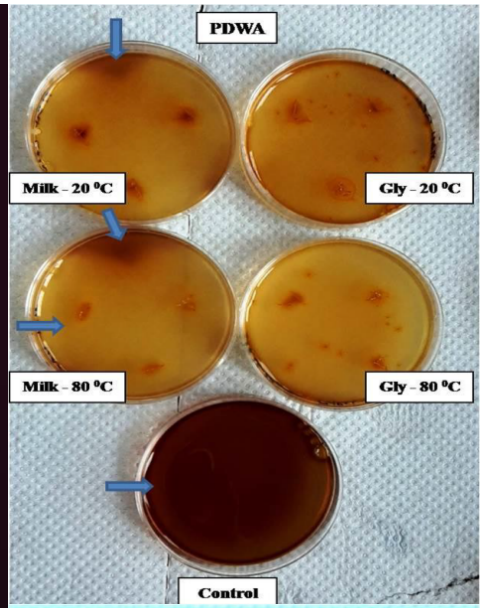
| Days | Microaerophilic cryocultures | Temperature / cryoprotectant | | | |
|---|---|---|---|---|---|
| | | –20°C milk | –20°C glycerol | -80°C milk | -80°C glycerol |
| Day 30 | Consortium GFM | √√√ | √√√ | √√√ | √√√ |
| | ConsortiumPDWM | √√√ | √√√ | √√√ | √√√ |
| | 1GFM | √√√ | √√√ | √√√ | √√√ |
| | 1PDWM | √√√ | √√√ | √√√ | √√√ |
| | 2PDWM | √√√ | √√√ | √√√ | √√√ |
| Day 90 | Consortium GFM | √√√ | √√√ | √√√ | √√√ |
| | ConsortiumPDWM | √√√ | √√√ | √√√ | √√√ |
| | 1GFM | √√√ | √√√ | √√√ | √√√ |
| | 1PDWM | √√√ | √√√ | √√√ | √√√ |
| | 2PDWM | √√√ | √√√ | √√√ | √√√ |

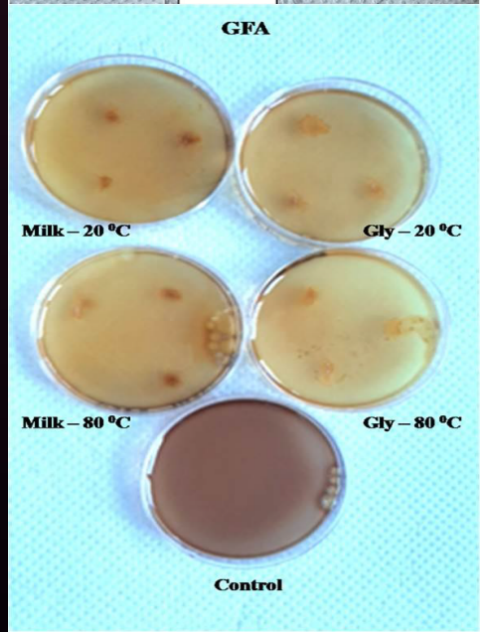


# Storage of anaerobic microbial population with cellulolytic activity (Day 30 and Day 90)

| Days | Anaerobic cryocultures | Temperature / cryoprotectant | | | |
|---|---|---|---|---|---|
| | | –20°C milk | –20°C glycerol | -80°C milk | -80°C glycerol |
| Day 30 | Consortium GFM | √√√ | √√√ | √√√ | √√√ |
| | ConsortiumPDWM | √√√ | √√√ | √√√ | √√√ |
| Day 90 | Consortium GFM | √√√ | √√√ | √√√ | √√√ |
| | ConsortiumPDWM | √√√ | √√√ | √√√ | √√√ |



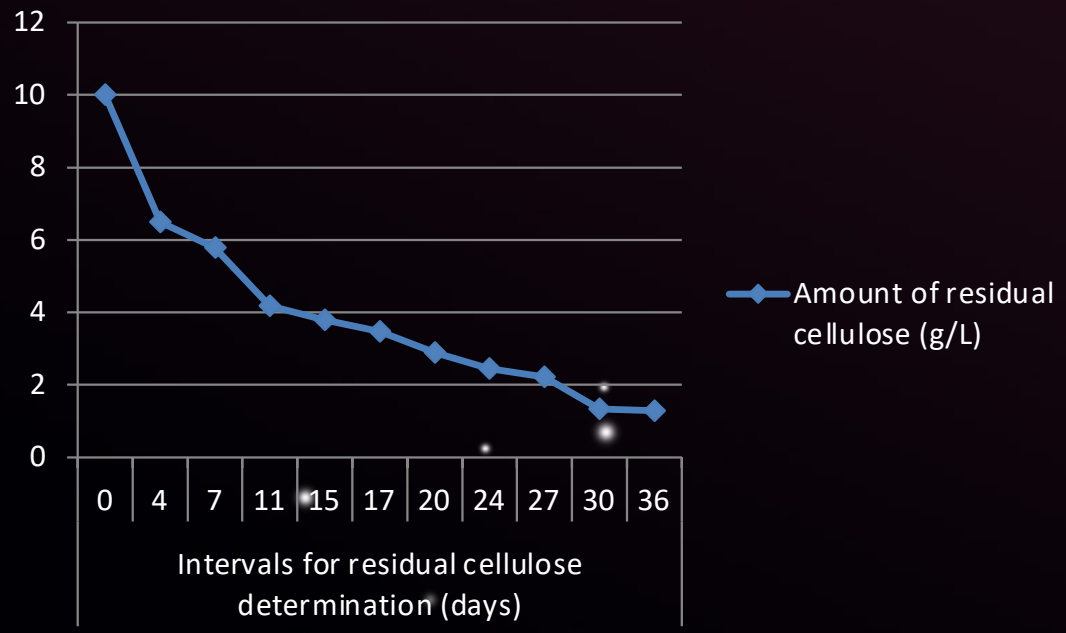
# Laboratory model (2 L working volume of bioreactor) of microaerophilic biodegradation




On the 5th day, the 35% of cellulose biodegradation was observed. On the 14th day of the experiment the cellulose was biodecomposed with about 41% and at on the 20th day - about 67%.

| Parameters | Intervals for residual cellulose determination (days) | | |
|---|---|---|---|
| | 5 | 14 | 20 |
| Amount of residual cellulose (g/L) | 6.48 | 11.88 | 3.33 |

# Laboratory model (4 L working volume of bioreactor) of anaerobic biodegradation




The results show that on the 7th day of the experiment the cellulose was biodecomposed with about 31,35% and about 54,8% on the 15th day. At the end of experiment (36th day) the cellulose was decomposed up to 84,6%.

# Parallel experiment with RPM and 2D clinostat placed in a 37 °C incubator (experiment of microgravity simulation)

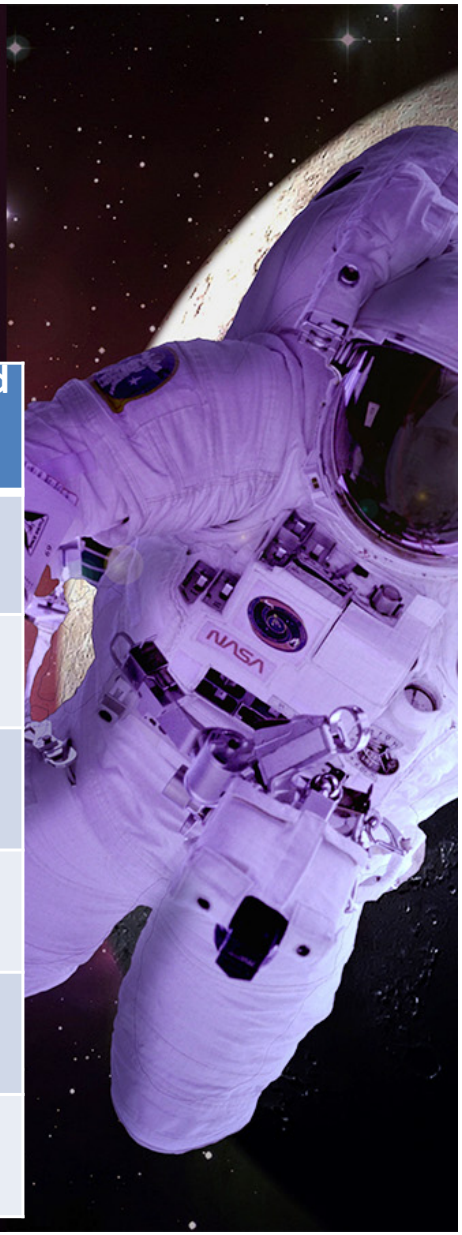
Magnitude and direction of the vibratory accelerations vary from 10-5 g to 10-3 g (from tens of µg to several mg, RMS), and 0,0033s<T<100s.

With a Random Positioning Machine (RPM) and a modified 2D Clinostat we simulated the vibratory accelerations aboard a spacecraft (International Space Station) due to combined effects of equipment, crews and spacecraft:

- pumps, fans, centrifuges, compressors, etc.;
- crews' movement (ergometer, traditional exercises);
- spacecraft structural modes.

# Residual cellulose after 21 days of incubation of samples loaded with different inoculum and gravity regimes

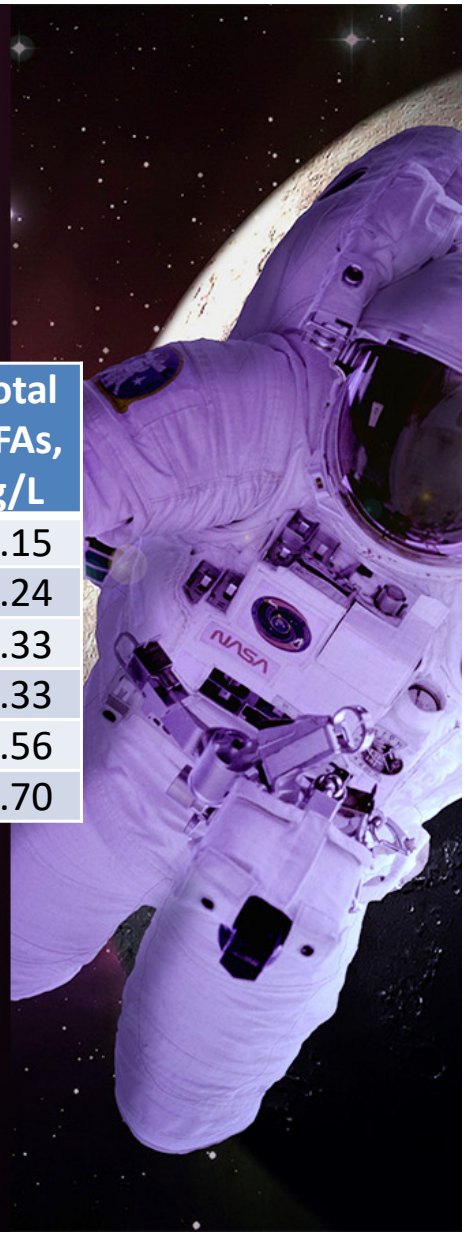
| Samples | Residual cellulose (mg/ml) | Decomposed cellulose (%) |
|---|---|---|
| ***Tube 1*** **(inoculum of stabilized consortium isolated from goat feces, under the Earth's gravity conditions)** | 3,914 | 60,86 |
| ***Tube 2*** **(inoculum of stabilized consortium isolated from goat feces, under simulated microgravity by RPM)** | in progress | in progress |
| ***Tube 3*** **(inoculum of stabilized consortium isolated from goat feces, under simulated microgravity by a clinostat)** | 3,363 | 66,37 |
| ***Tube 4*** **(inoculum of stabilized consortium isolated from partially decomposed wood, under the Earth's gravity conditions)** | 4,790 | 52,10 |
| ***Tube 5*** **(inoculum of stabilized consortium isolated from partially decomposed wood, under simulated microgravity by RPM)** | in progress | in progress |
| ***Tube 6*** **(inoculum of stabilized consortium isolated from partially decomposed wood, under simulated microgravity by a clinostat)** | 3,899 | 61,01 |



# Determination of volatile fatty acids (VFA)

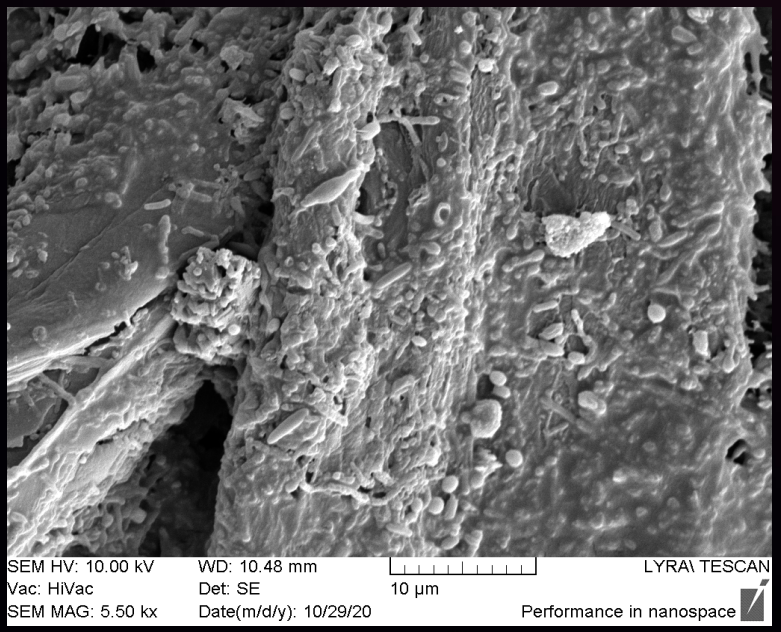
| Conditions | Cultivation, days | VFAs-component, g/L | | | | | | | Total VFAs, g/L |
|---|---|---|---|---|---|---|---|---|---|
| | | Ac | Prop | i-But | But | i-Val | Val | Cap | |
| Earth gravity | 18 | 1.84 | 0.34 | 0.21 | 0.30 | 0.29 | 0.09 | 0.08 | 3.15 |
| 2D clinostat microgravity | 18 | 1.81 | 0.37 | 0.21 | 0.41 | 0.27 | 0.09 | 0.08 | 3.24 |
| RPM microgravity | 18 | 1.87 | 0.38 | 0.21 | 0.42 | 0.28 | 0.09 | 0.08 | 3.33 |
| Earth gravity | 25 | 1.98 | 0.37 | 0.22 | 0.28 | 0.31 | 0.09 | 0.08 | 3.33 |
| 2D clinostat microgravity | 25 | 2.09 | 0.40 | 0.22 | 0.39 | 0.29 | 0.09 | 0.08 | 3.56 |
| RPM microgravity | 25 | 2.17 | 0.41 | 0.22 | 0.42 | 0.30 | 0.10 | 0.08 | 3.70 |

***Legend:*** Ac - Acetate, Prop – Propionate, i-But – iso-Butyrate, But – Butyrate, i-Val – iso-Valerate, Val - Valerate, Cap – Caproate



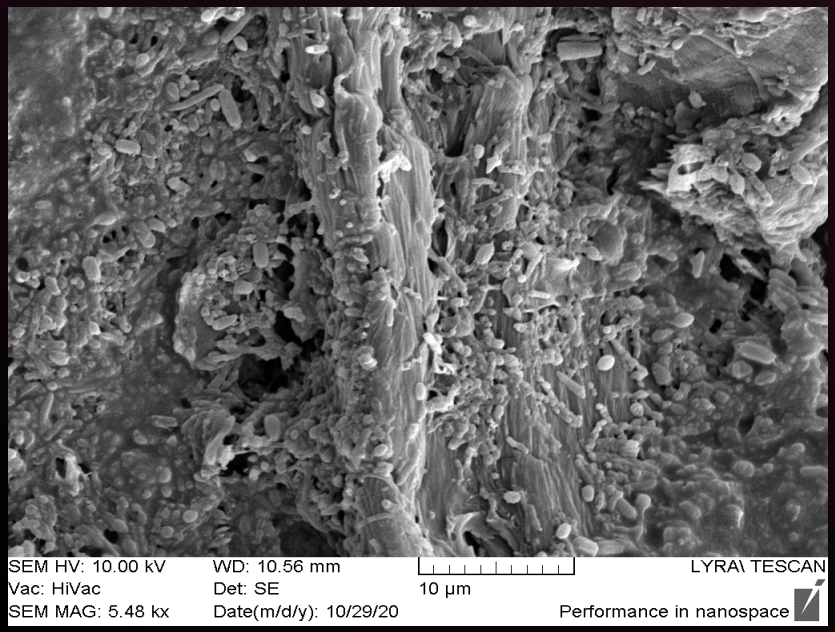
# Comparison of cellulose biodegradation between terrestrial and microgravity conditions by aerobic mesophilic bacterial consortia isolated from goat faeces

## Static conditions




SEM. MAG. 5.50 kx

## Microgravity conditions




SEM. MAG. 5.48 kx

# Dissemination of results

The President of the Republic of Bulgaria Mr. Rumen Radev visited IMIKB on September 29, 2020. Prof. Hr. Naidenski presented the project "Technology model for microbial degradation of cellulose containing wastes in life support system for manned space flights" and the cooperation of IMICB with ESA. The President was impressed and interested in the main achievements, as a former pilot of the Bulgarian Air Force.

# CONTRIBUTORS

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Thanks for your attention!!!