Executive Summary Report – Bacterma Project

BACTeRMA

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Figures and Tables

1 Introduction

Microbial growth on textiles poses challenges for space exploration on the Moon and future long-duration flights to Mars. Traditional laundry methods are impractical in space habitats, and storing space suits increases the risk of microbial growth. The BACTeRMA project, led by the Austrian Space Forum and cooperated with Terram Sequitur Vienna Textile Lab, aimed to develop biocidal textile processing techniques using bacterial metabolites. These substances, such as antimicrobial peptides, can prevent microbial contamination on space suits. The project aimed to identify factors that reduce microbial strains on textiles and test dying strategies based on bacterial metabolites as potential antimicrobial substances. By adapting and testing these techniques, the project aimed to provide suitable solutions for the inner layers of astronauts' suits during lunar and Mars exploration.

Microbial growth on space suits can lead to foul odors, health hazards, and damage to materials. Traditional silver or copper coatings are not suitable for space textiles due to health and environmental risks. The BACTeRMA project focused to overcome these challenges by utilizing bacterial metabolites. By treating space suit textiles with antimicrobial agents derived from these metabolites, to prevent microbial contamination and ensure the cleanliness and functionality of the suits. The project's research consortium focused to develop innovative techniques that can be applied to spaceflight textiles, particularly for the inner layers of astronauts' suits, ensuring the safety and comfort of future space missions.

After testing several potential future lunar space suit textile candidates on their suitability to serve as potential materials for a future lunar EVA (Extravehicular Activities) space suit, the BACTeRMA project aims to identify (novel) techniques that help to reduce microbial growth on the textile's surface that may pose significant risks.

Figure 1 A NASA spacesuit on the ISS stored in the Quest airlock. Image Credit NASA

2 References

2.1 Applicable Documents

AD	Name
AD1	ECSS-Q-ST-00-01, ECSS System, Classifying of terms
AD2	ECSS-M-ST-10C.Rev.1, Space Project Management, Project Planning and Implementation
AD3	ECSS-M-ST-80C, Space Project Management, Risk management
AD4	ECSS-E-ST-10-03C, Space Engineering, Testing
AD5	ECSS-E-ST-32-08C Rev.1, Space Engineering, Material
AD6	ECSS-E-ST-34C, Space Engineering, Environmental control and life support (ECLS)
AD7	ECSS-Q-ST-70-06C, Space Product Assurance, Particle and UV radiation testing for space materials
AD8	ECSS-Q-ST-70-21C, Space Product Assurance, Flammability testing for the screening of space materials
AD9	ECSS-Q-ST-70-29C, Space Product Assurance, Determination of offgassing products from materials and assembled articles to be used in a manned space vehicle crew compartment
AD10	AATCC147: Assessment of Textile Materials: Parallel Streak Method
AD11	DIN EN ISO3071: Textiles - Determination of pH of aqueous extract (ISO 3071:2020); German version EN ISO 3071:2020
AD12	DIN EN ISO 105E04: *Textiles — Tests for colour fastness — Part A04: Method for the instrumental assessment of the degree of staining of adjacent fabrics*
AD13	DIN EN ISO 10993-5: Biological evaluation of medical devices - Part 5: Tests for cytotoxicity: in vitro-methods (ISO 10993-5:1999); German version EN ISO 10993-5:1999
AD14	DIN ISO 20743, Textiles- Determination of antibacterial activity of textile products
AD15	DIN EN ISO 12947-2_2017-03A: Textiles - Determination of the abrasion resistance of fabrics by the Martindale method - Part 2: Determination of specimen breakdown (ISO 12947-2:2016); German version EN ISO 12947-2:2016
AD16	DIN EN ISO 12938-2:2020-03: Textiles - Bursting properties of fabrics - Part 2: Pneumatic method for determination of bursting strength and bursting distension (ISO 13938-2:2019); German version EN ISO 13938-2:2019
AD17	BACTERMA Final Report - BACT-021-FR

2.2 Referenced Documents

2.3 Acronyms

Abbreviation	Meaning
AM1	Antimicrobial 1: Violecin
AM2	Antimicrobial 2: Prodigiosin
AM3	Antimicrobial 3
BACTeRMA	Biocidal Advanced Coating Technology to Reduce Microbial Activity
CFU	Colonies Forming Units
ECLSS	Environmental Control and Life Support System
EMU	Extravehicular Mobilty
ESEM	Environmental scanning electron microscope
EVA	Extra Vehicular Activitiy
ISS	International Space Station
IVA	Intravehicular Activity
IP	Intellectual Property
LCVG	Liquid Cooling and Ventilation Garment
MBC	Minimum Bactericidal Concentration
MIC	Minimum inhibitory concentration
OeWF	Österreichisches Weltraum Forum (Austrian Space Forum)
PMMA	Polymethylmethacrylate
RhE	Reconstructed human epidermis
ROS	Reactive Oxygen Species
RQ	Requirements
SMAC	Spacecraft Maximum Allowable Concentration
SSF	Solid-state fermentation
SPE	Solar Particle Event
TN	Technical Note
UV	Ultra Violet
VOC	Volatile Organic Compounds
VTL	Vienna Textile Lab
WP	Work Package
ZOI	Zone of Inhibition

3 Scope of the Document

The present document describes the findings of the “BACTeRMA-Biocidal Advanced Coating Technology for Reducing Microbial Activity”, providing a brief overview of the whole program, major findings, conclusions, and further study areas.
This executive summary report provides a concise overview of the findings of a comprehensive study on the antibacterial properties of textile treatments (AM1 and AM2) for potential use in space applications.

4 Aim of the Study

The purpose of the study was to evaluate the effectiveness of the AM treatment in conferring antibacterial properties to the textiles, as well as assessing their biocompatibility, flammability, resistance to radiation, abrasion, bursting, and offgassing properties. The findings contribute to the development of cleaner and safer environments for astronauts during long-duration space missions.

The scenario related to the application of an antimicrobial substance on textile are observed and discussed. Several suitable fibers are described and the most relevant for their application as spacesuit’s innermost layer are selected. The fabrics made from the selected fibers will be dyed using antimicrobial substances produced by microorganisms (bacterial secondary metabolites). The most interesting substances are described and selected to be applied on textile. The dyed textiles will go through a series of tests to evaluate their antimicrobial activity, their toxicity towards humans and will be exposed to regolith, radiation, and perspiration.

The project aims to present the potential biocidal treatments (detailed above) for human planetary surface operations and long-term space flight applications, in particular inner layers of astronaut space suits for lunar (and later Mars) exploration.

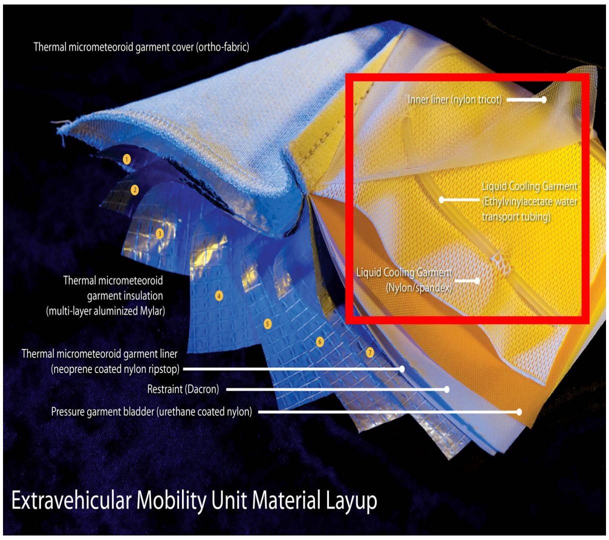

Figure 1 and 2: 1-Textile layers of NASA's EMU. Red rectangle indicates the aimed innermost layer of this project. These layers are mostly made of Nylon tricot; Nylon/spandex; Nylon/bladder. 2- AM1 treated Merinos textile body suit samples (BACT-021-HW1)

5 Scenario definition

This section aims to define the different possible scenario related to the application of antimicrobial finish **on the suit innermost layer** by considering different environmental parameters and scenario definitions, see Table 1:

Table 1 Scenario definitions of BACTeRMA

TEST SCENARIOS	Choice of the antimicrobial substance	1. The strains of bacteria are selected, then fermented and the dye is extracted in order to dye the textile. 2. Unwillingness of the bacteria for producing the dye or contamination of the culture resulting in delays. If antimicrobial effect is not powerful enough, there is the possibility to double dye, or dye with higher concentration.
	Choice of the textile	1. Different textiles are chosen, the dye is fixed on each fabric in a satisfying/dissatisfying way. 2. The properties of the textile are affected by the antimicrobial finish.
	Testing the antimicrobial activity	1. The antimicrobial dye has a high/low antimicrobial activity for the space application and there is a need to design with another already known antimicrobial agent. 2. The antimicrobial substance is durable/not durable.
	Testing the toxicity	1. Perspiration fastness: the antimicrobial may be leaching on the skin causing micro-organism depletion on the skin or the antimicrobial is not affecting the skin. 2. pH: the pH is/ is not skin friendly and cannot be used for underwear. 3. Skin irritation/corrosion: the antimicrobial has negative/ne effect on the skin. 4. Cytotoxicity: the antimicrobial is toxic/not toxic for human skin cells. Toxicity: the degradation products of the antimicrobial are toxic/not toxic for human skin.
	Testing the robustness	1. The textile chosen is robust/not robust enough. The antimicrobial dye is/not affecting the properties of the textile.
	Effect of the antimicrobial on the target microorganisms	1. Limitation of the growth of odor generating micro-organisms on the fabric and improvement of the clothes' lifetime. 2. Limitation of the growth of pathogens and improvement in the health of the astronauts, decrease in skin infections and other infections. 3. The dye has a high antimicrobial potential, destroys the astronauts' microbiome and lead to irritations and diseases. 4. No effect on the growth prevention of odor generating micro-organisms. 5. No effect on the growth prevention of pathogens. 6. Limitation of the production of resistant bacteria. 7. Production of resistant bacteria.
SPACE SCENARI	Effect of the microgravity	1. The antimicrobial has a powerful effect on the virulent pathogens. 2. The antimicrobial has a low effect on the virulent pathogens. 3. The results of antimicrobial tests are different on earth than on orbit. Antimicrobial effect is not high enough to counteract the more virulent and resistant pathogens in space.

	Effect of the regolith	1. The regolith enhances a powerful oxidative reaction, the antimicrobial dye is locally destroyed. 2. Presence of moisture or sweat in the textile form hydrogen peroxide, Fenton's reagent locally or fully destroys the antimicrobial dye. 3. The skin of the astronaut may be altered by the oxidative reactions. 4. Gas may be produced by the oxidative reaction between the dye and the regolith. 5. The quantity of regolith spread on the fabric is too low to cause any reaction.
	Effect of the radiations	1. The dose of radiation received is altering the antimicrobial. 2. The dose of radiation has no effect on the antimicrobial. 3. The radiations enhance catalytic degradation of the antimicrobial with regolith and perspiration.

6 Definition of environmental parameters and selected materials

6.1 Environmental parameters

Space exploration presents significant challenges, necessitating the development of intricate life support systems to ensure the safety and sustainability of manned missions. To determine the requirements for space suit materials, both past (e.g., Apollo missions) and future scenarios (e.g., proposed Mars or lunar missions) are examined to understand the tasks performed during surface extravehicular activities (EVAs). Projects like the Gateway space station in lunar orbit prioritize efficiency and longevity by minimizing suit exchanges and ensuring durability. Testing methods are designed to mimic real-use conditions by drawing on parameters from the International Space Station's life support systems (ECLSS). While this project focuses on the innermost layer of the suit, the spacecraft and suit environments are still considered, albeit with expectations of not being exposed to extreme environmental parameters.

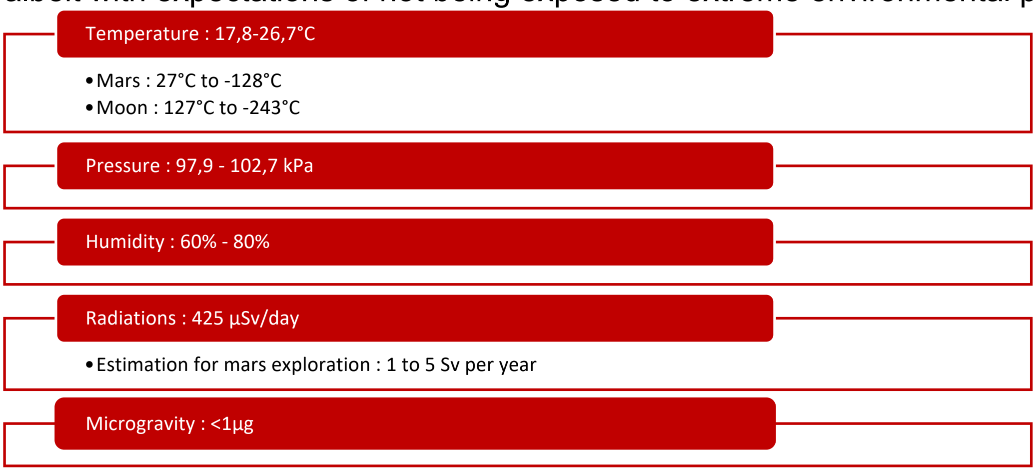

Figure 3. Environmental parameters at ISS. All data from ECLSS

The storage time might vary depending on the mission. The planned long-stay missions on Mars would be approximatively 500 days on Mars and 360 travel time. The spacesuit should therefore resist 20 640 hours at max. of storage time.

6.2 Materials

The study focuses on investigating the innermost layer of the spacesuit and exploring potential new fibers. Non-mixed fibers, namely Nylon (spandex), Merinos, and Lyocell (Tencel) , are used for antimicrobial testing to simplify interpretation. Two antimicrobial substances, AM1 (violacein) and AM2 (prodigiosin), are examined. The preferred test microorganisms include S.aureus, Candida albicans, E.coli, and P.aeruginosa, which are also commonly found on the International Space Station (ISS).

The project considers two planetary environments, Mars and the Moon, where astronauts are expected to have direct surface contact. Although the innermost layer of the spacesuit does not have direct contact with regolith, fine-grained and abrasive regolith dust particles may migrate into the suit layers. Therefore, the effects of regolith simulants (JSC-1 Martian and CAS-1 Lunar) on treated textiles are observed by exposing them to different combinations of textile samples with added regolith simulants.

The treated textiles undergo X-Ray radiation exposure at the Medaustron research facility. The radiation levels used simulate extreme environments based on the minimum and maximum estimated dosages for a long-distance round-trip to Mars (1Sv) or 2-3 years of gateway suit storage conditions, accounting for the occurrence of one solar flare (5Sv). The exposure tests aim to achieve the maximum dose the innermost layer of the spacesuit would receive. Additionally, the treated samples are exposed to combinations of regolith, radiation, and perspiration to simulate mixed exposure conditions.

7 Requirements

Inner Layer Materials that follow the outermost layer will have their committed functions. (e.g., one material for thermal insulation, one material for radiation protection, and one material for impact protection). Based on general environmental parameters on the ISS and the exposure level of the innermost layer space suit, we defined the test requirements following:

Table 2 Requirements list of the project

Name	DESCRIPTION
RQ1	Demonstrated compatibility with the expected environmental conditions for 2500 hours with temperature range 21-26 °C (storage and in-suit conditions) and humidity between 50-70%
RQ2	Demonstrated compatibility of antimicrobial dyes/activity on selected textiles (qualitative and quantitative).
RQ3	Demonstrated compatibility for one-long distance long trip (e.g., Mars) with 1Sv or for 2/3 years gateway suit storage conditions with 5Sv (1 Solar Flare) radiation environment

RQ4	Demonstrated fatigue integrity of the textile after AM treatments
RQ5	Demonstrated fatigue integrity of the treated textile after exposing radiation, regolith and sweat (The radiation might enhance catalytic degradation of the antimicrobial with regolith and perspiration).
RQ6	Demonstrated resistance of antimicrobial activity in space environment (exposing radiation, regolith, and perspiration)
RQ7	Demonstrated fatigue integrity over the expected suit life (120 cycles/hour, 2500 hours).
RQ8	Demonstrated resistance to wear by abrasive regolith (considering lunar & mars environment) for exposure of EVA suit over 2500 hours.
RQ9	The material shall not off-gas toxic substances (e.g., the oxidative reaction between the dye and the regolith).
RQ10	The material shall be non-flammable.
RQ11	Demonstrated antibacterial activity of the substance: qualitative and quantitative.
RQ12	Demonstrated antibacterial activity of the textile: qualitative and quantitative.
RQ13	The material shall be biocompatible in terms of pH and cytotoxicity.
RQ14	Perspiration fastness: make sure the perspiration is not altering the dye or staining on the skin. The antimicrobial may be leaching on the skin causing micro-organism depletion on the skin or the antimicrobial is not affecting the skin.

8 Result Assessment

The study aimed to evaluate the effectiveness of these treatments in inhibiting microbial growth, determine their minimum inhibitory concentration (MIC), assess their antibacterial activity on textile materials, and evaluate their biocompatibility.

Antibacterial Testing:

AM1

- Minimum inhibitory concentration (MIC) test showed inhibition of S.aureus but not E.coli.
- Antibacterial activity on the textile was not strong enough according to industrial standards.
- Further improvement is needed, such as lowering treatment temperature and using purer substances.

AM2

- Showed antibacterial effect against S.aureus in disc diffusion assay and MIC test.
- Antibacterial effect on the textile was not strong enough according to industrial standards.
- Improvement is required to enhance antibacterial activity, especially against E.coli.
- Increasing substance uptake on the textile and exploring synergistic combinations with other natural antibacterial are potential approaches.

Biocompatibility Testing:

- The pH of the aqueous extracts from the treated textiles was within the acceptable range and biocompatible for human skin.
- No cytotoxic effects were observed in the in vitro cytotoxicity test.
- The colour fastness to perspiration resulted in excellent grades for all tested fabrics, except for nylon treated with AM2 and acidic perspiration, which experienced a change in shade.

The study demonstrated that AM1 treatment effectively inhibited the growth of S. aureus but had limited effect on E. coli. AM2 treatment showed antibacterial activity against S. aureus but not E. coli. The treatments exhibited varying effectiveness on different textile materials. The treatments were found to be biocompatible within the tested parameters.

Flammability Testing:

- AM-treated samples showed distinct behaviour compared to untreated samples, leaving behind rough ash accumulation or molten layers.
- AM treatment increased the ignition time and reduced flammability, particularly in merino samples.
- AM treatment has the potential to improve flame retardancy and material resistance to high temperatures.

Radiation Testing:

- The AM treatment on the textiles, as well as the interaction with regolith simulant with or without water, did not significantly affect the shielding properties after radiation.
- The AM-treated samples demonstrated successful application of radiation.

Abrasion and Bursting Testing:

- NYLAM samples (nylon treated with AM) showed the highest abrasion resistance and durability among all tested materials.
- Pure nylon samples exhibited high strength and resistance to bursting.

Offgassing Testing:

- No gassing of toxic substances related to AM2 treatment was detected in any of the samples.
- All samples were deemed safe for use based on offgassing properties.

9 Conclusions

The AM treatment showed promising antibacterial effects against S.aureus but lacked sufficient activity against E.coli. Further research and development are necessary to optimize the treatment method, including temperature, substance purity, and uptake on the textiles to optimize the antibacterial efficacy of the treatments and explore their potential for space applications.

The AM treatment demonstrated potential for enhancing flame retardancy and resistance to high temperatures. All tested materials exhibited good biocompatibility, strength, and durability. The absence of toxic offgassing properties makes the AM-treated textiles suitable for various applications.

Table 3 Summary of the AM test results.

Test	AM1	AM2
Disc diffusion assay	Positive	Positive, on *S.aureus*.
Minimum Inhibitory Concentration	Active against S.*aureus*	Active against *S.aureus*.
Parallel streak test	Not observed	Observed on *S.aureus*.
Antibacterial activity of textile	Insufficient	Insufficient
Cytotoxicity	Non-cytotoxic	Non-cytotoxic
Perspiration Fastness	Good	Excellent
pH of textile	Excellent	Excellent

Note: In the "Disc diffusion assay" and "Minimum Inhibitory Concentration" tests, "positive" indicates the presence of antibacterial activity, while "active against S.aureus" specifies the specific microorganism affected. The "Parallel streak test" indicates the absence or presence of antibacterial activity, with the focus on S.aureus. "Insufficient" in the "Antibacterial activity of textile" test refers to the lack of significant antibacterial activity.

The project's second objective was to evaluate different textile materials for space exploration applications. Tests were conducted to assess flammability, radiation resistance, abrasion resistance, bursting strength, and offgassing properties. The materials displayed diverse characteristics and suitability for specific uses. The findings demonstrate the potential of advanced material treatments to enhance flame retardancy and improve overall textile performance in space environments.

Table 4 Overview of the test results

Test	Sample	Result
Flammability Test	Merinos	After combustion, rough accumulation of ash residue
	Lyocell	Slight reduction in flammability
	Nylon (Spandex)	Molten residue attracting soot, forming droplets that burned with minor flames, leaving hardened remnants
Radiation Test	All	No damage or color differentiation observed after irradiation
Abrasion Test	Merinos	Abrasion resistance: Lower durability compared to Nylon
	Nylon	Abrasion resistance: Highest durability
Bursting Test	Merinos	Potential use in applications requiring strength and durability
	Nylon	Maximum pressure over 404kPa, indicating high strength and durability
Offgassing Test	All	No gassing of toxic substances detected

The flammability test results revealed that Merinos and Spandex exhibited distinct behaviour during combustion, with the former leaving behind a rough accumulation of

ash residue and the latter forming molten residue and droplets. Lyocell, on the other hand, showed a slight reduction in flammability, suggesting improved flame retardancy. These findings indicate that advanced material treatments, such as AM (antimicrobial) treatments, could effectively enhance the flame retardancy of textiles for space exploration.
In the radiation test, all textile samples demonstrated excellent resistance to irradiation, as there was no visible damage or colour differentiation observed. This indicates their potential suitability for use in space environments where exposure to radiation is a concern. Furthermore, the abrasion test showed that Nylon exhibited the highest abrasion resistance and durability, making it a promising choice for applications requiring robust textile materials.
The bursting test results revealed that both Merinos and Nylon exhibited potential for use in applications requiring strength and durability. Nylon displayed the highest bursting strength, making it a favourable option for environments with high pressure demands. However, it should be noted that the AM2 treatment resulted in a reduction in strength for both materials, although they remained within the range of durable material strength.
The offgassing test demonstrated the safety of all tested samples, as no gassing of toxic substances was detected. This suggests that the materials, including those treated with AM2, can be used without posing a risk to human health or the environment.

Table 5 Summary of performance test results

Test	**AM1**			**AM2**		
	Merinos	**Lyocell**	**Spandex**	**Merinos**	**Lyocell**	**Spandex**
Flammability Test	Good	Slightly Good	Slightly Good	Good	Slight good	Slight good
Radiation Test	All samples exposured radiation equally			All samples exposured radiation equally		
Radiation Shielding Test	No major shielding effect determined			No major shielding effect determined		
Abrasion Test	N.A.			Good	N.A.	Very good
Bursting Test	N.A.			Very good	N.A.	Very good
Off-gassing Test	N.A.			No-gassing-(Very Good)	N.A.	No-gassing-(Very Good)

Note: N.A." stands for "Not Applicable" indicating that the specific test is not applicable to the corresponding material.

In conclusion, this study provides valuable insights into the performance of textile materials for space exploration applications. The results indicate that advanced treatments, such as AM treatments, have the potential to enhance flame retardancy. Nylon emerges as a highly durable and versatile material, suitable for various demanding applications. As mentioned above, it is recommended to explore further

underlying mechanisms of material treatments and their impact overall performance in space environments.
The findings of the study showed promising results in terms of antimicrobial activity, biocompatibility, and other properties of the treated textiles. The antimicrobial substances derived from bacterial metabolites demonstrated the potential to limit the growth of odor-generating microorganisms and pathogens, thereby improving the cleanliness and health of astronauts during space missions.

In general, further research and testing are necessary to refine and optimize the techniques developed in the BACTeRMA project. The project's findings contribute to the ongoing efforts to create cleaner and safer environments for astronauts, not only for lunar exploration but also for future missions to Mars.
The BACTeRMA project represents an important step forward in the development of biocidal textile processing techniques for space applications. The project's findings have the potential to significantly improve the functionality and safety of space suits, enhancing the overall success and sustainability of manned space missions.