Microbial Growth in Insulation Blankets under Simulated Environmental Conditions

CFR project

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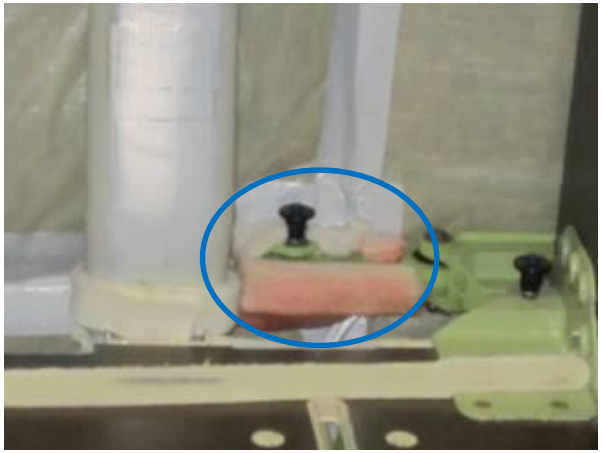
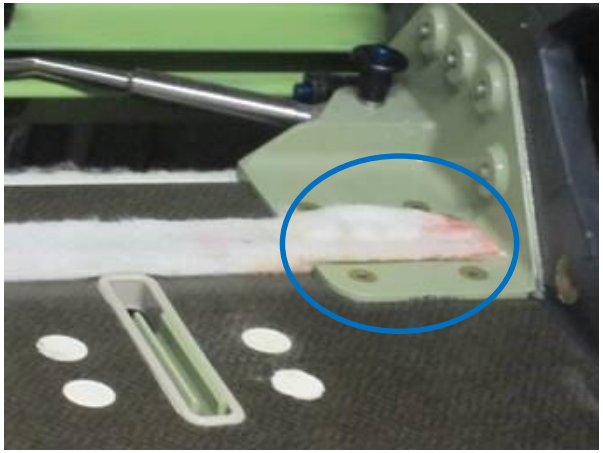
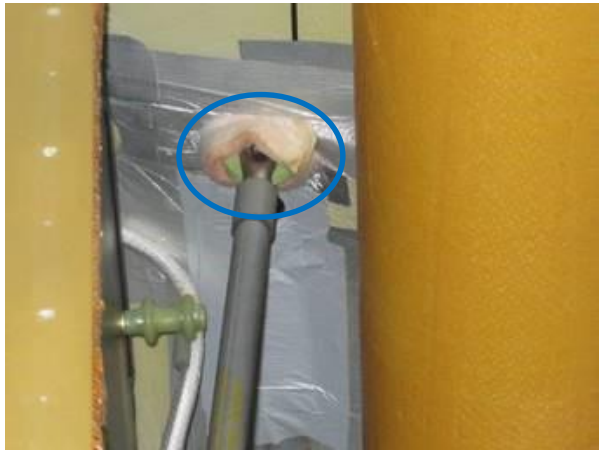
Introduction

The blankets (multiple layers usually packed within a bag) for air planes, due to moisture trapped within, discolored by the growth of microbial. The questions are:

1. What is the contaminant microbes in the contaminated blankets?
2. Are the contaminant microbes harmful to humans?

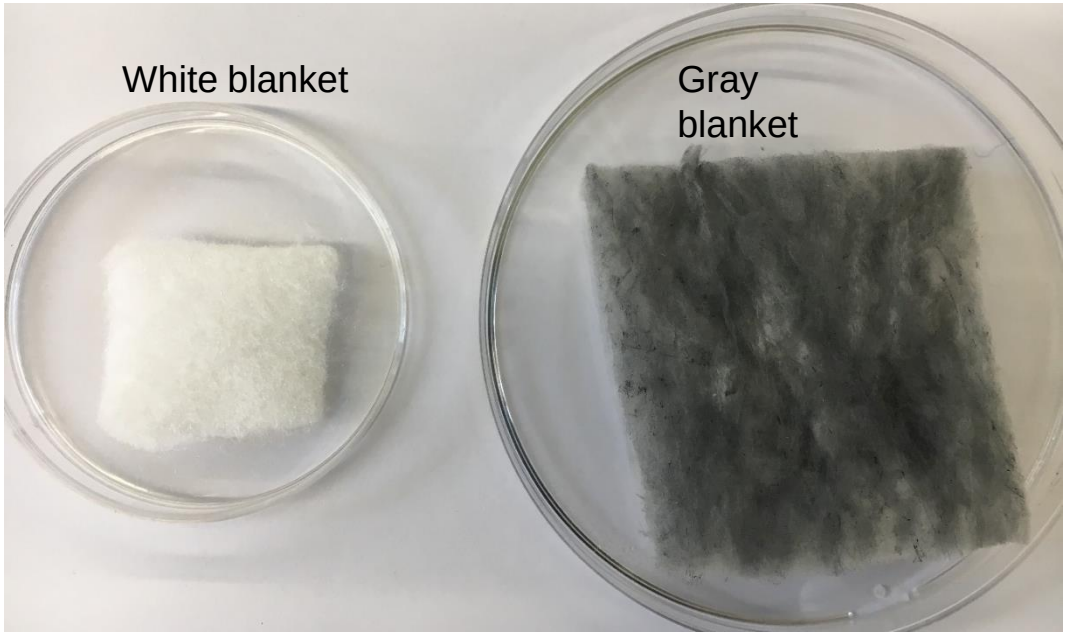
Previous studies identified following fungi as possible causes for the discolor:

- *Fusarium fujikuroi* (white mold colonies)
- *Rhodotorula mucilaginosa* (pink-yeast like colonies)

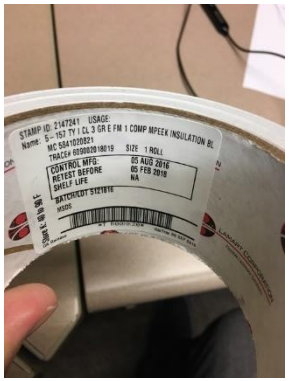




Materials

All the materials are provided by Boeing.

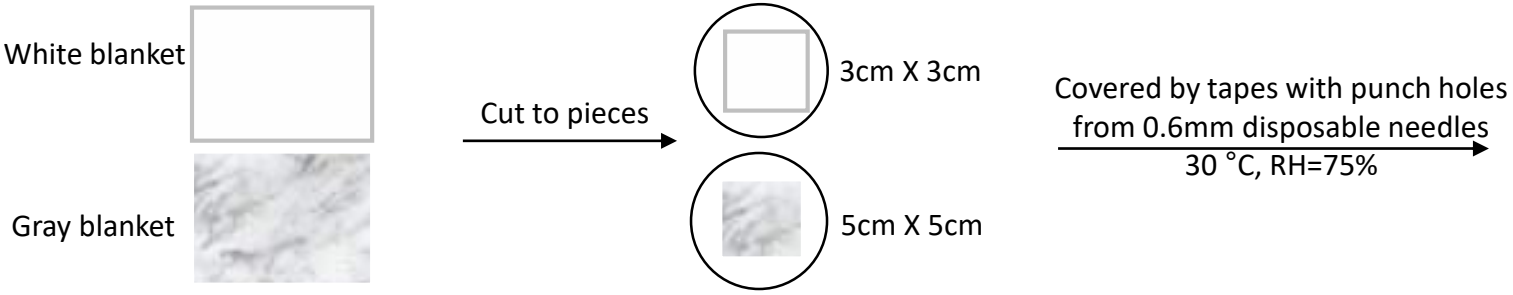


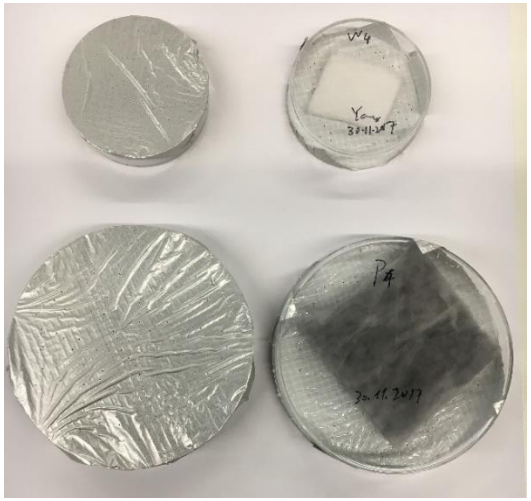
Tapes

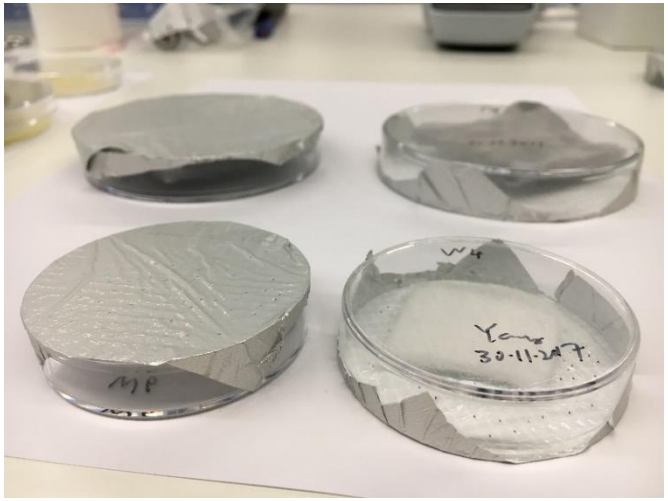


Results

Mimic the contamination in a real scenario







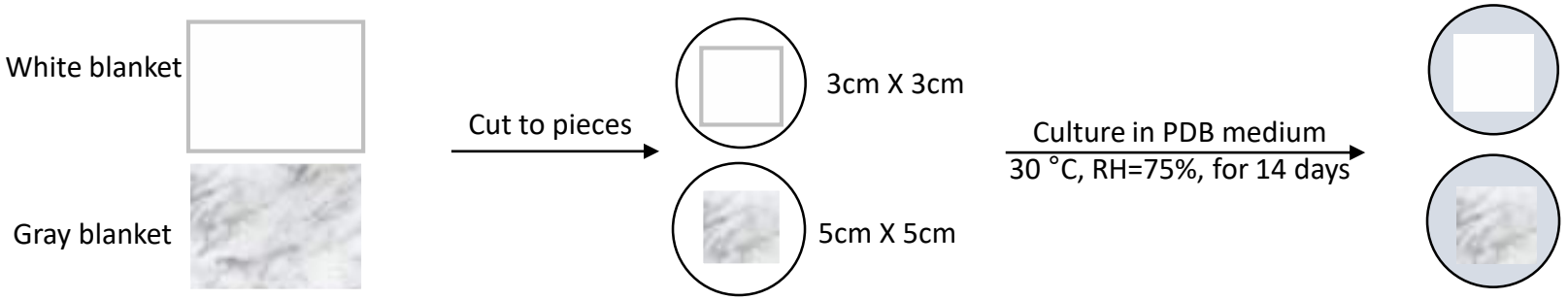
Culture in climate chamber from 30.11.2017

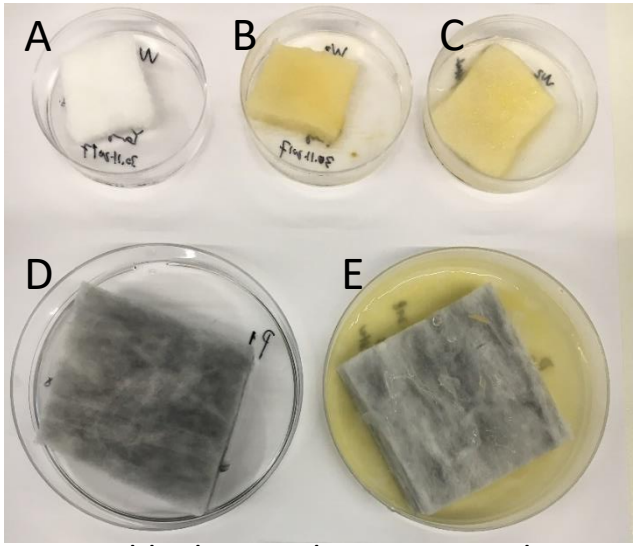
Results: The color of each blanket did not change significantly in 135 days. One possible reason is that the blankets was not contaminated by specific microbes. Another possible reason is that the blankets had insufficient nutrition to support microbial growth.

Next: continue culturing and check the color change time by time.

Culture blanket in PDB medium

Aim: As the color of blankets did not change during the environment mimicking experiment, culture media for microbial growth was used in the 2nd series of experiments to speed up the microbial growth in blankets. From references[1,2], fungi is a main reason in the blankets contamination. Therefore, the fungi culture medium, potato dextrose broth (PDB medium, liquid medium made from potato infusion and dextrose), was used in the experiment.





A, D: blankets without PDB medium
B, C, E: blankets with PDB medium.

Results: The color of blankets without PDB medium did not change significantly in 14 days. However, the color of blankets with PDB medium, especially the white blanket, changed to yellow, which looked like color change induced by microbial contamination.

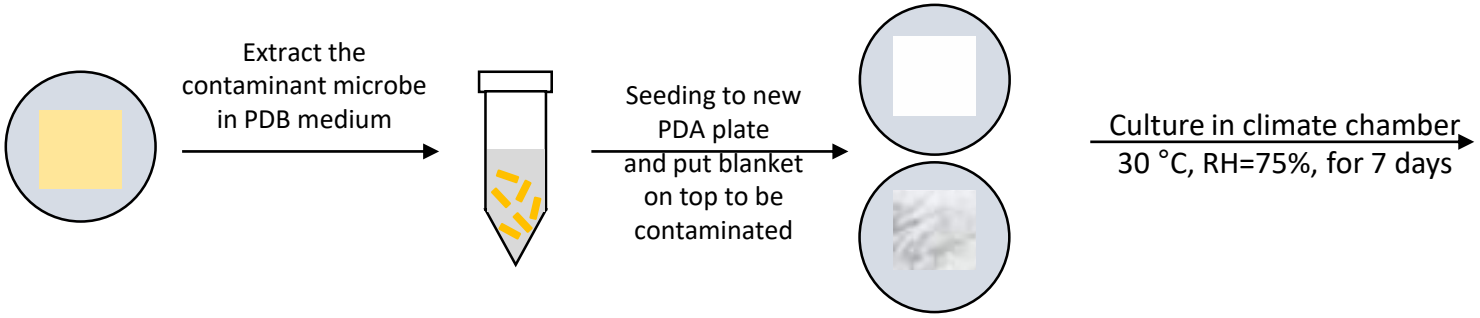
Next: subculture these contaminated microbe in new blankets and see the color changes.

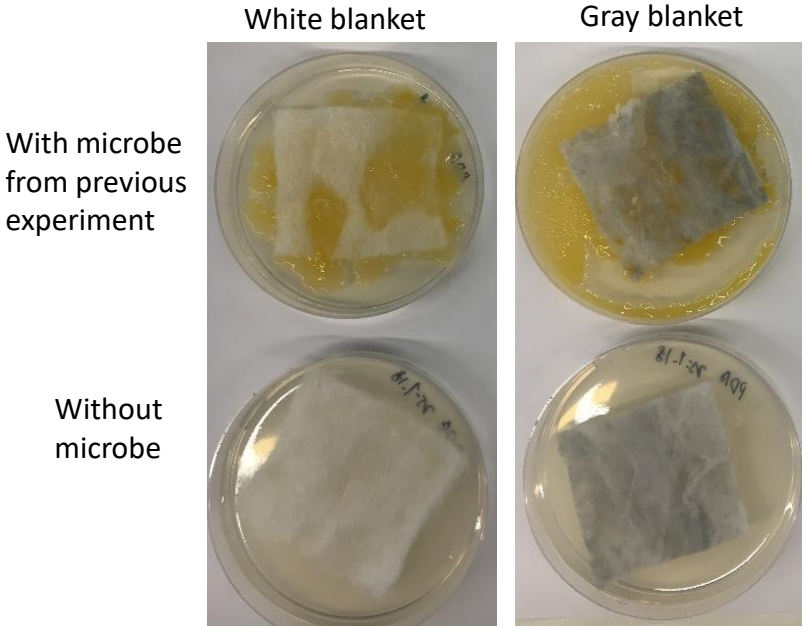
We found that the fibers from white blanket were hydrophilic materials, whereas the fibers from gray blankets were hydrophobic materials.

1. Bioresource Technology, Volume 58, Issue 3, 1996, Pages 217-227.
2. World Journal of Microbiology and Biotechnology, 2014, Volume 30, Issue 11, pp 2811–2819.

Subculture microbe with blanket in PDA plate

Aim: In the previous experiment, we observed the color changes in whiter blankets. To identify which microbe induced this color change, the microbe from the Exp 18-2 was subcultured in PDA plate to form colonies. Potato dextrose agar (PDA) is a semisolid medium (with 1.5% agar) including the same nutrition such as the PDB medium.
Subculture: taking a few microbiota samples and seeding in new agar plate for culturing.





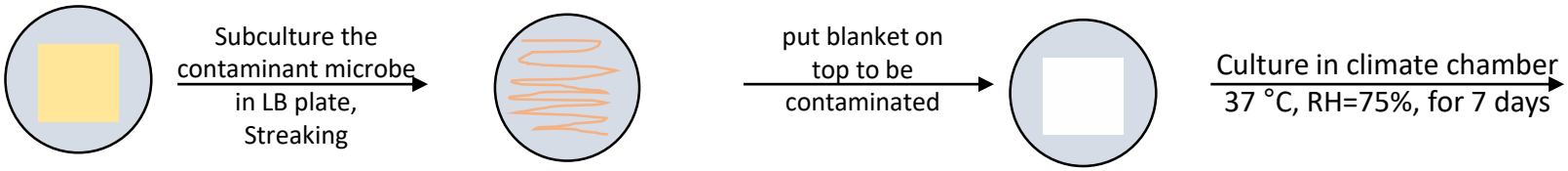
Results: In the subculture process, isolated microbe induced the same color change phenomenon in white blankets. Yet, it is not clear whether the color changed in the gray blankets because of the strong gray color background. Microbe might have difficulties to grow on hydrophobic materials. Besides, from the phenotype of colony, the microbe looks like bacteria and not fungi.

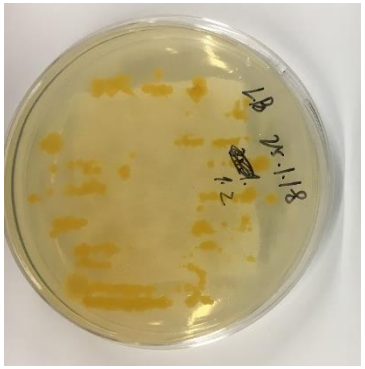
Next: subculture microbe in medium for bacteria.

Subculture microbe with blanket in LB plate

Aim: in the previous study, the microbe looked like bacteria. Therefore, the microbe was cultured on Luria-Bertani (LB) medium, which is the most widely used medium for bacteria growth.

LB medium: Tryptone, Yeast extract, Sodium chloride, agar.





White blanket

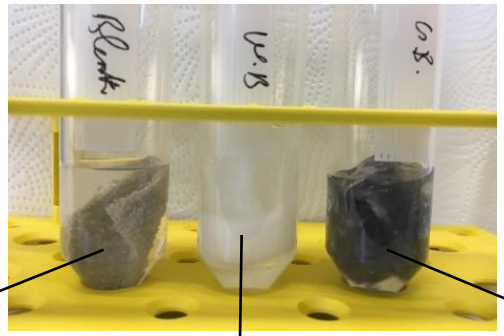
Results: The microbe can form typical bacterial colony in LB plate.

Next: identify the source of bacteria.

The isolated microbe was renamed as: 18-3.

Identification the bacteria source

Aim: the isolated bacteria were from white blanket culturing. Yet, it was difficult to identify the source of this bacteria, which might be from environment in Zurich or US. Therefore, this experiment extracted the bacteria from each blanket with water and cultured in LB plate. A piece of cloth from current ETH-Zurich lab was used as control.



- Putting piece of blanket/cloth in 15 mL sterilized water and stirring for 30 min.
- Spreading 0.1 mL of extracted solution from each sample in LB plates.
- Culture in climate chamber, 37 °C, RH=75%, for 7 days

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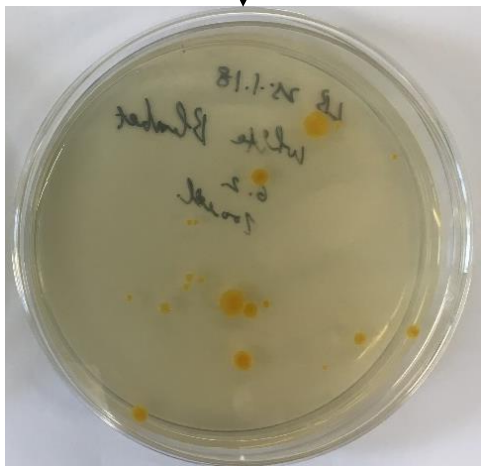
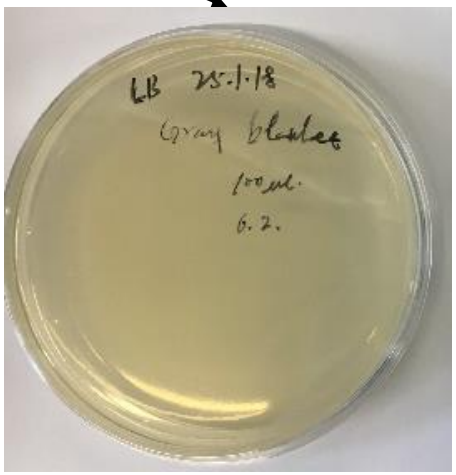
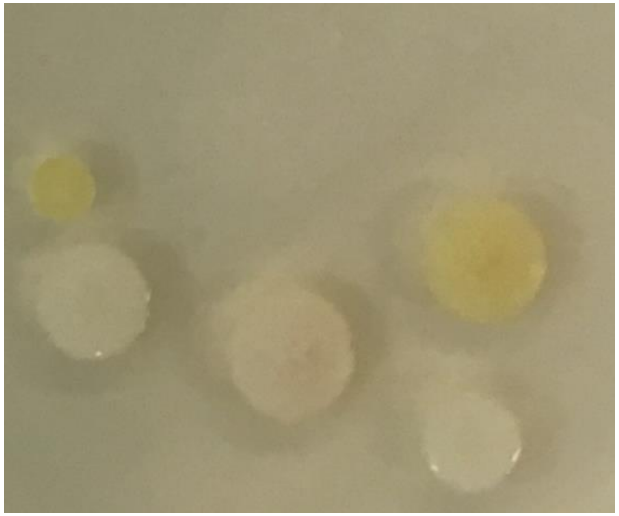


White blanket



Gray blanket



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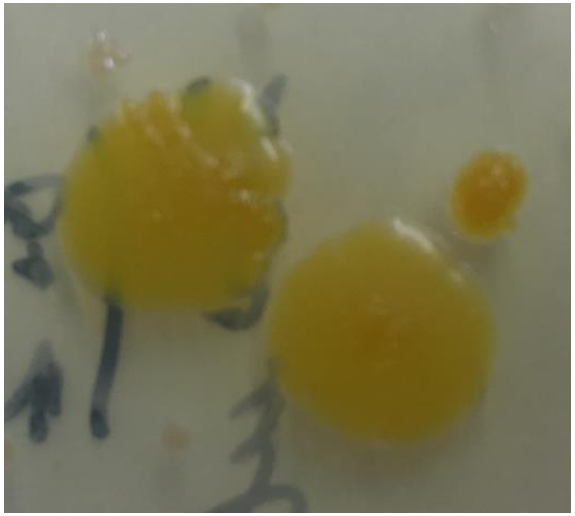


White blanket

Results: The main colonies phenotype in the white blankets plate were similar as the colonies in previous experiment. The ETH cloth plate had multiple colonies with different appearances which was significantly different from white blankets. This suggests that the bacteria which can induce blanket color change is from white blanket, the original environment or during transport. No colony is cultured in the gray blankets plate.

The isolated microbe was renamed as: **18-5**.

Identification the isolated bacteria - Imaging

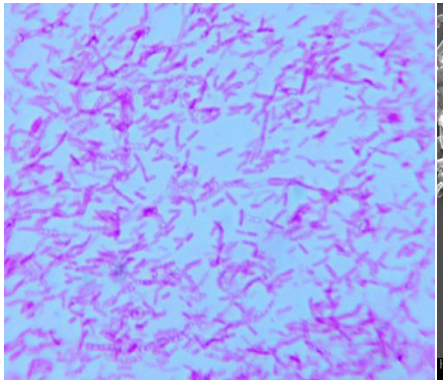
Aim: To identify the isolated bacteria by gram staining and SEM imaging. As the isolated bacteria showed different appearances in LB and PDA media, the isolated bacteria were cultured in both LB and PDA medium for imaging.

Methods:

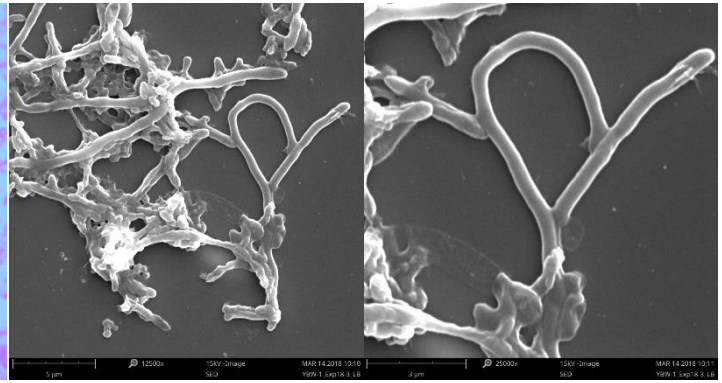
- Gram staining.
- SEM: fixation by 2.5% glutaraldehyde in PBS buffer for 1 h, gradient dehydration with ethanol, air drying, sputter coating with Pt (10 nm), imaged by Phenom ProX.

Bacteria: 18-3, 18-5.

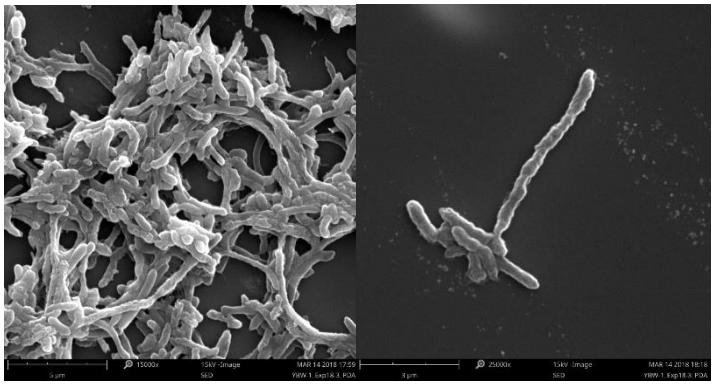
Isolated microbe: 18-3



18-3 Gram staining, X100

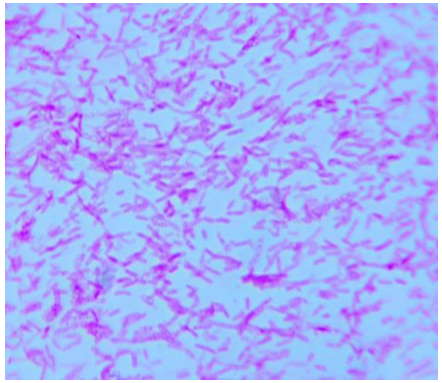


SEM, 18-3 in LB medium

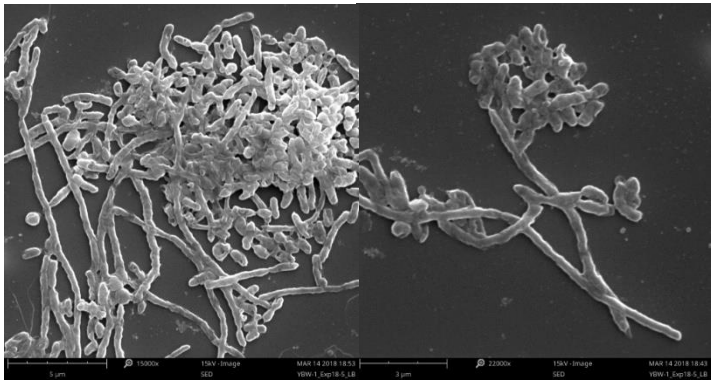


SEM, 18-3 in PDA medium

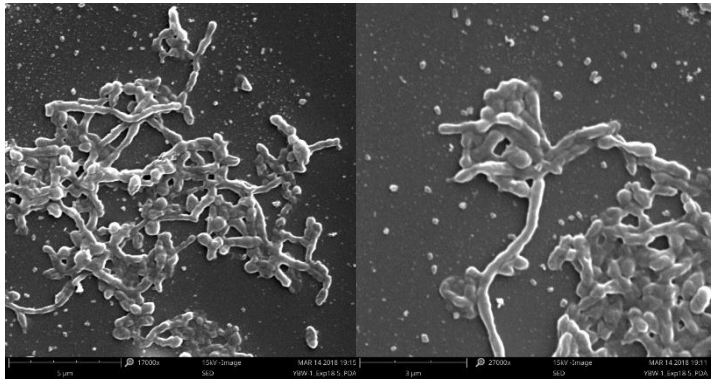
Isolated microbe: 18-5



18-5 Gram staining, X100



SEM, 18-5 in LB medium



SEM, 18-5 in PDA medium

Results:

- Isolated bacteria were gram-negative bacteria.
- SEM imaging showed that isolated bacteria were 600-800 nm in diameter and several micrometers in length. Some bacteria have spores.

Identification the isolated bacteria – 16S rRNA sequence

Aim: To identify the isolated bacteria by 16S rRNA sequencing.

Bacteria: 18-3, 18-5.

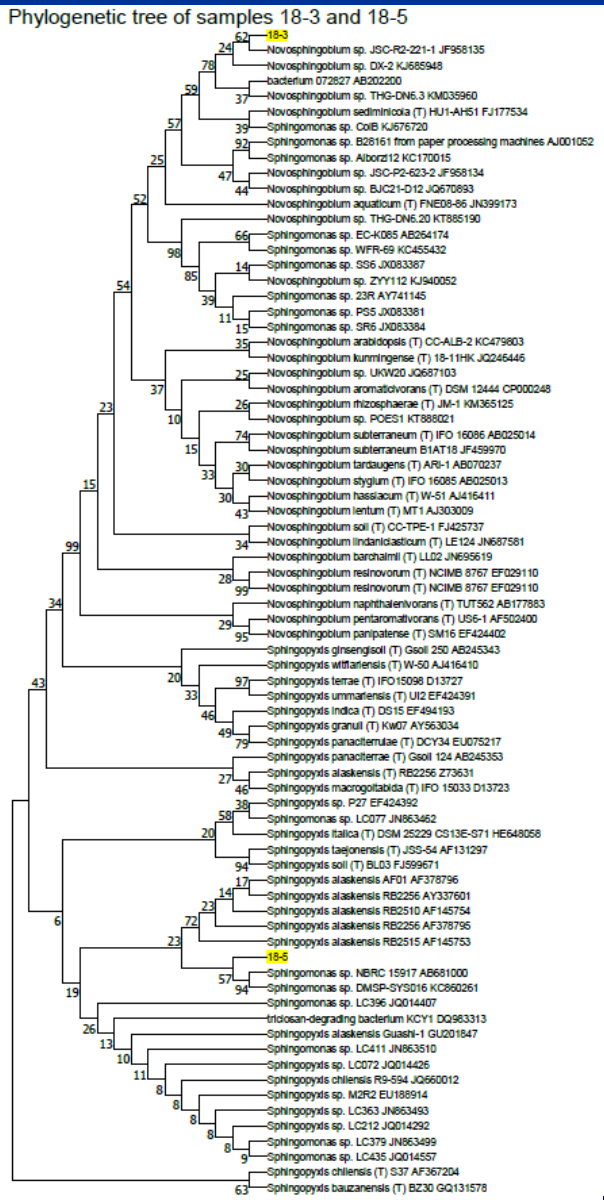
Methods: 16S rRNA sequencing.

- DNA extraction from single colonies.
- PCR amplification of the 16S rRNA.
- DNA-sequencing of the obtained PCR products.
- The obtained consensus sequences were compared with public databases using the blastn algorithm (NCBI Genbank) as well as the algorithms provided by the ribosomal database project (http://rdp.cme.msu.edu).

Analysis results:

Microbes	*Closest species matches*	*Classified by CCOS as:*
18-3	*Novosphingobium sediminicola*	*Novosphingobium sp.*
18-5	*Sphingopyxis alaskensis* *Sphingopyxis chilensis*	*Sphingopyxis sp.*

- **Sample 18-3** might be a representative of a new species of the genus *Novosphingobium*.
- **Sample 18-5** can not clearly be attributed to a distinct species of the genus *Sphingopyxis* because the 16S rRNA sequencing does not provide sufficient resolution on species level. The 18-5 might also be a representative of a new species, but additional tests should be performed.



- Both *Novosphingobium sp.* and *Sphingopyxis sp.* belong to the Family of *Sphingomonadaceae*.
- Most of *Novosphingobium* were isolated from water, plant and soil[1]. *Novosphingobium* is a genus of Gram-negative bacteria that includes *N. taihuense*, which can degrade aromatic compounds such as phenol, aniline, nitrobenzene and polycyclic aromatic hydrocarbons (PAHs)[2].
- Most of *Sphingopyxis* were isolated from soil[3]. Some *Sphingopyxis* speices were reported to have the capacity to degrade the azo dye[4,5] and poly(vinyl alcohol)[6].

1. http://www.bacterio.net/novosphingobium.html
2. Lyu Y, Zheng W, Zheng T, et al. Biodegradation of polycyclic aromatic hydrocarbons by Novosphingobium pentaromativorans US6-1[J]. PloS one, 2014, 9(7): e101438.
3. http://www.bacterio.net/sphingopyxis.html
4. Miran W, Nawaz M, Kadam A, et al. Microbial community structure in a dual chamber microbial fuel cell fed with brewery waste for azo dye degradation and electricity generation[J]. Environmental Science and Pollution Research, 2015, 22(17): 13477-13485.
5. de los Cobos-Vasconcelos D, Ruiz-Ordaz N, Galíndez-Mayer J, et al. Aerobic biodegradation of a mixture of sulfonated azo dyes by a bacterial consortium immobilized in a two-stage sparged packed-bed biofilm reactor[J]. Engineering in Life Sciences, 2012, 12(1): 39-48.
6. Yamatsu A, Matsumi R, Atomi H, et al. Isolation and characterization of a novel poly (vinyl alcohol)-degrading bacterium, Sphingopyxis sp. PVA3[J]. Applied microbiology and biotechnology, 2006, 72(4): 804.

Conclusions

- Two bacteria, *Novosphingobium sp.* and *Sphingopyxis sp.*, were isolated from the white blankets, which can induce the color change of white blankets to pale yellow.
- It is difficult to confirm during which step the bacteria deposited on the blankets, in US or in the transport period.
- The gray blankets are difficult to be contaminated with bacteria. The *hydrophobic fiber* may be the reason.
- The environment mimicking experiment did not work very well. The low nutrition content on the blankets may be the reason. The accelerated contamination experiment with microbial culture medium was very useful in the current project.

Questions

- Is the color change observed in our experiments the same as that in aircrafts?
- Do the isolated bacteria exist in actual aircrafts?
- Are the isolated bacteria harmful?
- Are the white blankets more easily contaminated than gray blankets in aircraft?

Outlook

- Isolating target species from original contaminated blankets.
- Collecting bioaerosol samples from aircraft by portable air samplers, e.g. Andersen impactor.
- Risk analysis of the isolated microbes.
- Studying the effect of temperature and RH on the growth of the bacteria with relevance to aviation.
- Investigating the color changes on blankets by mimicking the contamination in a real scenario similar to that in the actual flight.

Thanks!

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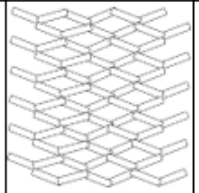
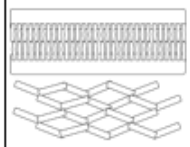
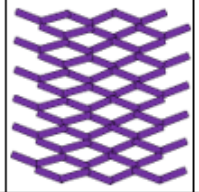
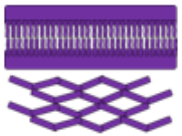
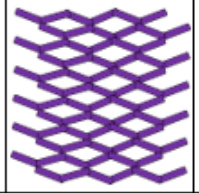
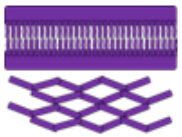
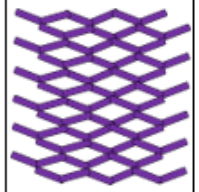
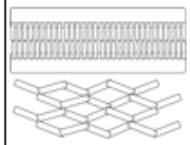
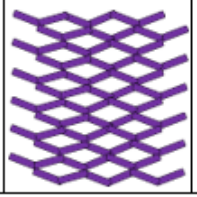
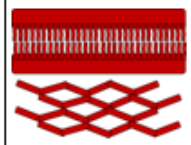
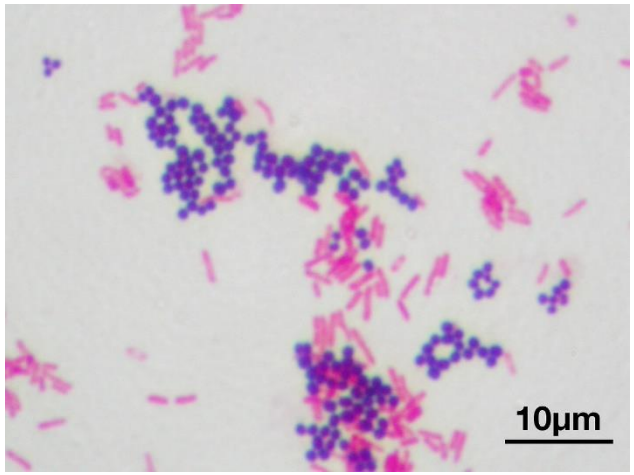
Gram staining

Gram Staining Procedure		Gram Positive Cell Wall		Gram Negative Cell Wall	
Process of test	Appearance of Cells	Effect of Step	Effect on Cell Wall	Effect of Step	Effect on Cell Wall
Step 1: Begin with heat fixed cells		**Step 1:** Cell wall remains clear.		**Step 1:** Cell wall remains clear.	
Step 2: Flood slide with crystal violet dye for 1 min.		**Step 2:** Peptidoglycan cell wall is flooded with crystal violet and appears purple.		**Step 2:** Cell wall is stained purple from the crystal violet dye.	
Step 3: Add iodine solution for 1 min.		**Step 3:** A crystal violet – iodine complex is formed within the peptidoglycan cell wall trapping the purple stain.		**Step 3:** A crystal violet-iodine complex is formed but does not adhere to the cell wall due to the thin layer of peptidoglycan.	
Step 4: Wash slide with alcohol for 20sec.		**Step 4:** The crystal violet – iodine complex is trapped with the peptidoglycan cell wall and doesn't wash out.		**Step 4:** The crystal violet – iodine structure is washed out of the thin peptidoglycan layer.	
Step 5: Counter stain with safranin.		**Step 5:** As the peptidoglycan cell wall remains stained purple the red safranin has no effect.		**Step 5:** The red safranin stains the washed gram negative cells.	



Staphylococcus aureus: gram-positive cocci, in purple
Escherichia coli: gram-negative bacilli, in red.