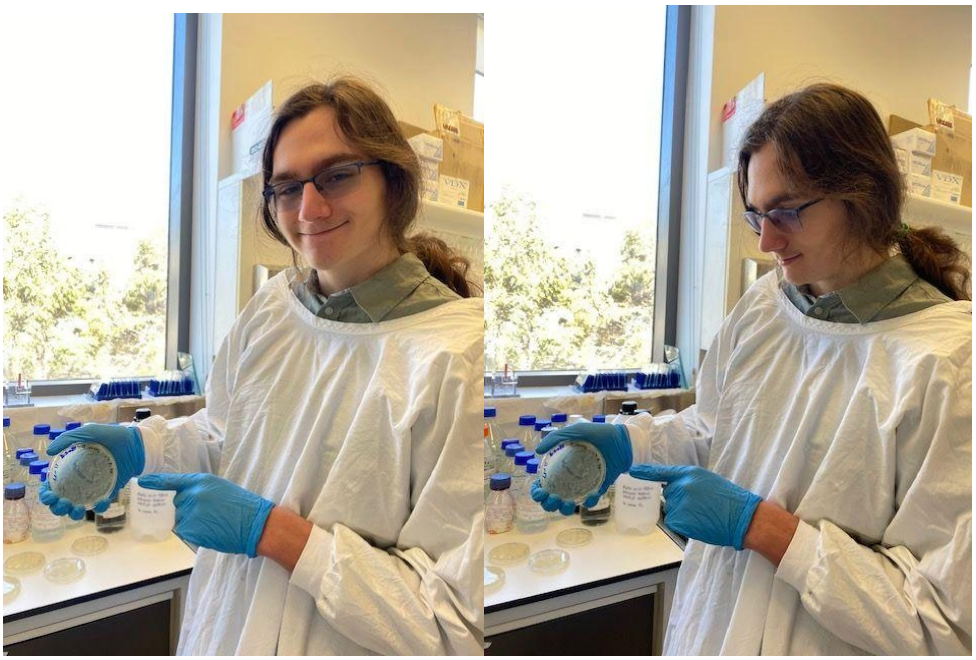
ANU Research Report: Bacterial Isolates from Kitchen Sponges



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by Harvey Del Gaizo

Aim

During my time at the ANU our team conducted an experiment on three of the workplace's kitchen sponges, examining the amount of bacterial organisms per gram of sponge. This investigation was conducted because of recent concerns of possible cross contamination when eating on plates that had been washed with these sponges. If our results concluded an overwhelming amount of foreign bacterial specimens we would report our findings to the School requesting a more frequent change of the kitchen sponges to avoid the health hazard.

Procedure and equipment

This experiment was conducted within the Leyton laboratory at the ANU, to ensure a sterile environment and to utilise all available equipment needed for our investigation.

For the experiment we followed a basic procedure for this exact test using the tools listed below:

- sterile scissors
- 1 x control sponge
- 1 x kitchen sponge from each floor (Levels 1-3)
- multiple LB agar plates
- balance
- single use (sterile) plastic pipettes
- 1 x vortex
- Phosphate-Suffered Saline (PBS) (sterile)
- a container of 80% ethanol
- multiple (sterile) test tubes (per sample and sample solution)
- multiple (sterile) centrifuge test tubes
- benchtop centrifuge
- sterile sample spreader sticks.

To begin our experiment, my supervisor, Dr. Denisse Leyton, and I collected 3 used kitchen sponges from within the Linnaeus Building, ANU Research School of Biology (RSB) storing them into separated, sterile containers including a fresh, newly opened control sponge, to see just how "gross" the kitchen sponges were.

Each sponge was then brought into the laboratory, slicing 1 gram of sponge from each sponge with sterilised scissors and transferred into their respective tubes; *L1, L2, L3*, and *Control*. Each was then filled with 5 mL of PBS and left overnight in the fridge.

When returning in the morning we extracted a total of 1 mL of PBS from each sample, noting that *L1's* sample had a distinct colouration compared to the other samples, a possible indicator of higher microorganism content. Because of the higher likelihood for more colony forming units (CFU) within this sample, after the dilution process for each sample, we used the undiluted (neat) sample and dilutions 10^{-1}, 10^{-3}, and 10^{-5} for *L1* and used the neat sample and dilutions 10^{-1} for the other respective samples (*L2* and *L3*). We spread 0.1 mL of

samples *L1-L3* in duplicate with each set of plates eventually placed into different environmental conditions; 25°C (room temperature) and 37°C (body temperature) and left overnight.

When reviewing the growth on each individual agar plate, as theorised, *L1's* sample appeared to contain the most amount of foreign microorganisms for both temperatures. The neat sample of all plates except that of the new (unused) control sponge produced confluent growth across the agar where individual colonies were so close together that they could not be counted with any accuracy (see Figure 1).

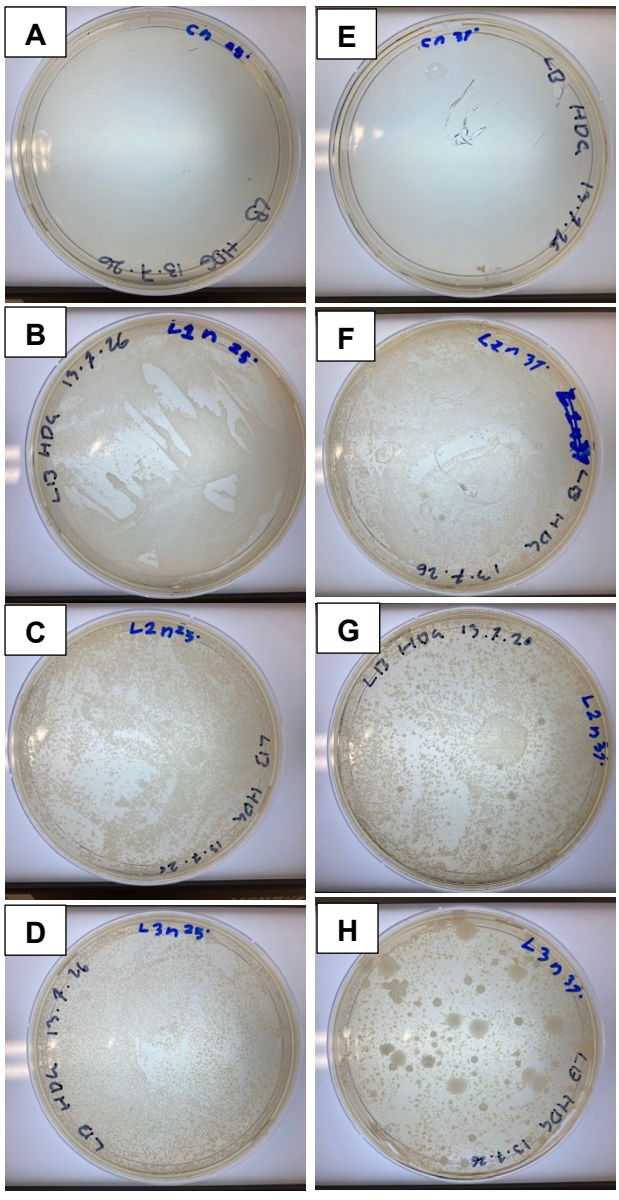

Figure 1. Photos of LB agar spread plated with microorganisms isolated from Linnaeus Building kitchen sponges. LB agar spread plated with 0.1 mL neat samples taken from the new control sponge (A), level 1 sponge (B), level 2 sponge (C), and level 3 sponge (D) and incubated at 25°C. LB agar spread plated with 0.1 mL neat samples taken from the new control sponge (E), level 1 sponge (F), level 2 sponge (G), and level 3 sponge (H) and incubated at 37°C.

To accurately examine how many microbes lived on 1 gram of sponge (equivalent to 1 mL of PBS extracted from the sponges) we counted all the visible colonies within the highest diluted samples and calculated the CFUs (see Table 1). The Linnaeus Building kitchen sponge contaminated with the most microorganisms was that from *L1* incubated at 25°C with 3.74 million CFUs per 1 gram of sponge.

Table 1. Colony Forming Units (CFUs) per 1 gram of Linnaeus Building kitchen sponges.

Sample area	No. of colonies at 25°C	CFU/1g	No. of colonies at 37°C	CFU/1g
New control kitchen sponge	0 (neat)	0	1 (neat)	1
Level 1 kitchen sponge	374 (10^{-3} dilution)	3.74×10^6	121 (10^{-3} dilution)	1.21×10^6
Level 2 kitchen sponge	1312	1.312×10^5	1400	1.4×10^5
Level 3 kitchen sponge	1384	1.384×10^5	1216	1.216×10^5

Before concluding our experiment we decided to finally examine if these colonies were bacterial (as opposed to fungal) by Gram-staining some of the colonies. Gram-staining is a process which uses chemicals, like crystal violet, to react with the bacteria's membrane turning the sample into either a purple colour for Gram-positive or a pinkish colour for Gram-negative.

The results of the Gram-staining showed a mixture between Gram-positive and negative bacteria within the samples suggesting that the Linnaeus Building kitchen sponges are contaminated with Gram-positive skin flora such as *Staphylococcus* and *Streptococcus* species and Gram-negative bacteria perhaps from food, e.g., *E. coli* (see Figure 2).

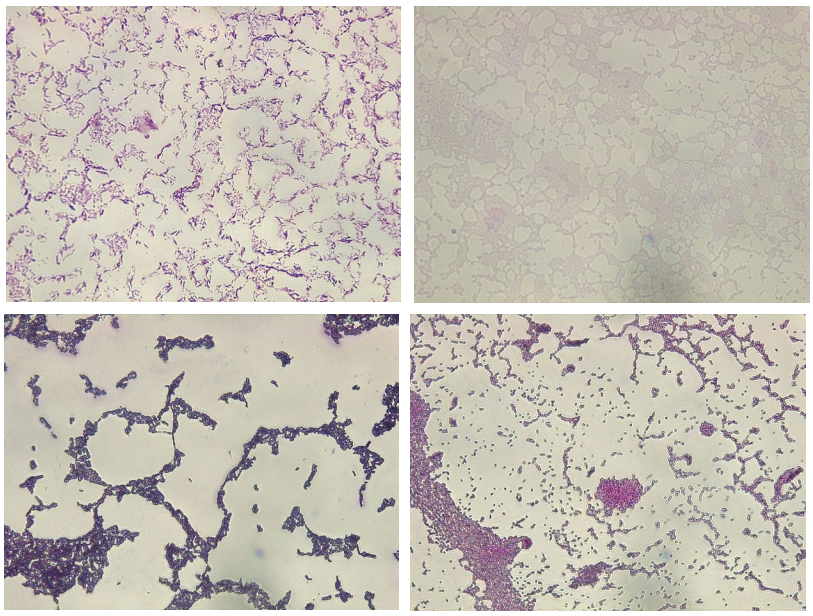

Figure 2. Gram-stained bacteria isolated from Linnaeus Building kitchen sponges. Gram-positive bacteria stain purple and Gram-negative bacteria stain pink.

Conclusion

The results of our experiment support the concerns regarding the hygiene of these kitchen sponges are most definitely valid. Our bacterial counts revealed millions of microbes (mainly bacteria) within the sampled sponges, and the Gram-staining results showed that a large majority of the bacteria present are possibly harmful Gram-negative bacteria. Overall, this

experiment demonstrated not only how unhygienic and “gross” these sponges are, but also the genuine potential for harmful bacteria to be transferred to anyone who comes into contact with them.

Recommendation: replace kitchen sponges more regularly.

Acknowledgements

Though this experiment was undoubtedly “gross” it was simultaneously amazing! Working within the lab was an unforgettable experience and I am very grateful to have had the chance to work at the ANU and with Denisse as my supervisor. A big thank you to Melanie, Mrinalini, and Yiming from the BTLC for providing the Gram-staining reagents and imaging equipment.