University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

   Surname: Barber Forename: Jack

   E-mail address: 2248286B@student.gla.ac.uk

2. Supervisor

   Surname: Douce Forename: Gillian

   E-mail address: gillian.douce@glasgow.ac.uk

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   Isolation and identification of soil bacteria producing novel antimicrobial substances that are effective against *Clostridium difficile*.

   3.2 Project Lay Summary (copied from application):

   Antimicrobial resistance is a serious issue whereby bacteria can become resistant to the effects of antibiotics. Clostridium difficile is a pathogenic bacterium, prominent in hospitals, to which limited treatments are available.

   To date, the most effective antimicrobial agents have been identified from organisms that reside within soil. This is because this is a highly competitive environment and organisms that are able to kill the competition for nutrients will survive. The aim of this project is to determine whether any soil bacteria produce novel antimicrobial substances that can also kill C. difficile.

3.3 Start Date: 28th May 2018 Finish Date: 13th July 2018

3.4 Original project aims and objectives (100 words max):

The aim of this project is to analyse soil samples from the local area in an attempt to identify bacteria which produce novel antimicrobials effective against *C. difficile*.

The isolation and identification of such bacteria could provide a new source of antibiotics that can be used to treat *C. difficile* infection. Expanding the number of effective antibiotics would reduce the risk of *C. difficile* becoming resistant to its (currently limited) repertoire of antibiotics. Furthermore, by understanding how these antimicrobial compounds work we could gain a greater understanding of the pathogen and design more effective treatments against it.

3.5 Methodology: Summarise and include reference to training received in research methods etc. (250 words max):

Standard Soil Culturing

Soil samples were recovered from University grounds and a serial dilution was performed to a final bacterial dilution of 10^{-6}. Each dilution was plated on a range of media and incubated in a variety of conditions to maximise recovery. Training was received for working with anaerobic bacteria.

Colonies from these spread plates were isolated and plated onto two further plates, which were overlaid with soft agar containing either *E. coli* or *C. difficile* respectively. Training was received for handling *Clostridium difficile*. To identify novel antimicrobial-producing bacteria, colony PCR using 16S primers was performed on colonies which produced a zone of lysis in the *C. difficile* overlay. The 16S DNA PCR product was run on an agarose gel, then purified and sent for sequencing. Results were analysed using a BLAST search, and Gram staining was used as a qualitative technique to reinforce any findings.

Spent media was harvested from antimicrobial-producing colonies and was used for spotting on *C. difficile* spread plates to determine if the compound with antimicrobial action was excreted into the spent media.

Culturing With an iChip Device

Aided by existing literature, a novel methodology was developed for culturing soil bacteria using an iChip, or isolation chip (Figure 1, also described in section 3.7). Using this methodology, three iChips were buried in the soil of the studentship supervisor's garden. One iChip was recovered every week for three weeks. The recovered bacteria were plated out and, after a period of incubation, were tested for antimicrobial action against both *E. coli* and *C. difficile* overlays. The variety of colonies cultured using the iChip culturing method and the standard culturing method (see above) were compared to test the feasibility of the iChip method.

3.6 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

Standard Soil Culturing

As can be seen in Figure 2, there was limited variation in colony morphology of the bacteria cultured using standard culturing techniques. The vast majority of colonies were either mucosal (usually cream-coloured) or filamentous in structure, suggesting a limited variety of soil bacteria were recovered when standard culturing methods were used.

Of the colonies tested for antimicrobial action against *E. coli* and *C. difficile*, one colony produced a zone of lysis in an *E. coli* overlay only, while another (Figure 3) produced a zone of lysis in both *E. coli* and *C. difficile* overlays. In keeping with the aim of the project, it was decided that only the second of these colonies would be used for colony PCR. To test that amplification of the 16S region was successful the PCR product was run on a 0.8% agarose gel (Figure 4) that showed bands at roughly 400bp (which was expected with the primers used). The results of Sanger sequencing of the purified PCR product, when processed using a BLAST search, showed the unknown antimicrobial-producing colony belonged to the genus *Bacillus*. A Gram stain of the *Bacillus* sp. showed spore-forming, gram-positive, rod-shaped bacteria (Figure 5), providing further evidence the unknown organism was of the genus *Bacillus*.

Spent media spotted onto spread plates of *C. difficile* showed that the antimicrobial compound produced by the *Bacillus* sp. was excreted into the spent media (Figure 6).

Culturing With an iChip Device

Bacteria cultured using the iChip culturing method showed a much wider variation in colony morphology (Figure 7) compared to the standard technique, indicating the iChip method allows for the recovery of a far greater range of soil bacteria.

Compared to the standard culturing technique (which recovered two antimicrobial-producing bacteria from a sample of soil) the iChip method yielded a greater number of antimicrobial-producing bacteria, with seven recovered in total. However, these seven organisms only produced zones of lysis in *E. coli* overlays, i.e. none were effective against *C. difficile*.

3.7 Discussion (500 words max):

Most microbial populations (many of which produce useful antimicrobials) cannot be cultured using artificial media, as they do not receive the nutrients required to allow them to grow *in vitro* (Amann, Ludwig and Schleifer, 1995). One solution is to cultivate microbes *in situ* as opposed to in the lab, which can be achieved with the use of an iChip device (Kaeberlein, Lewis and Epstein, 2002).

An iChip consists of multiple small diffusion chambers set into a piece of plastic, with each chamber being inoculated with one bacterium (on average) from a sample of soil (Berdi et al., 2017). When buried in soil, the bacteria in the iChip are provided with all the nutrients they require to grow (Aoi et al., 2009). A wider variety of soil bacteria can, therefore, be cultured by using such a device instead of standard culturing techniques, and a higher proportion of these soil bacteria are likely to be novel (Nichols et al., 2010).

There was a much wider variation in colony morphology of bacteria cultured using the iChip method compared to the standard method. If not for time and budget limitations, 16S sequencing could have been performed on colonies cultured using both techniques. The percentage of identity shared with known bacterial species could then have been calculated to determine the phylogenetic novelty of colonies cultured using the two techniques. The indicated increase in the variety of bacteria cultured when the iChip method was used is promising for research into novel antimicrobials. This is because these bacteria are unlikely to have been cultured previously and so are more likely to produce novel antimicrobials than any bacteria cultivable using standard petri dish techniques, the secondary metabolites of which have been overmined (Nichols et al., 2010). iChip devices are cheap to produce, quick to assemble and could easily be adapted for automation, all of which add to the feasibility of using such a device for research in the field of environmental microbiology (Berdi et al., 2017).

One issue encountered with this technique was that the soil the iChips were buried in was dry due to the weather conditions of the previous week. This may have led to the drying out of the agar in some of the diffusion chambers, reducing the number of bacteria recovered.

The *Bacillus* sp. isolated from soil using the standard method of culturing was shown to produce an antimicrobial compound effective against *C. difficile* that was excreted into spent media. Further research into this antimicrobial would involve the isolation of this compound from spent media to identify its structure. The mechanism of action of the compound could then be discerned, which may provide valuable information on the interaction of *C. difficile* with antimicrobials and allow for more effective targeting of treatments against the pathogen.

As described above, most bacteria recovered from soil using standard culturing techniques (and any secondary metabolites produced) are already well characterised. It should be noted, therefore, that as the soil *Bacillus* sp. was cultured using such a technique, it is highly unlikely to be a novel species or to produce a novel antimicrobial compound effective against *C. difficile* – a probability of 10^{-7} according to Nichols et al. (2010). However, the protocol defined here can be easily applied to bacteria culturing using an iChip device, which would be much more likely to produce such a novel antimicrobial substance.

4. Reflection by the student on the experience and value of the studentship (300 words max):

The studentship has provided me with invaluable experience of a research-based career, including the variety of work and standards expected of research scientists. I'm particularly thankful for the advice of my supervisor, Dr Gill Douce, who has, during the planning and execution of the studentship, ignited within me a passion

for research. I am also thankful to the members of her research group, who answered all my questions, passed on their vast practical knowledge and made my experience in the lab that much more enjoyable. I was able to shadow my colleagues, a Master's student and a PhD student, while working on my own project, and this has given me an idea what these academic degrees involve.

Working with experts in the field of microbiology has provided me with a range of new skills and knowledge which I look forward to applying to my undergraduate years at university and beyond. These skills ranged from laboratory basics, such as making my own agar plates and making bacterial stocks, to completely new techniques, such as using kits for silver stains and DNA purification, working in an anaerobic chamber and amplifying the 16S region of the bacterial genome using PCR.

Working with people outside of the research group I was a part of showed how the different members of a research institute interact. I learned that there are a variety of roles in such an institute, all of which are necessary to allow the University of Glasgow's Institute of Infection, Immunity and Inflammation to produce the ground-breaking research that it does. I also had the opportunity go to the university's School of Engineering to meet with Nikolaj Gadegaard, who fabricated the iChips for my project and who took the time to show me his lab and tell me about his work in bioengineering.

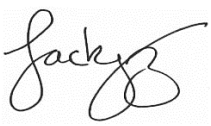
As a whole, the studentship has been an unforgettable experience that has allowed me to learn many new skills and has reinforced my desire to pursue a career in scientific research.

5. Dissemination: (note any presentations/publications submitted/planned from the work):

I plan to submit my work to an undergraduate journal (such as the University of Glasgow's own multidisciplinary undergraduate research journal, *[X]position*).

I may also have the opportunity to present my research at the 2019 Microbiology Society annual conference, at a session for undergraduates undertaking 'Antibiotics Unearthed' projects.

6. Signatures:

Supervisor	Date	Student	Date
	3/8/18		3/8/18

References

Amann, R., Ludwig, W. and Schleifer, K. (1995). Phylogenetic Identification and In Situ Detection of Individual Microbial Cells without Cultivation. *Microbiological Reviews*, 59(1), p.144.

Aoi, Y., Kinoshita, T., Hata, T., Ohta, H., Obokata, H. and Tsuneda, S. (2009). Hollow-Fiber Membrane Chamber as a Device for In Situ Environmental Cultivation. *Applied and Environmental Microbiology*, 75(11), pp.3826-3833.

Berdi, B., Spoering, A., Ling, L. and Epstein, S. (2017). In situ cultivation of previously uncultivable microorganisms using the ichip. *Nature Protocols*, 12(10), pp.2232-2242.

Kaeberlein, T., Lewis, K. and Epstein, S. (2002). Isolating "Uncultivable" Microorganisms in Pure Culture in a Simulated Natural Environment. *Science*, 296(5570), p.1127.

Nichols, D., Cahoon, N., Trakhtenberg, E., Pham, L., Mehta, A., Belanger, A., Kanigan, T., Lewis, K. and Epstein, S. (2010). Use of Ichip for High-Throughput In Situ Cultivation of "Uncultivable" Microbial Species. *Applied and Environmental Microbiology*, 76(8), pp.2445-2450.

Appendix

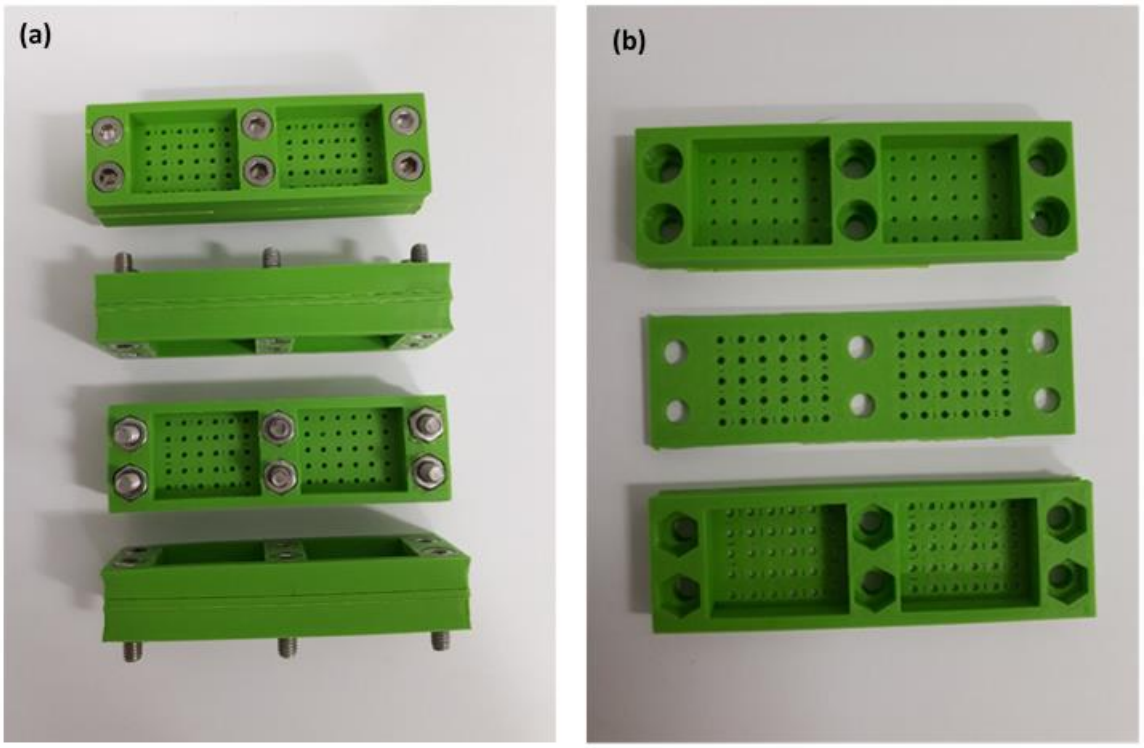


Figure 1: Structure of an iChip device. (a) shows four different views of the iChip. (b) shows the three plastic components of an iChip device. The small holes of the middle component of image (b) are filled with agar that has been mixed with bacteria from a sample of soil. 0.2 µm filters are cut to the correct size then placed over the small holes (on both sides) of the this component to form miniature diffusion chambers. This will allow soil nutrients to pass through into these small diffusion chambers when the iChip is buried but will not permit new soil bacteria to contaminate the agar. The larger holes are left free for screws to hold the iChip together. The iChip can then be assembled and deployed. In total, this procedure can take someone without previous experience assembling iChips as little as 2-3 hours.

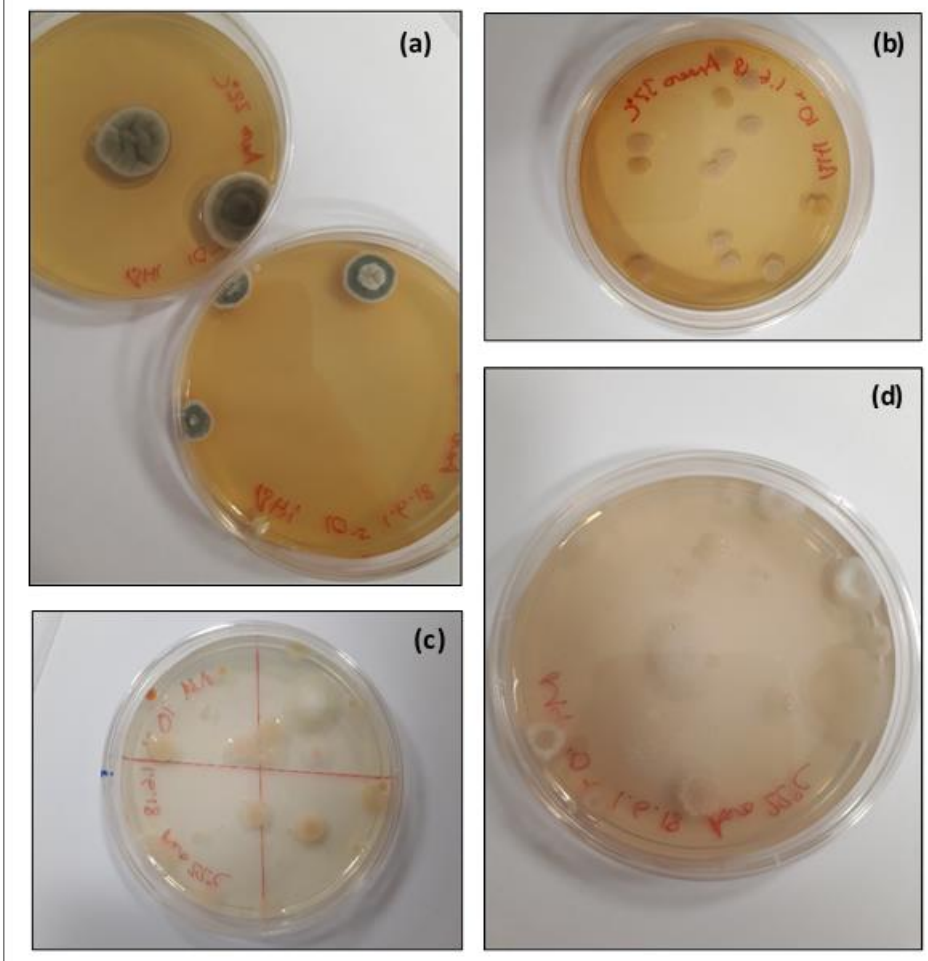


Figure 2: Variation in colony morphology of microorganisms cultured using a standard culturing technique. (a) and (b) show colonies grown on BHI agar, (c) shows colonies grown on nutrient agar and (d) shows colonies grown on M9 minimal media.

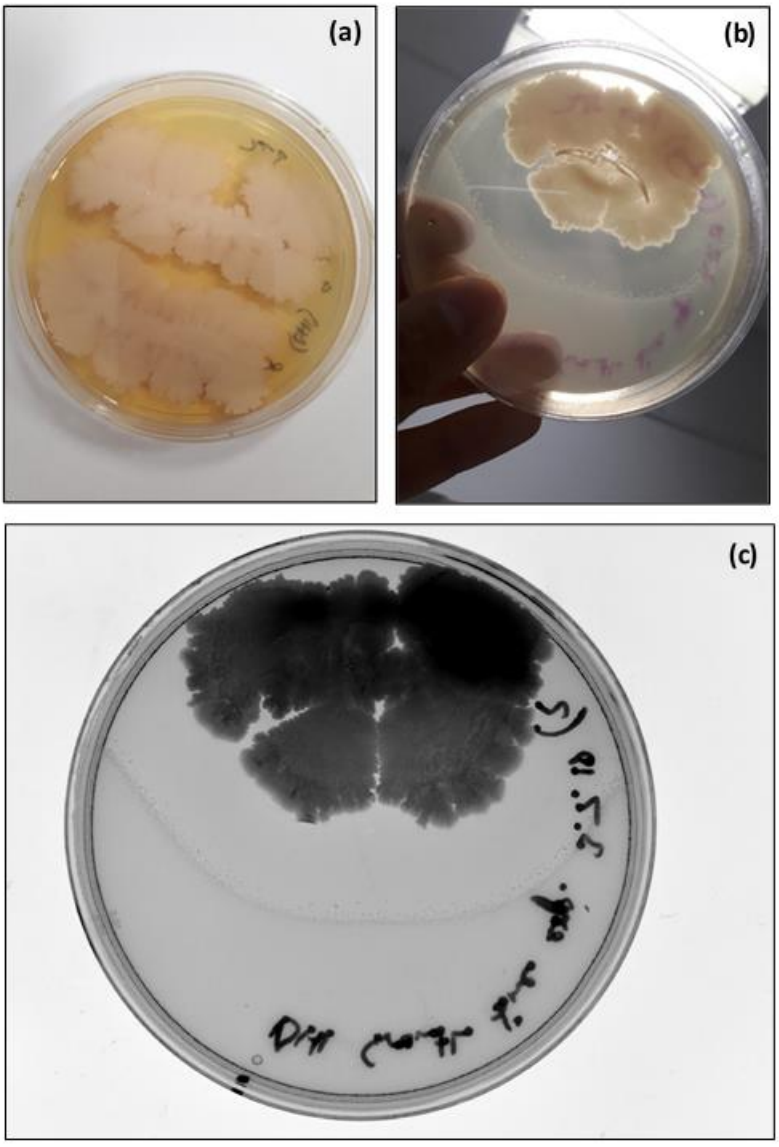


Figure 3: *Bacillus* sp. identified using 16S sequencing and its effectiveness against an overlay of *Clostridium difficile*. (a) shows a BHI agar plate containing a pure culture of the *Bacillus* sp. (b) and (c) both show a clear zone of lysis in an overlay of *C. difficile*, with the *Bacillus* organism present at the top of the plate in both images. Image (c) was taken using a ChemiDoc imagining system.

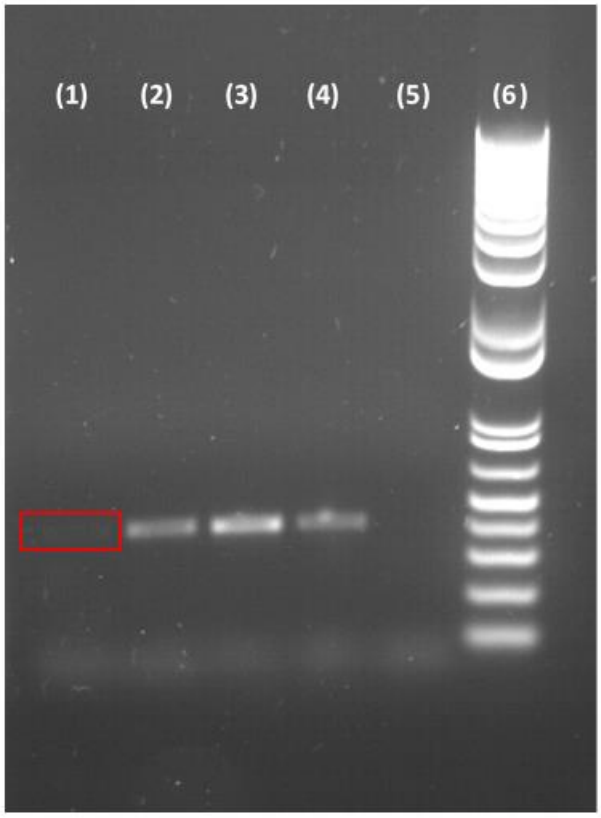


Figure 4: Agarose gel electrophoresis of the PCR product produced after colony PCR of the *Bacillus* sp., with a negative control. Lanes (1-4) contain 16S DNA of the *Bacillus* sp. amplified using colony PCR, with bands at around 400bp. Note that a very faint band is in fact present in lane (1), which is shown in a red box. Lane (5) contains a negative control, with no colony DNA to act as a template for the PCR reaction. Lane (6) contains a DNA ladder.

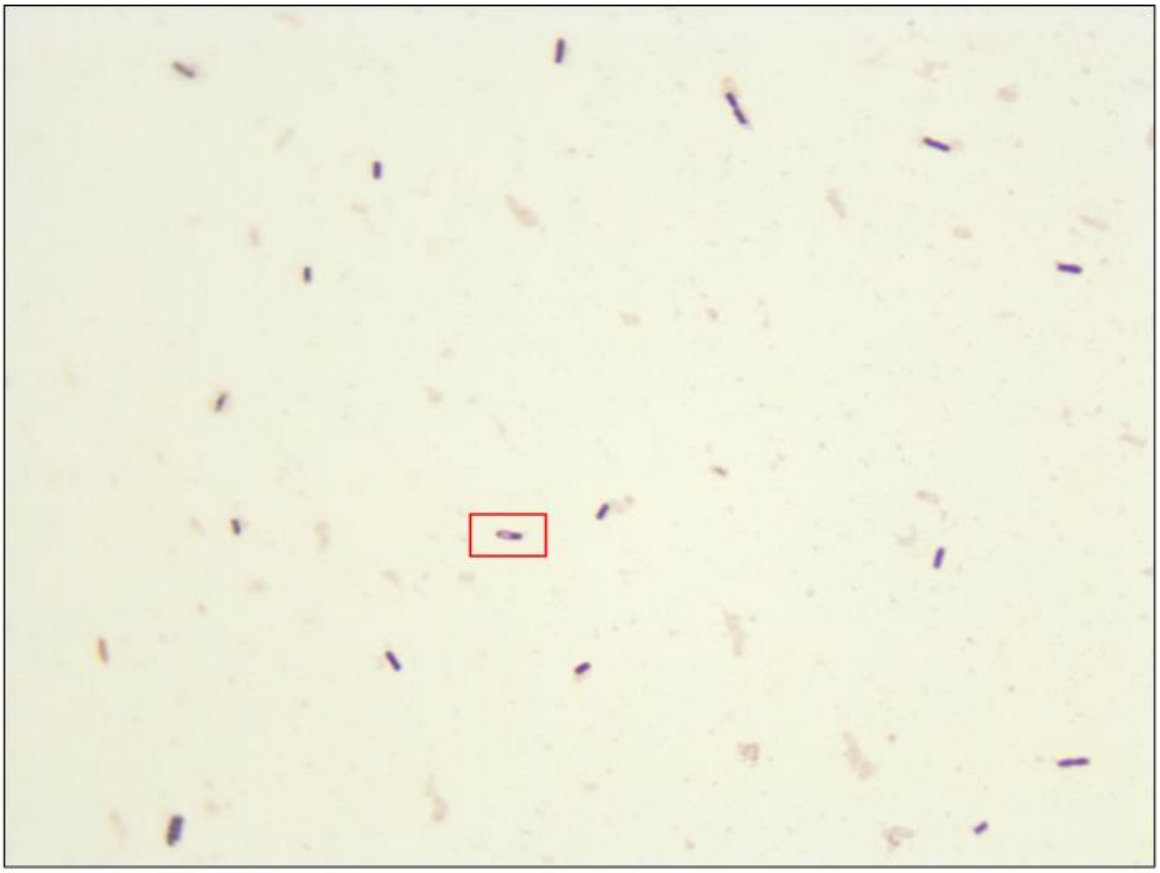


Figure 5: Microscopy of Gram stained *Bacillus* sp. Gram-positive rod-shaped bacteria are visible, which equated with 16S sequencing results. Note that there is a bacterium forming a spore (shown in a red box, with the spore appearing as a clear spot within the purple-coloured bacterium) which is an environmental response associated with bacteria including those of the genus *Bacillus*.

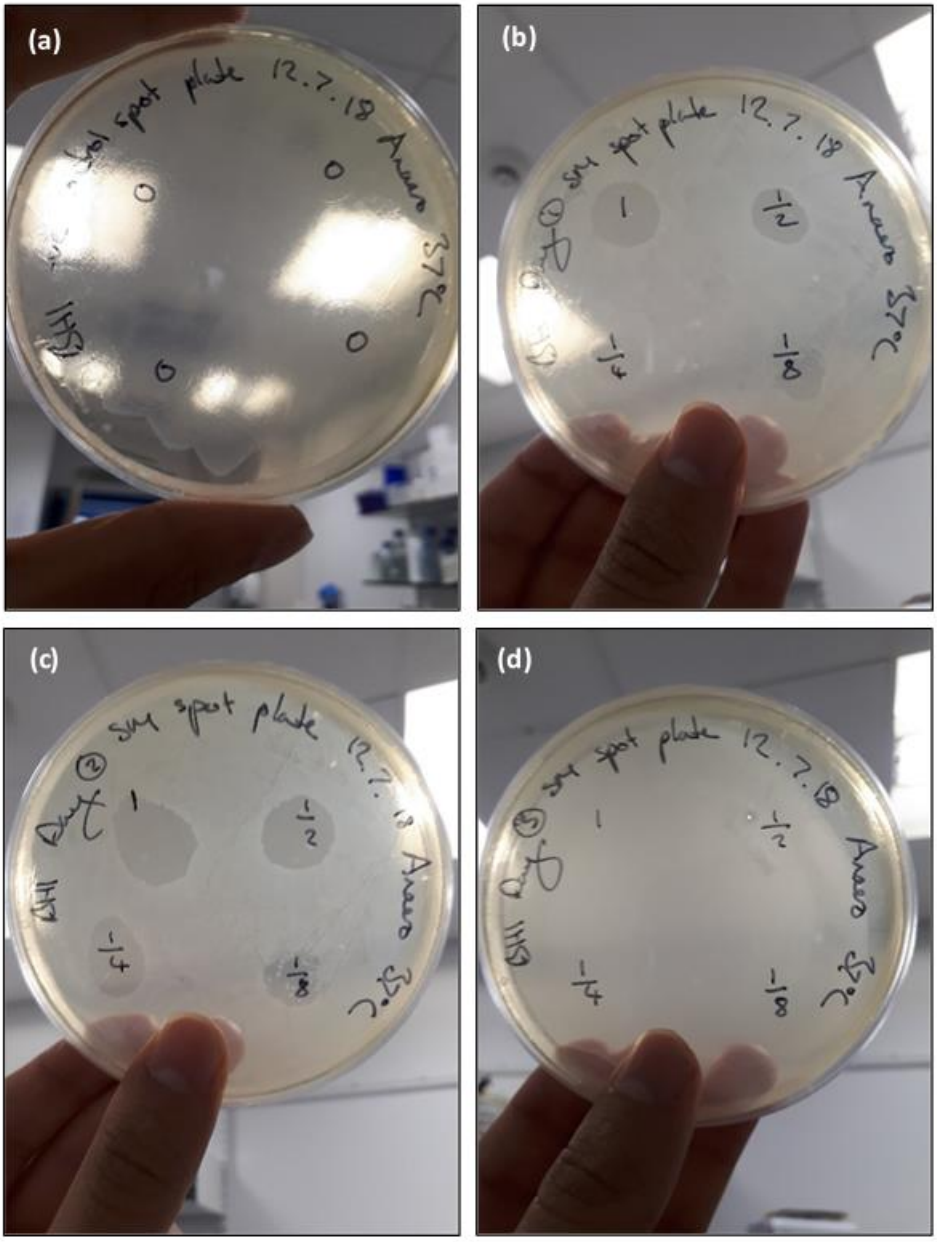


Figure 6: Spent media harvested from the *Bacillus* sp. spotted onto *C. difficile* spread plates to examine whether any antimicrobial compound produced by the *Bacillus* sp. was excreted into spent media. (a) shows a negative control (spent media concentration of 0). (b) shows spent media of four concentrations (1, ½, ¼ and ⅛) harvested after one day of *Bacillus* sp. growth which were spotted onto a *C. difficile* spread plate. (c) and (d) show the same as (b) but with spent media harvested after two and three days of *Bacillus* sp. growth respectively. Note that while spent media harvested after one and two days appears to inhibit the growth of *C. difficile*, spent media harvested after three days appears to have no effect on *C. difficile* growth. This is thought to be the result of experimental error.

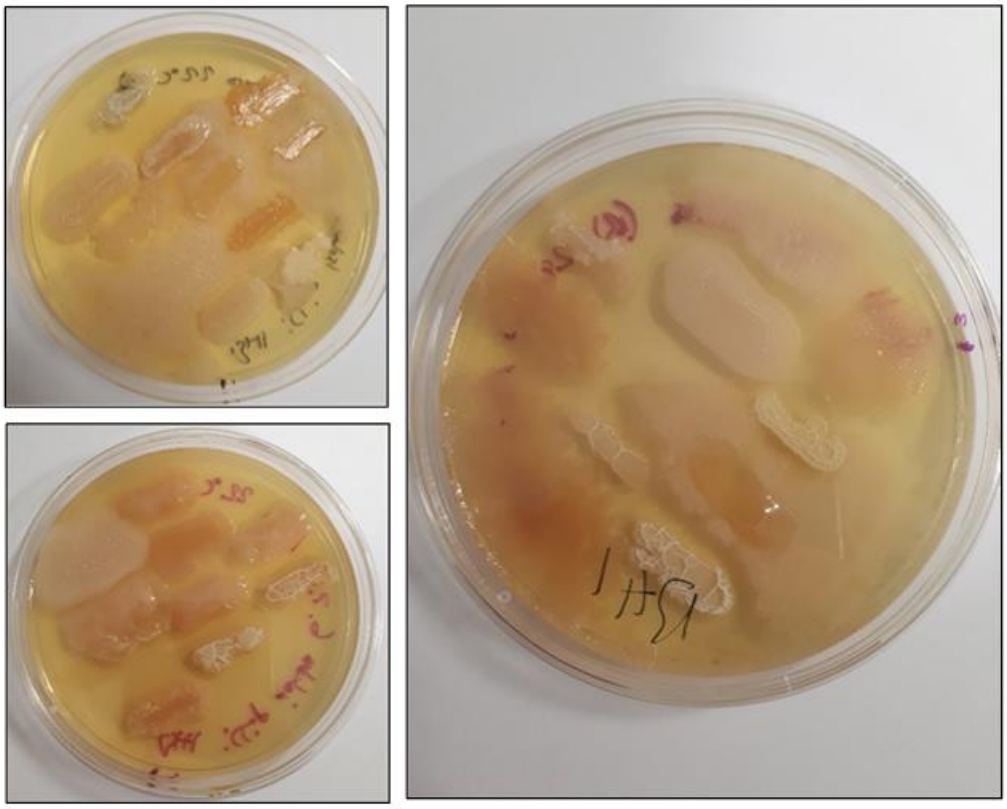


Figure 7: Selection of colonies cultured using the iChip method to illustrate the variety of colony morphologies of bacteria recovered. Compare to Figure 1, which shows the variety of colony morphologies of bacteria recovered from soil using a standard technique.