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優勝作品專輯

作品編號 **070011**

參展科別 **微生物學**

作品名稱 **Bacteria with Headphones**

得獎獎項

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1 ABSTRACT

1.1 Inspiration for the project

I first found out about the Young Scientist competition last year, in 2021. I thought it was an interesting and challenging opportunity; something that could take students out of their comfort zone.

I researched for some inspiration online and found a website with all the different types of project ideas.

While reading about the experiments, I stumbled upon a project relating the music to bacterial growth. It sounded ridiculous at first, but I soon realized it was the right project for me. It intertwined music and biology- form of art I've been involved in since the age of 9, and one of my favorite subjects and a field I want to work in in the future.

1.2 Effects of music on living beings

External stimuli such as sound influence all living beings every day. A study was conducted in Spain in 2007 investigating the milk production in cows that were exposed to Mozart. It was shown that milk production in these cows increased to 30 – 35 liters of milk per day. Additionally, it has been claimed that people's health condition and intelligence has become better when listening to Mozart's music. (Makiello, 2012).

It has also been shown that sound affects the cell growth in microbes. In Piyasena *et al.*'s experiment in 2003, ultrasound has been used for sterilizing and killing off unwanted bacteria due to the thinning of the cell membranes and other factors (Piyasena *et al*., 2003). A study investigating the effect of Indian classical music on microbial growth, production of certain metabolites and antibiotic susceptibility, proved that all the bacteria, except *S. marcescens,* showed better growth under the exposure of music (Sarvaiya *et al*., 2017).

2 INTRODUCTIONS

2.1 Escherichia coli

For my project I have used *Escherichia coli* as it was easily available at school. *E. coli* is 'the first choice for researchers to investigate numerous basic biological processes which are essential for life and is the most extensively used organism in molecular genetics' (Wei Yunlin *et al.*, 2014). Initially, I tested the growth of *Bacillus subtilis* too, but it did not grow well compared to the *E. coli*. *E. coli* also has 'simple nutritional requirements' and 'rapid growth rate' with a rate of cell division of an average of 'once in every 30 minutes.' This is because it reproduces via binary fission and so its 'population undergoes exponential growth' (Wei Yunlin *et al.*, 2014).

2.2 Music

To make sure that I do not repeat the same project as other scientists that have used the *E. coli* breed, I have tried to use several different genres and styles of music. I have tested classical music, hip- hop, jazz and EDM. These genres of music differ in styles and vibrations.

According to (Hongbo *et al.*, 2008) sound at a proper frequency and intensity can promote cell division. A study in 2009 showed that all the chosen frequencies, (1 kHz, 5 kHz, and 15 kHz) have increased the number of bacteria, with bacteria responding the most to the frequency of 5 kHz. The researchers have therefore concluded that 'the degree of their respond is different to a different sound frequency' (Ying et al., 2009). From this we can infer that the frequency of different genres is what could make the difference in bacterial growth.

Another factor in music that could influence the bacteria are different tempo measures like Allegro,

Presto and Adagio (Bauman, 1991), or the musical mood and lyrics as shown 'the more varied music's wave components are' including meaning of lyrics and the mood, 'the stronger the effects of microbial substrate utilization would be' (Pornpongmetta & Thanuttamavong, 2010).

3 TARGETED OUTCOME

3.1 Hypotheses

In this experiment I wanted to investigate the effect of different genres of music on *E. coli*. I used 2 different volumes as an additional condition. My experiments showed that classical music and hip hop had the greatest impact on the bacteria. Frequency for classical music is about 440 Hz as classical instruments are tuned at this frequency, so in this case, frequency could play an important role in bacteria's growth.

3.2 Variables

The independent variable is the music including all the different genres (classical, hip hop, jazz and EDM) and the volume of the music.

The dependent variable is the growth of bacteria.

The control group did not listen to any music.

I had several controls including the duration of exposure to music (about 8 hours a day for 4 days a week), the volume used (either full volume or half of the volume), the incubator they grew in, the music (different genre every week).

4 METHODOLOGIES

4.1 Materials

(All of the equipment was sterile)

Materials for pouring the plates:	Materials for spreading plates:
• 250ml beaker filled with water • Hot plate • Melted agar medium in a glass bottle • Ethanol for disinfection • Paper towels • Bunsen burner • Striker • Plastic Petri dish • Cloth	• Paper towels • Bunsen burner • Striker • L-shaped cell spreader • Plastic pipette • Marker • Bacteria culture (*Escherichia coli*)

4.2 Procedure

4.2.1 Procedure for pouring plates

1.) Fill a 250ml glass beaker with water about halfway full (enough to cover the agar and heat it up).
2.) Heat up the hot plate.
3.) Take out the agar from the fridge and place it in the 250ml beaker.
4.) Heat until the agar has completely melted (approximately about 20 minutes).
5.) While the agar is melting, set up the area for pouring plates.
6.) Disinfect the area thoroughly using paper towers and ethanol and make sure to put ethanol away once the area has been sterilized.
7.) Place the Bunsen burner in the middle of the sterilized area and turn it on using a striker.
8.) Place the Petri dishes around the Bunsen burner.
9.) Once the agar has melted, carefully take it off the hot plate from the beaker using cloth for protection from the heat.
10.) Sterilize the agar bottle by putting the top over the Bunsen burner and turning it around a few times. Remove the top part of the Petri dish and pour agar. Make sure it is about 1- 2cm deep.
11.) Put the top part back on but do not cover completely- make sure to leave some space. This is to prevent

condensation and contamination by molds.
13.) Repeat steps 10 and 11 with the rest of the Petri dishes.
14.) Wait for the agar to cool down. Once it has solidified, cover the Petri dishes completely with the top part and put them in a fridge.
15.) Sterilize the agar bottle again, then turn off the Bunsen burner, put it away and disinfect the area once again with ethanol and paper towels.

4.2.2 Procedure for spreading plates

1.) Disinfect the area thoroughly using paper towers and ethanol and make sure to put ethanol away once the area has been sterilized.
2.) Place the Bunsen burner in the middle of the sterilized area and turn it on using a striker.
3.) Take the Petri dishes (with agar) from the fridge and place them around the Bunsen burner.
4.) Label the bottom part of each Petri dish with a marker (I wrote the date, bacteria culture, control/music, initials of my name, and FJSL).
5.) Open the bottle with the bacteria culture and sterilize it by rotating the top part over the Bunsen burner a few times.
6.) Sterilize the cell spreader and the plastic pipette with the Bunsen burner as well.
7.) Remove the top part of the Petri dish, add one drop of the bacteria culture using the pipette and then spread it using a cell spreader
8.) Close the Petri dish and flip it over so that the agar is turned upside down.
9.) Repeat steps 7 and 8 for all the Petri dishes.
10.) When finished, throw away the pipette and the cell spreader, put the plates into the incubator, sterilize the bottle with bacteria culture once more before closing it, turn off the Bunsen burner and sterilize the area once again.

4.2 Procedure for setting up the music:

1.) Download the songs on the mp3 player and select the option that repeats the songs once the playlist has reached the end. (The mp3 players used Lenco Xemio-760BT with 8GB and mpman MPFOL15 with 4GB)
2.) Connect the headphones to the mp3 player and put them over the plates in an incubator at around 8:00h.
3.) Take the headphones off at around 16:00h so that the bacteria were exposed to the music for around 8 hours.
4.) Repeat step 3 for another 3 days in the week, then change the music type and/or volume the following week.

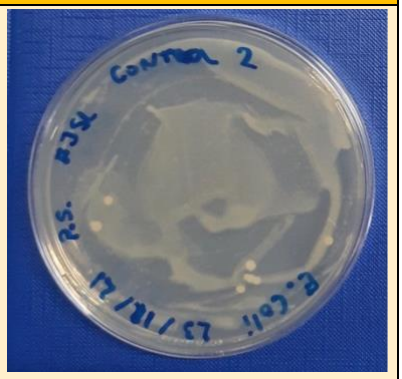
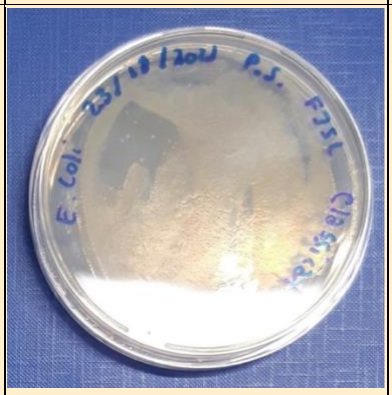
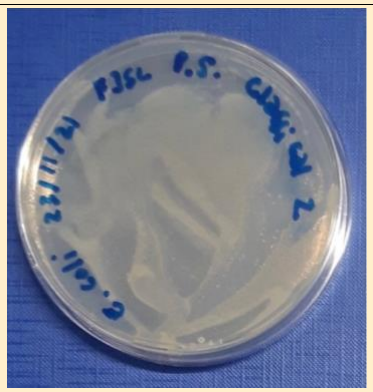
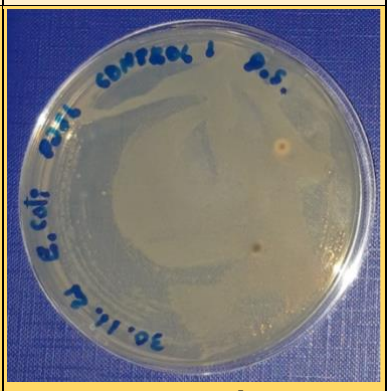
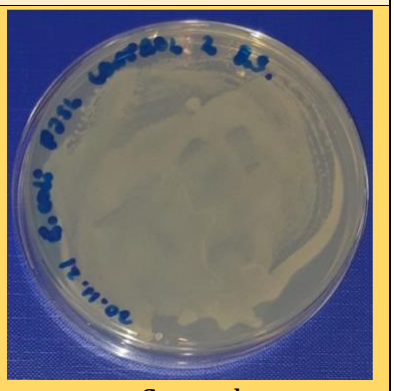
4.2.4 Timeline and details of the project

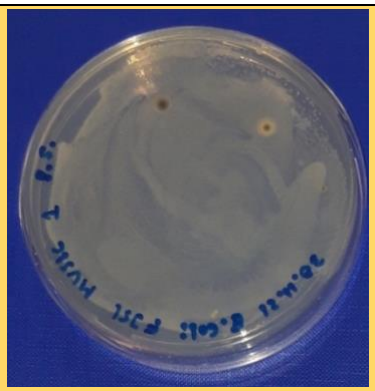
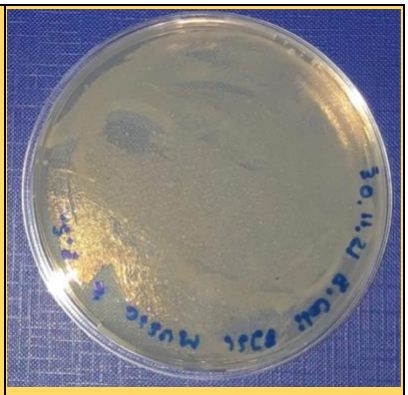
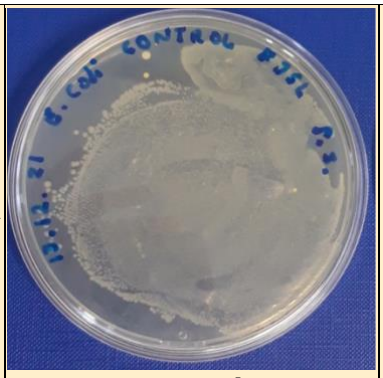
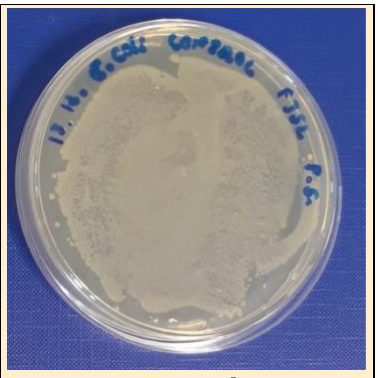
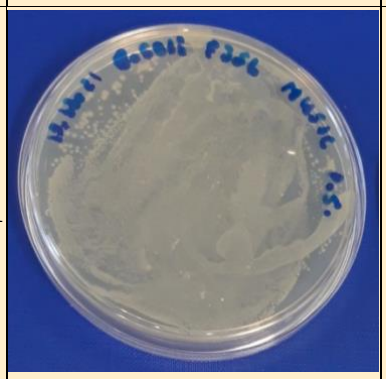
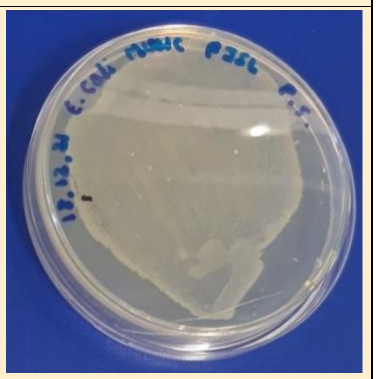
Experiment	Growth period	Genre of music	Volume	Temperature/°C	Additional info.
1.)	23.11.2021 – 26.12.2021	Classical	15/30	21	Earbuds used
2.)	30.11.2021 – 03.12.2021	Classical	30/30	21	Headphones used
3.)	13.12.2021 – 16.12.2021	Hip hop	15/30	21	Headphones used

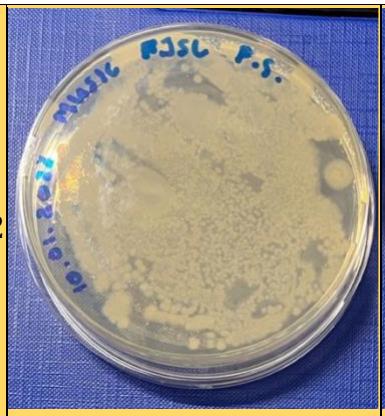
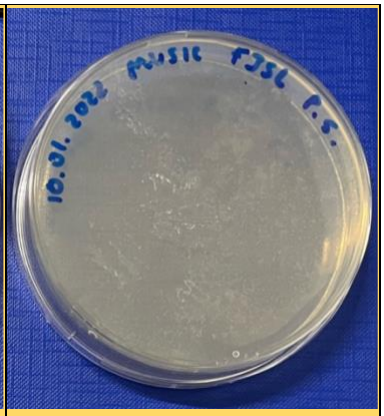
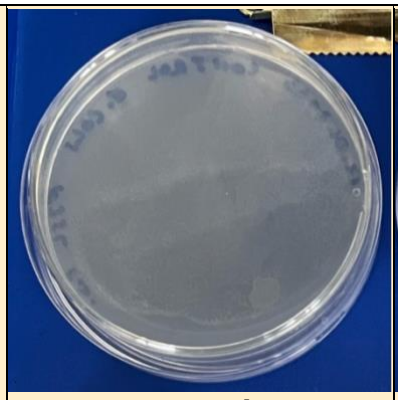
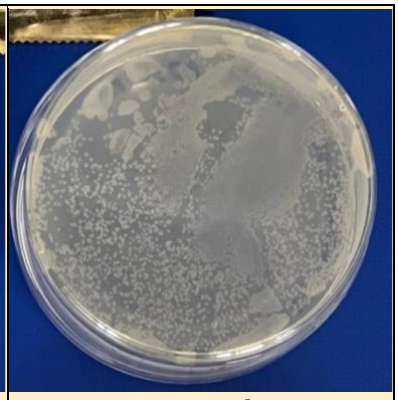
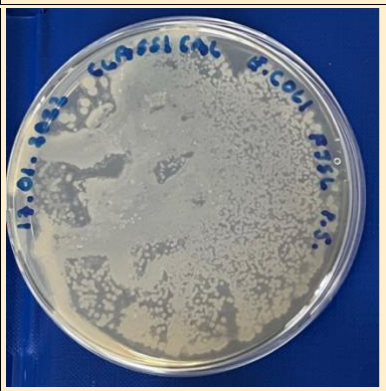
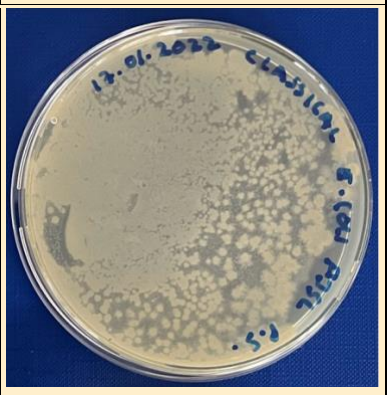
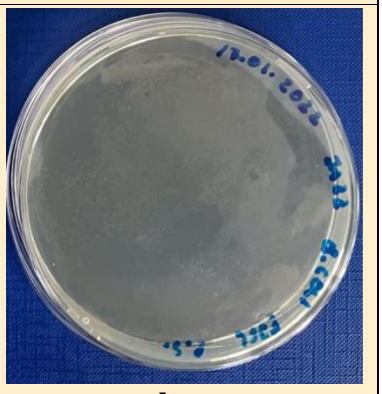
4.)	10.01.2022 – 13.01.2022	Hip hop	30/30	36	Headphones used
5.)	17.01.2022 – 20.01.2022	Classical, jazz	Classical: 30/30 Jazz: 15/24	36	Headphones used
6.)	01.02.2022 – 04.02.2022	EDM, jazz	EDM: 15/30 Jazz: 24/24	36	Headphones used
7.)	(To be finished after the midterm)	EDM, classical	EDM: 30/30 Classical: 15/30	36	Headphones will be used

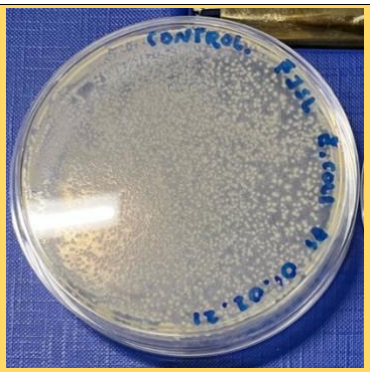
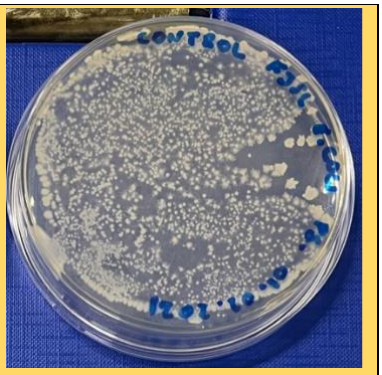
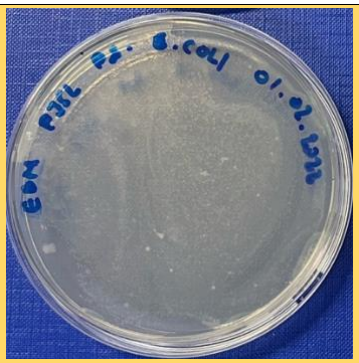
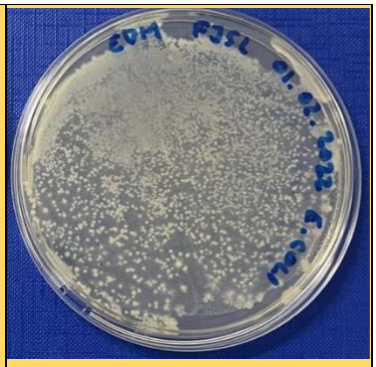
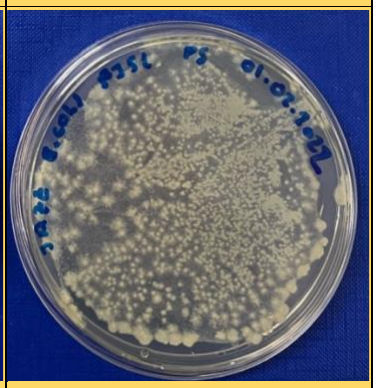
5 OUTCOMES

5.1 Results:

Growth period	Condition 1	Condition 2
1.) 23.11.2021 - 26.12.2021	 Control	 Control
1.) 23.11.2021 - 26.12.2021	 Classical music	 Classical music
2.) 30.11.2021 – 03.12.2021	 Control	 Control

2.) 30.11.2021 – 03.12.2021	 Classical music	 Classical music
3.) 13.12.2021 – 16.12.2021	 Control	 Control
3.) 13.12.2021 – 16.12.2021	 Hip hop	 Hip hop
4.) 10.01.2022– 13.01.2022	 Control	 Control

4.) 10.01.2022 – 13.01.2022		
	Hip hop	Hip hop
5.) 17.01.2022– 20.01.2022		
	Control	Control
5.) 17.01.2022 – 20.01.2022		
	Classical	Classical
5.) 17.01.2022 – 20.01.2022		
	Jazz	Jazz

6.) 01.02.2022– 04.02.2022	 Control	 Control
6.) 01.02.2022 – 04.02.2022	 EDM	 EDM
6.) 01.02.2022 – 04.02.2022	 Jazz	 Jazz

5.2 Discussions and possible improvements

The experiments 1 and 2 were a 'test run' for the project and were used to figure a lot of things out. For experiment 1, I used earbuds for the experimental group and as there was no difference between the 2 conditions, I decided to try it out again but with headphones this time (experiment 2). Headphones have a bigger surface area so I thought they could therefore stimulate the bacteria more effectively. There were also some technical difficulties with the format when we tried downloading the music onto the mp3 player.

This made a slight difference in experiment 2. Control group seems to have a little less colony than the experimental group. Therefore, in experiment 2 classical music did slightly increase the growth.

In experiment 3, hip hop condition did slightly better as we can see in the first plate, but not much difference can be seen in the second plate compared to the control groups. However, the first plate in the hip hop condition in

experiment 4 did much better compared to the control groups. This could be due to the louder volume. Higher temperature could have also played the part in the increased growth in all the following experiments. Perhaps headphones themselves warmed up the plates a bit because they cover quite a bigger area compares to earbuds. I am not sure why there was no growth at all in the second plate, but it could be due to the lack of sample when I added it in the first place.

For the fifth experiment, the same problem happened with complete lack of growth for one of the control and jazz conditions. However, we can see that classical music conditions did significantly better than the second plate in the control group. Jazz condition did better than the control group as well, but not as good as classical music.

In the sixth experiment we can spot that the difference between jazz and control conditions is not significant even though I have used a louder volume. EDM condition did about the same as the control group and one of the plates here did not work out either.

Possible improvements here could be to standardize the procedure even further by using the same mp3 players and the same headphones because the technical differences possibly played a part in the results. Equally, the same temperature should have been used for all the experiments. It would be good to repeat all these at least 3 times to ensure reliable results. There was contamination in a few plates so to prevent that from happening, we need to make sure to take all the necessary measurements to work in a sterile environment (although I have sterilized the environment the best I could, so I am not sure where the contamination came from). Another major improvement could be using liquid medium and therefore measuring the results from the broth culture using a spectrophotometer or colorimetric assays in order to get quantitative data which is more accurate and precise than qualitative data I have collected.

5.3 Explanations

There are several different explanations as to why music affects growth. Kotwal *et al*., describes the music as 'sound waves which are mechanical in nature' and explains that it 'acts as a mechanical stress' (Kotwal *et al*., 2016). Additionally, music can be seen as 'a disturbance transported through a medium via particle interaction' (Sarvaiya *et al.*, 2017).

These mechanical stimuli may have several different effects on the bacteria including the transfer of energy and 'promoting the absorption of nutrients and combination of DNA in the "S" phase, as well as synchronizing the cell cycle' (Zhao et al, 2003).

Music can also modulate traffic in the membranes and speed up the metabolic activity (Apodaca, 2002), and membrane fluidity can increase and make changes in the protein structure of plasma membranes (Zhao et al., 2002).

Other results of music on bacteria include increased production of pigments e.g., violacein and prodigiosin and a change in intracellular concentration of calcium.

The repeated expansion and contraction which may be sensed by neighboring cells, essentially causes the cells to grow. It is however not known what mechanisms bacteria use to 'hear' the music- 'cells do not possess receptors on the membrane which can sense mechanical pressure' (Sarvaiya *et al.*, 2017).

5.4 Conclusions

Ultimately, although the procedure was not extremely standardized at first (controls were added on throughout the experiment), I did get some results. The condition that did the best would be classical music in experiment 5, followed by hip hop in experiment 4, so some musical genres indeed to influence bacteria. Therefore, music does indeed improve the growth of bacteria. Ying *et al*. found that 'sound treatment at all selected frequencies have increased the viable cells compared to control samples' (Ying *et al.*, 2009) and results from Kotwal *et al.*, agree by stating that the music has enhanced bacterial growth in their experiment (Kotwal *et al*., 2016). Both experiments used *E. coli,* and both showed an increased growth in bacterial cells, just like my experiment did.

These results can perhaps be used on a larger scale. Sarvaiya *et al*. suggest that music could be used in fermentation industry or accelerating waste-treatment by microbes (Sarvaiya *et al.*, 2017).

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I would also like to thank the members of the Fondation Jeunes Scientifiques Luxembourg for giving students like me a chance to experience the world of research for the first time and encourage us to take on the scientific pathway.

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【評語】070011

The author was intrigued to analyze whether music affects living organisms' growth by the cultivation of the simplest bacteria，E. coli，with different music. The author also elucidated several explanations for why music affects cell growth. It was an interesting preliminary work. However，the experimental results did not provide evidence for the effects because most bacteria spread on agar plates seem to be too many to count. The author suggested using broth culture with a spectrophotometer or colorimetric assays to get better quantitative data. The result is strongly encouraged to be included.

Suggestions：

1. It is recommended to define the condition for disinfection，such as the percentage of ethanol. You may need an autoclave and a colony counter to help you effectively sterilize your equipment and count colonies.
2. The abstract should be a summary of the project rather than an introduction.