Impact of Attributes of Music on Growth of Escherichia coli

Colsen H. Nguyen, Paiton Ransibrahmanakul

Received August 29, 2024
Accepted January 24, 2025
Electronic access February 15, 2025

Background / Objective: Music accelerates bacterial growth. However, it is unclear what attributes of music have the greatest impact on growth. We examined the impact of specific attributes of music including tempo and sound intensity of musical genres (Heavy Metal music, Western Classical music, and Popular music) on the growth of E. coli.
Methods: We used various applications to measure the attributes of three different songs representing Heavy Metal, Western Classical, and Popular music. We conducted bacterial growth experiments in soundproof chambers and measured optical density of bacterial solutions at time 0, 60, 120, 150, and 180 minutes to determine the musical genre with the greatest growth. We analyzed the musical genres to determine which musical attributes were associated with growth.
Results: We found that Heavy Metal music accelerated E. coli growth more than Western Classical or Popular music. We did not find that frequency or pitch influenced bacterial growth. However, the dose of sound intensity greater than 80dB and tempo were associated with increased bacterial growth.
Conclusions: This is the first study to show a correlation between sound dosimetry and tempo on bacterial growth.

Introduction

Music has been described to have both positive and negative effects on cells and living organisms. Studies have examined various genres of music including Western classical music (Amadeus Mozart), Indian classical music (Gayatri Mantra, raag Miyan-Malhar), and Heavy Metal (Metallica).[1] Each of these music genres has been shown to increase bacterial growth. However, not all sound increases growth. White noise, a heterogenous, wide-ranged mixture of sound frequencies, was found to have a negative influence on mice and the gut microbiota of mice, specifically a decreased proportion of Firmicutes in the microbial composition of the gut.[2] While each of these songs has a range of frequencies, specific single sound frequencies have also been studied and shown to increase bacterial growth.[3] Different biomechanical processes have been proposed for the ability of sound to increase growth, including increased oscillation of cells, mechanical stress on the membrane of cells, and different pressure distributions in the membranes of different cell types. Current research in sonomechanobiology suggests that cells respond to vibrations via mechanosensing mechanisms like proteins embedded at the cell extracellular matrix interface that are sensitive to membrane stiffness changes or mechanosensitive ion channels. These mechanosensors trigger intracellular changes including calcium signaling, phosphorylation cascades, and DNA transcription factor binding that results in the ability of the cell to transcribe the detected and transmitted mechanical cues into complex transcriptomic and signaling events[4] While the prevailing research conclusions are that bacterial growth is stimulated by specific frequencies of sound, other attributes of music have not been studied. Music contains a variety of attributes including melody (high frequencies that are the most recognizable), bass (low frequencies that drive the song forward), sound intensity (amplitude of sound waves that affects how loud or soft the song is), and tempo (the speed of the song). Each of these proposed biomechanical processes of stimulation of growth may be influenced by different characteristics of music. We propose to describe the correlation between different musical attributes on bacterial growth. For example, it is unclear if higher frequencies (melody) or sound intensity may have a greater impact or correlation with bacterial cell growth. A better understanding of the attributes of music and their impact on bacterial growth may inform further research into specific biomechanical mechanisms to stimulate growth.

Methods

The complete experiment was divided into two parts. The first part was an evaluation of the music attributes that we chose. The second part was an evaluation of how each song performed in facilitating bacterial growth. After determining which song accelerated bacterial growth the most, we split that top growth-stimulating song into its components, or stems, to determine which specific attributes of that song has the greatest impact on bacterial growth. We compared the instrumental/vocals stem to the bass/drums stem because the instrumental/vocal stem contains higher frequencies and the bass/drum stem contains lower frequencies. We analyzed the E. coli growth for the three songs and for the two stems by periodically measuring the turbidity of the bacterial solution and converting it to bacterial count.

Evaluation of Music

The three songs, representing respective genres, that we used for our experiment were "Master of Puppets" by Metallica (Heavy Metal), "Symphony No. 9" by Ludwig van Beethoven (Western Classical), and "Wildest Dreams" by Taylor Swift (Pop). To determine how one genre affected bacterial growth more than others, we first analyzed each of them. These songs have different ranges of frequencies in hertz (Hz), tempos in beats per minute (bpm), and sound intensity in decibels (dB). We used the app Decibel X (https://apps.apple.com/us/app/decibel-x-db-sound-level-meter/id448155923) to measure the sound intensity and frequency, Pulse (https://apps.apple.com/us/app/pulse-metronome-tap-tempo/id1097323003) to measure the tempo, and FL Studio (https://www.image-line.com/fl-studio) to separate a song into its musical stems: instrumental/vocals and bass/drums.

Soundproof Chamber

Soundproof chambers were made with storage boxes lined with soundproof foam panels (Amazon, ASIN: B08QN6B2FD). Each chamber was large enough to contain an incubator. Each incubator (Amazon, ASIN: B0BPYDQ3VD) was large enough to contain a test tube rack for the test tube containing the E. coli bacterial solution as well as a Bluetooth speaker. The speakers (Amazon, ASIN: B00D89H1NO) were calibrated to deliver a similar volume in decibels.

Bacterial Growth

E. coli strain ATCC 25922 was ordered from Biomerieux and grown 24 hours prior to the experiment on a blood agar plate (Hardy Diagnostics 5% sheep blood) at 37° C. Nutrient broth (Hardy Diagnostics peptone and beef extract) was also prepared before the experiment. To make the E. coli bacterial solution, 8 mL of the nutrient broth was placed into a test tube using a 1 mL Eppendorf variable volume pipette. Then, two colonies of E. coli from the agar plate were taken and placed into the nutrient broth test tube and vortexed. Next, the E. coli bacterial solution was pipetted into four different test tubes, 2 mL each. We measured the baseline turbidity of each test tube by pipetting 125 μL from each test tube into a 96 microwell plate with a 1 μL Eppendorf variable volume pipette. Each of the tubes was prepared from the same solution. Using a spectrophotometer, we measured the turbidity of the bacterial solutions at 600 nm. (baseline measurement). We placed paraffin on the tubes and put the tubes into the incubators in the soundproof chambers. Three soundproof incubators played music with Bluetooth speakers and the fourth was in silence (control).

Bacterial Growth and Turbidity Measurement

After an hour of growing the bacteria, we took the test tubes out of the incubators. We vortexed each of them and pipetted 125 μL of each of the solutions into a 96 microwell plate using the 1 μL Eppendorf variable volume pipette. We replaced paraffin on the test tubes and placed them back into the incubators. We measured the turbidity of the bacterial solutions in the microwell plate using a spectrophotometer run at 600 nm. We repeated this process at the 120-minute, 150-minute, and 180-minute marks.

Conversion from Optical Density (OD) to Bacterial Count

We used the E. coli Cell Culture Concentration from OD_{600} Calculator by Agilent[5]. This calculator takes the OD_{600} value and gives the number of bacterial cells/mL. The conversion is based on the Beer-Lambert Law that relates the concentration of a sample to the attenuation of light as it passes through the sample, and it is the most common measurement in microbiology laboratories to assess microbial growth in a solution[6]. We graphed the number of bacterial cells of E. coli/mL over time in response to the musical selections.

Best Fit Line for Growth Rate

Using the LINEST function from Microsoft Excel, we graphed the best fit line using the existing data points for each experiment[7].

Results

Music Attributes

We chose three broad genres of music, Heavy Metal, Western Classical, and Popular Music, because of the diversity in the music attributes in order to better distinguish how different attributes may contribute to bacterial growth. The heavy metal song, "Master of Puppets," had the fastest tempo, followed by the Popular music song, "Wildest Dreams," and the Classical piece, "Symphony No. 9." Each of the songs had overlapping sound frequency ranges, particularly between 3000-4000Hz (Table 1, Figure 1). While each of the musical genres had overlapping frequencies, our Pop music selection had the greatest range of frequencies, followed by Classical music. Heavy metal had the shortest range of frequencies. Heavy metal had the fastest tempo followed by Pop and Classical (although Classical had a wide range of tempos throughout the piece). When examining sound intensity, although each of the songs had varying levels of loudness throughout the song, Heavy Metal had the overall greatest loudness as compared to Classical and Pop music. To normalize the amount of sound intensity, we measured the dose of loudness over 80dB as projected over 1 hour and found that Heavy Metal would have the greatest dose, followed by Pop and Classical. Of note, while the Classical music piece had a greater dose over one playthrough, it was much longer than the Pop music piece. As a result, the Pop music piece would have had a greater projected dose over 1 hour compared to Classical (Table 1).

We also examined the varying sound intensities of each of the music selections over a range of frequencies (Figure 1). While Heavy Metal and Pop music had two separate peaks of sound intensity (likely representing the bass at the lower frequencies and the melody at higher frequencies), Classical music had a more even distribution of sound intensity over a wider range of frequencies. The instrumental/vocals stem and the bass/drums stem of the Heavy Metal sample were similar in tempo, sound intensity, dose, and projected dose over 1 hour, but were different in frequency ranges (Table 1). As expected, the instrumental/vocals stem frequencies were higher than the bass/drums stem frequencies.

The Heavy Metal music sample had a very fast tempo with a driving, punching beat (Table 1). This resulted in a greater sound intensity, characteristic of the Heavy Metal genre. Another characteristic of Heavy Metal that is included in our sample is distortion, a way to modify the sound of an electric instrument to last longer at a similar intensity. This makes it feel louder without increasing volume. This also contributed to the greater sound intensity. The Classical music sample had variations in tempo and different sound intensity throughout the piece, including slow and quiet as well as fast and loud. The Pop music sample had a moderate tempo with no distortion and a natural decay in sound over time with each note played. This contributed to a lower dose at the same volume as the other samples.

Bacterial Growth

Heavy Metal Music showed the greatest slope (fastest rate) in bacterial growth followed by Classical Music and Popular Music as compared to silence (Table 3, Figure 3) over 180 minutes of growth. All of the musical selections had a greater slope of growth as compared to silence. When we separated the music stems of the heavy metal song, "Master of Puppets" we found nearly identical slopes of growth in comparing the instrumental/vocals stem as compared to the bass/drums stem (Table 3, Figure 4).

Conclusions

Music has been shown to increase bacterial growth in 7 different organisms including Xanthomonas, Chromobacterium, Serratia marcescens, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus mutans, Saccharomyces cerevisiae, and Candida albicans in response to Indian classical music (raag Miyan-Malhar).[8]. Our results conclude that Heavy Metal music had the fastest rate of bacterial growth over time of E. coli as compared to Classical or Pop music. Each of the musical selections had greater rates of growth compared to silence. To better understand the attributes of music that may have greater impact on bacterial growth, we compared the attributes of each of the music selections and found that tempo likely had the strongest correlation to bacterial growth. Additionally sound intensity may also play a role as the projected dose of sound intensity over 80dB also correlated with bacterial growth. This is the first study to find a correlation between tempo and sound dosimetry and bacterial growth. In comparing the instrumental/vocals stem and bass/drums stem of Heavy Metal music, we found that both stems showed comparable rates of E. coli growth. While each of the stems showed a range of frequencies, they did not overlap. This suggests either that there are two or more frequency ranges that stimulate E. coli growth equally or that frequencies may not be the primary driver of growth. Each of the music stems, however, had the same tempo and similar projected sound intensity, and it is possible that these attributes of music may have had more of a driving force in growth than frequency. In previous studies, Shaobin, et al studied three frequencies in a sine tone including 1000Hz, 5000Hz, and 15,000Hz with each increasing E coli growth.[3]. These findings were later supported by Gu, et al showing E coli biomass and growth rate at 1.7x and 2.5x compared to control at 8000Hz (Table 2).[9]. While these experiments used continuous sine waves at the designated frequencies, music is not a constant single sound. Additionally, studies suggest that sound intensity at 100dB slowed growth rate compared to 80dB.[9] The previous study proposes that there may be limitations to a single sound frequency and intensity in bacterial growth. From a mechanobiology standpoint, this may suggest that a single mechanosensing mechanism in cells may have limitations with respect to cell growth. Our study builds upon existing knowledge in the field of mechanobiology. We found that the dose of sound intensity above 80dB was associated with growth, and this parameter may have had the most significant impact on differential growth between the genres. The maximum sound intensity never went above 90dB at any time during our experiment. A faster tempo and sound intensity were associated with faster growth. From a mechanobiology standpoint, our results suggest that there may be other mechanosensing mechanisms corresponding to tempo and sound intensity.

Our study had some limitations. Although a single solution of E. coli was prepared, there was a slight difference in baseline optical density between the test tubes. It is unclear if the minor baseline variations could have impacted growth. Errors in optical density readings could have occurred when taking a sample that has a higher or lower concentration of E. coli than the average of the tube. As we serially obtained the samples from each of the incubators, there may have been slight variation in the amount of time each of the test tubes was in the incubator. Another limitation is that our experiments were not performed in duplicate or triplicate to reduce the amount of variability in measurements. Finally, there may be additional factors like melody or harmony that we did not specifically isolate or study that may have an impact on bacterial growth. This may impact the broader application of these findings. Mechanoreceptors on bacterial cell walls play a role in mechanotransduction, the cellular process by which mechanical stimuli are transducted into biochemical signals.[4]. The activation of these receptors by sound may play a role in accelerating bacterial growth. We demonstrate that doses of sound intensity greater than 80dB and faster tempo may both play a role in activating mechanoreceptors.

Augmenting bacterial growth can have significant impacts. According to the World Health Organization, there are 48.9 million cases of sepsis annually and at least 11 million die as a result, representing 20% of all deaths.[10] The clinical microbiology lab aids in the diagnosis and management of sepsis by growing bacteria and testing growth against various antibiotics. The average turn-around time from collection of a blood culture to an actionable result was 2.71 days.[11] Improving the efficiency of this process by enhancing bacterial growth in the microbiology lab may have a positive impact on patient outcomes by giving actionable results sooner. Industrial microbiology is a field using bacterial growth for the production of everything from cosmetics to beer.[12]Studying ways to improve bacterial growth may have significant implications on efficiency and production.

We conclude that Heavy metal music accelerates the growth of bacteria greater than classical or popular music. The dose of sound intensity greater than 80dB and a faster tempo is associated with faster growth. More research is needed to further delineate how music impacts bacterial growth, but our study suggests a line of experimentation beyond the frequencies of sound and the inclusion of sound intensity and tempo may be more in line with the biomechanical mechanisms thought to underpin the basis of accelerating bacterial growth.

Acknowledgements

The authors wish to thank the clinical microbiology lab supervisor, Susan Simmers, and her staff at VA Northern California Health Care System for their support and Mrs. Nicole Brousseau of Christian Brothers High School for being our sponsor for this project.

References

1 N. Kotwal, J. Damani and J. Damani, *International Journal of Recent Scientific Research*, 2016, **7**, 13205–13208.

2 Z. Zhang, Y. Wu, S. Zhou, P. Fu and H. Yan, *Microorganisms*, 2023, **11**, 2272.

3 G. Shaobin, Y. Wu, K. Li, S. Li, S. Ma, Q. Wang and R. Wang, *Colloids and Surfaces B: Biointerfaces*, 2010, **78**, 367–371.

4 L. A. Ambattu and L. Y. Yeo, *Biophysics Reviews*, 2023, **4**, 021301.

5 A. Technologies, *Bacterial Optical Density (OD) Calculator*, 2025, https://www.agilent.com/store/biocalculators/calcODBacterial.jsp, Accessed: February 8, 2025.

6 J. A. Myers, B. S. Curtis and W. R. Curtis, *BMC Biophysics*, 2013, **6**, 4.

7 M. Support, *LINEST function*, 2025, https://support.microsoft.com/en-us/office/linest-function-84d7d0d9-6e50-4101-977a-fa7abf772b6d, Accessed: February 8, 2025.

8 N. Sarvaiya and V. Kothari, *J Appl Biotechnol Bioeng*, 2017, **2**, 212–219.

9 S. Gu, Y. Zhang and Y. Wu, *Peer J*, 2016, **4**, e1920.

10 W. H. Organization, *Sepsis*, 2025, https://www.who.int/news-room/fact-sheets/detail/sepsis, Retrieved January 4, 2025.

11 Y. P. Tabak, L. Vankeepuram, G. Ye, K. Jeffers, V. Gupta and P. R. Murray, *Journal of Clinical Microbiology*, 2018, **56**, 1–8.

12 H. Onyeaka and O. C. Ekwebelem, *International Journal of Environmental Science and Technology*, 2023, **20**, 4635–4648.

Appendix

Table 1: Characteristics of Music

Genre	Tempo	Equivalent Continuous Sound Pressure Level (Leq)	Frequency Range of Frequencies Above 80 dB	Dose (above 80dB)	Projected Dose (over 1 hour)
Heavy Metal	210 bpm	84.1 dB	3K-5K	2.40%	141.20%
Classical	55-130 bpm	83.0 dB	500 - 4K	2.30%	106.30%
Pop	140 bpm	83.3 dB	800 - 6K	0.80%	117.00%
Instrumental and Vocal Stem	210 bpm	83.6 dB	1.25K - 3K	2.30%	140.10%
Bass and Drums Stem	210 bpm	84.2 dB	3.15K - 5K	2.20%	133.90%

Table 2. Previous Study Examining Frequencies on Bacterial Growth

Study	Frequency	Sound Intensity	Power Level	Comments
Gu, et al	2000Hz	80dB; when above 80dB biomass decreased and growth rate slowed – noted that logarithmic phase growth was extended at 80dB compared to 100dB	55dB; when power level exceeded 61dB both growth rate and biomass were reduced	Increased growth rate and biomass of E coli;
Gu, et al	8000Hz	80dB; when above 80dB biomass decreased and growth rate slowed – noted that logarithmic phase growth was extended at 80dB compared to 100dB	55dB; when power level exceeded 61dB both growth rate and biomass were reduced	Increased growth rate and biomass of E coli; found Increased protein and RNA at 6 hours

Figure 1.

A. Frequency Response Peaks of Heavy Metal ("Master of Puppets")

B. Frequency Response Peaks of Classical ("Symphony No. 9")

C. Frequency Response Peaks of Pop ("Wildest Dreams")

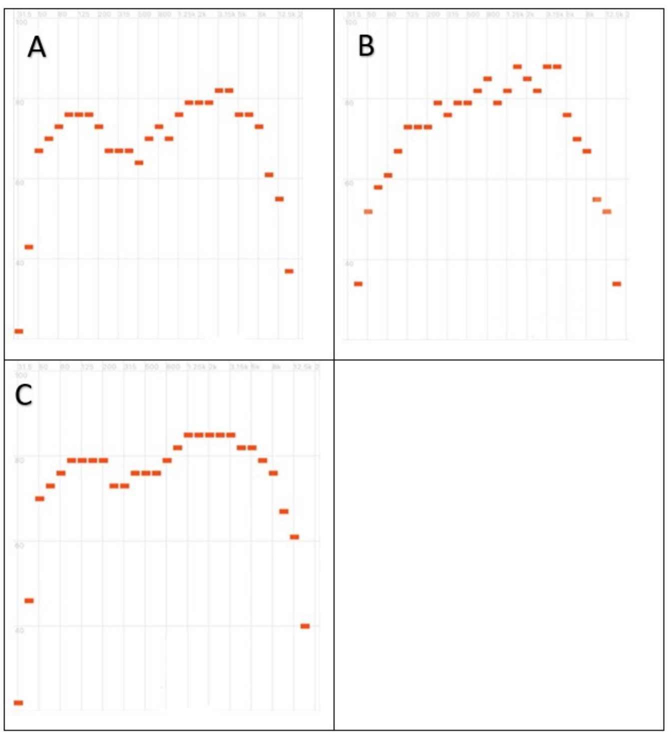
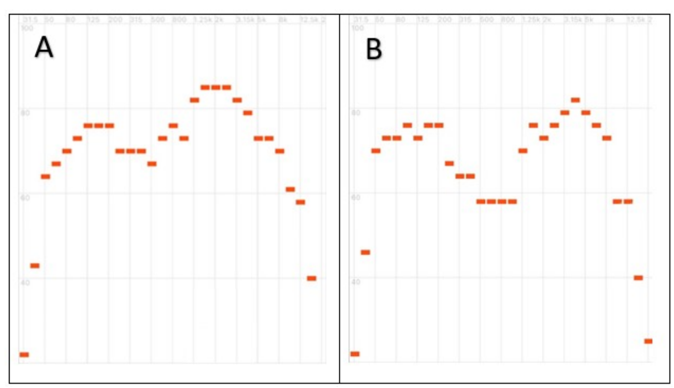


Figure 2.

A. Frequency Response Peaks of Instrumental and Vocal Heavy Metal ("Master of Puppets")

B. Frequency Response Peaks of Bass / Drums Heavy Metal ("Master of Puppets")

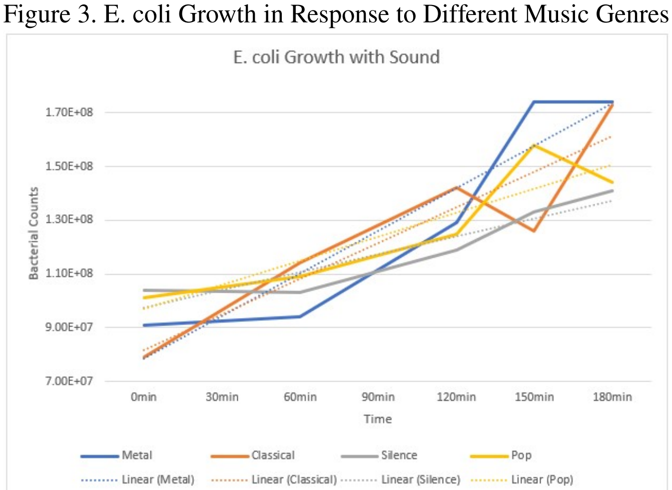


Note. This figure, as well as the other Frequency Response Peaks figures, are all taken directly from the Decibel X app. The x-axis is frequency, and the y-axis is decibels. The orange rectangles represent the sound level peaks for each frequency in the song.

Table 3. OD_{600} Values and Bacterial Count

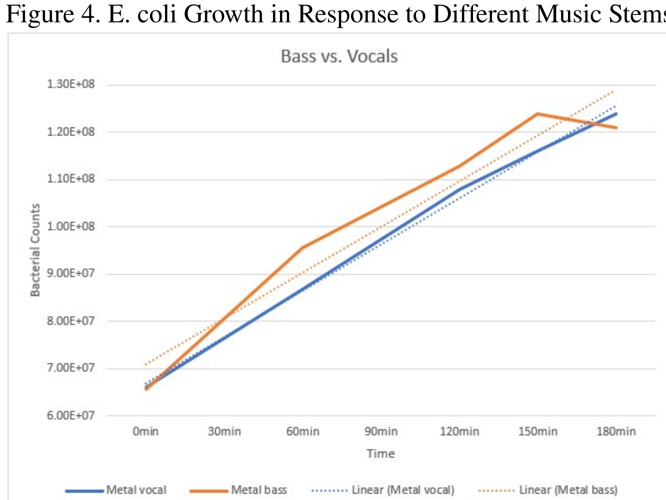
Genre	Time 0	60 min	120 min	150 min	210 min	Growth Rate
Heavy Metal OD600 Value	0.1134	0.1175	0.161	0.218	0.217	2.464E+07 bacteria/min
Heavy Metal Bacterial Count	9.08E+07	9.40E+07	1.29E+08	1.74E+08	1.74E+08	
Classical OD600 Value	0.099	0.143	0.1775	0.158	0.216	1.996E+07 bacteria/min
Classical Bacterial Count	7.92E+07	1.14E+08	1.42E+08	1.26E+08	1.73E+08	
Pop OD600 Value	0.126	0.136	0.156	0.197	0.1805	1.35E+07 bacteria/min
Pop Bacterial Count	1.01E+08	1.09E+08	1.25E+08	1.58E+08	1.44E+08	
Instrumental and Vocals Stem OD600 Value	0.0825	0.1085	0.137	0.145	0.155	1.452E+07 bacteria/min
Instrumental and Vocals Stem Bacterial Count	6.60E+07	8.68E+07	1.08E+08	1.16E+08	1.24E+08	
Bass and Drums Stem OD600 Value	0.082	0.1195	0.141	0.1555	0.151	1.392E+07 bacteria/min
Bass Drums Stem Bacterial Count	6.56E+07	9.56E+07	1.13E+08	1.24E+08	1.21E+08	
Silence OD600 Value	0.1305	0.129	0.1485	0.1665	0.1765	1.04E+07 bacteria/min
Silence Bacterial Count	1.04E+08	1.03E+08	1.19E+08	1.33E+08	1.41E+08	

Figure 3. E. coli Growth in Response to Different Music Genres



Note. The Heavy Metal song "Master of Puppets" showed a greater effect on the growth of the E. coli over 3 hours, followed by the Classical piece "Symphony No. 9," and the Pop song "Wildest Dreams."

Figure 4. E. coli Growth in Response to Different Music Stems



Note. The instrumental/vocals stem and the bass/drums stem of "Master of Puppets" showed similar effects on bacterial growth rate.