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Effect of Different Sound Intensities on the Growth of *Escherichia Coli*

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ABSTRACT
All living beings are surrounded by sound waves. It has been found that these sound waves can impact organic entities. Plants' metabolism, as well as the permeability and selection of the plasma membrane, can be improved by sound waves, impacting their development. Animals can be affected by sound waves as well. A ton of works were done on the impacts of music on non-hear-able cells. Consequences for development, apoptosis, invulnerable framework protein exercise in creature, plant and bacterial cells have been shown. These impacts are of physiological sort and requires atoms and physiochemical systems. The goal of this research was to see how different forms of music and sound frequencies affected the growth of Escherichia coli. When the test organism was exposed to music of various genres and sound frequencies, significant growth rates were seen. The experiment's findings revealed that not only did music boost bacteria's growth rate, but also the genres of Heavy Metal, Meditation, Hip-Hop, Classical, and Piano. In wastewater treatment companies, where sewage-degrading bacteria are inoculated in the sewage, this effect can be used on a bigger scale. The bacteria's efficiency can be increased greatly when the tanks are exposed to music, enhancing the wastewater treatment plant's efficiency. This reduces the amount of sludge delivered during wastewater treatment, saving money on transportation.
Keywords: *Isolation of pure culture of E.coli ; Ultrasonication ; Cell Biomass Measurement ; Biochemical Identification*

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INTRODUCTION

Sound is a pressure wave that travels in a circular pattern across matter. Sound waves are an important part of the environment. Almost all living species in the natural environment are exposed to sound waves and interact with them. By frequency, sound waves are divided into three categories: infrasound (10^-4-20Hz), audible sound (20*10^4 Hz), and ultrasound (2*10^4-10^12 Hz). Ultrasound's biophysical mechanics and biological impacts have been intensively studied in recent decades (14). Microbes have been demonstrated to be affected by mechanical waves, because of the thinning of cell membranes, localized heating, and creation of free radicals. Ultrasound has been utilized to sterilize and destroy undesirable germs through a variety of physical, mechanical, and chemical actions emerging from acoustic vibrations, ultrasound can inactivate bacteria and de-agglomerate bacterial clusters or flocks. However, bacteria cells such *as Escherichia coli, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa* adhered to and thrived on a polyethylene surface at a higher frequency of ultrasound. The use of ultrasound technology for large-scale microbiological cleaning or production is relatively costly. If this technology is to be used, it must first examine the amount of energy inputs and their cost. A more cost alternative method based on audible sound waves may sound feasible, and there have been some indications that this is the case (16).One of the environmental elements, audible sound, has a wide distribution in the natural world. When sounds of varying tone, pitch, loudness, or frequency are mixed together, it makes music when it is combined in a specific way. All living beings appear to be affected in some way by sound (audible/otherwise random sound) in a multitude of ways (specified sound pattern in the form of music) and at various levels. The use of audible sound has been found to boost colony formation. ability of *E. coli*, as well as to counteract salt's inhibitory effect stress on E. coli development in

the presence of higher levels of sodium chloride. Music influences the usage of microbial substrate by aerobic bacteria from a municipal waste water treatment plant. Low-intensity pulsed sound has been found to impact morphology and genetics of prokaryotic life forms have also been discovered. Sound is said to have an effect on people. According to Wang B (15),H+ in plasma membrane Chrysanthemum callus ATPase activity Sound stimulation can have an effect on nucleic acid synthesis and Chrysanthemum protein has also been reported to be impacted via means of sound waves(13).

The effect of audible sound of specific intensities on *Escherichia coli* is examined in this research. The effect is determined by comparing their cell density measured with a UV-Spectrophotometer to that of their corresponding control samples (not exposed to sound).

MATERIAL AND METHODS

Preparation of bacterial culture

Laboratory strain of *Escherichia coli* was obtained and cultured on Eosin-Methylene Blue (EMB) agar medium (HI media) at 37˚C for 18 hours.

Media Preparation: Sterile Nutrient broth

Nutrient broth was chosen as the medium for growth of the test organism. Nutrient broth powder (HI media) was taken and 0.65g of it was suspended in 50 ml of distilled water in 100 ml Erlenmeyer flask. 12 such 50 ml flask were prepared, 1 for blank solution, 10 for test and 1 for control experiment. The powder was dissolved completely to produce a uniform, homogenous solution. The broth was sterilized in an autoclave at a pressure of 15 lbs. at 121°C for around 15 minutes.

Inoculation

Under sterile conditions, 18 hours old culture suspension of *E.coli* was inoculated into 50 ml of sterile nutrient broth in a 100 ml Erlenmeyer flask. The absorbance of the flasks was adjusted to 0.1 at 540 nm using a calibrated colorimeter.

Experimental setup

Each flasks containing Nutrient broth inoculated with *Escherichia coli* was placed in sound-proof chambers. This sound-proof chambers contained one mobile phone connected with Bluetooth speaker, which played music of a particular genre continuously. The music genres chosen were: Classical – Mozart Sonata KV 331, Hip-Hop music track – Smooth Soul, Meditation music – Acoustic motivation, Piano – Dreamy piano and Heavy metal- Metal force.Two different sound intensities was in the range of 50-80 dB and80-100 dB.Control flask was also placed in separate sound-proof chamber in which no music was played. The flasks containing these bacterial cultures were exposed to different sound intensities for 4 hours at room temperature.

Measurement of Growth

After a period of 4 hours, the absorbance of the solutions in all of the conical flasks was measured at 540nm, using a UV-Spectrophotometer. A cuvette containing only the sterile nutrient broth was used as the blank.

Study of Biochemical Characteristics

One of the Flask containing bacterial growth after exposure to different sound frequencies was chosen and different biochemical tests: Indole test, Catalase test, Oxidase test, Citrate utilization test, Methyl red test, Vogues-Proskauer test, Carbohydrate fermentation in TSI agar slant and Carbohydrate fermentation tests for Lactose; Sucrose; Mannitol, were performed in order to study the biochemical characteristics of *Escherichia coli* after sound exposure by comparing with the standard biochemical chart of *E.coli.*

RESULTS AND DISCUSSION

Isolation and Screening of Test organism

Pure culture of *Escherichia coli* was isolated on nutrient agar medium and then streaked on Eosin methylene blue agar. The green Metallic sheen was observed on the EMB plate after incubation of 24 hrs at 37°C. The pure culture of Escherichia coli was inoculated into sterile nutrient broth for the experimental setup .The effect of sound intensities were determined by giving sound exposure for 4 hrs and comparing with the control flask.

Experimental results and Characterization of test organism after sound exposure for 4 hrs

Aftersound exposure, the Biochemical testing of sound treated culture of *Escherichia coli*was performed in order to determine the biochemical characteristics of the organism after sound exposure and the biochemical test results are shown in table 2.Theoptical density of control flask (Without sound exposure) and test flasks(with sound exposure) was measured after 4 hrs using UV visible spectrophotometer at 540 nm as shown in table 1. It was observed that music enhanced the growth of the bacterial culture of *Escherichia coli* with the varying sound intensities in dB ranges of all the different music tracks played. When compared to the absorbance readings obtained from the control setup

(wherein no music was played), the absorbance readings from the experimental setups, in which different forms of music with varying intensities ranges were played to the bacterial culture of Escherichia coli, had greater values.

The comparison between the growth rate of *Escherichia coli* due to the effect of different sound intensities exposed to different sound tracks has been shown in figure 3 in the graphical form. For Classical music, the cell biomass was increased with the increased sound intensity, 158% at50-80 dB and 152% at80-100 dB. For Hip-hop music, the organism had shown increased growth rate by 217% at 50-80dB and 176% at 80-100 dB in comparison with the control test results. Which means, Hip-Hop music has certain effect on the growth of *Escherichia coli.* The graph indicates the effects of Heavy metal music has increased the growth rate 400% at 50-80 dB sound intensities wherein as the sound intensities increased at80-100 dB, the decrease in optical density was observed247%.The significant difference in the growth rate of *E.coli* can be due to the interactions between the mechanosensitive channels which are present in the cell membrane of bacteria and sound waves., formation of reactive molecules leading to the inhibition or death of the organisms. In contrast, In contrast, there was a large drop in absorbance level for meditation and piano music as compared to the other music tracks. In meditation music, the absorbance value for 50-80 dB was 94% and increased absorbance value 205% at 80-100 dB. For piano music, at 50-80 dB the optical density was 82% and 152% for 80-100 dB in comparison with the absorbance value for control set. As a result, we observed that the genre of music enhanced the *E. coli* growth with the increased sound intensity for Classical, Heavy metal, Hip-Hop music tracks.

Music has an unavoidable impact on organisms' development and metabolic function. Because music contains mechanical sound waves, it works as a mechanical stressor for the test organism *E. coli*. Cell division can be aided by sound at the right frequency and intensity(5). Within the bacterial cells, it must have caused a cyclic oscillation. This type of alternative mechanical stress can generate motion within the cell's internal fluid, as well as probable plasma membrane deformation (14).Under sound stimulation, a cell's membrane fluidity can increase, causing changes in the protein structure of plasma membranes. Increased membrane fluidity stimulates cell division, growth, and metabolic activity by allowing substance transport and signal transmission across the membrane (17). Mechanical stimulation can transmit energy into the cell, which can benefit the cell by boosting food absorption, DNA combination in the "S" phase, and cell cycle synchronization (17)Sound and other mechanical inputs can alter membrane traffic and speed up metabolic activity.

The cytoskeleton, stretch-activated channels, integrins, phospholipases, tyrosine kinases, and cyclic adenosine monophosphate (cAMP) may all be involved in converting mechanical inputs into alterations in exocytosis or endocytosis (1). Zhao and his colleagues investigated the effect of sound stimulation on Chrysanthemum callus growth and discovered that it can boost the amount of soluble protein and sugar in the plant callus. Protein content is linked to the division and development of plant tissues. In the cell, soluble proteins are required for the accumulation of resources required for cell division, enzyme production, and metabolic activity. As a result, Zhao came to the conclusion that sound stimulation at a specific frequency and intensity could cause an increase in soluble protein and sugar content, which could serve as a foundation for cell proliferation and growth (17). According to certain studies, cells can feel mechanical stress through their extracellular matrix (ECM) and respond to changes in protein expression patterns (2). Sound stimulation stimulates lipid anabolism in the cell membrane, resulting in membrane lipid fluidity (17).

This might explain why *Escherichia coli* is affected by music. This is because Gram-negative bacteria have a higher content of lipopolysaccharide in their cell membranes (10). Heavy metal, Hip-Hop, and Classical genre of music enhanced the growth of *Escherichia coli* maximally as compared to other genres of music at 50-80 dB, whereas Meditation and Piano music tracks had shown enhanced growth of test organisms maximally at 80-100 dB.

CONCLUSION

In this study, effect of different sound frequencies on growth of *Escherichia coli* was studied. *E.coli* was chosen as model organism for conducting this research as it is easy to isolate at laboratory and having higher multiplication rate in less time and well studied organism. The effect of variable sound intensities was determined by using Ultrasonication, Cell Viability determination, Cell Biomass Measurement, and studying its Biochemical Characteristics after sound exposure. All 5 types of music genres had increased the absorbance values of the cells during growth. This implies that sound stimulation has a favorable effect on bacteria to some extent, resulting in proliferation. Due to the frequency and speed, the intensity of response was different for different genres of music played with different sound intensities. The model organism *E.coli* had shown significant increase in growth rate at 50-80 dB for Heavy Metal, Hip-Hop and Classical music tracks when compared with control set. Meditation and Piano music tracks had shown

decreased growth rate at 50-80 dB, when compared with the control set. At 80-100 dB, the decreased growth rate was observed for Heavy Metal and Classical Music tracks. Thus, It can be concluded that Model organism *Escherichia coli* is susceptible to Higher sound intensities for Heavy Metal and Classical music. Therefore, further research can be done to determine the effect of variable sound intensities on other microorganism.

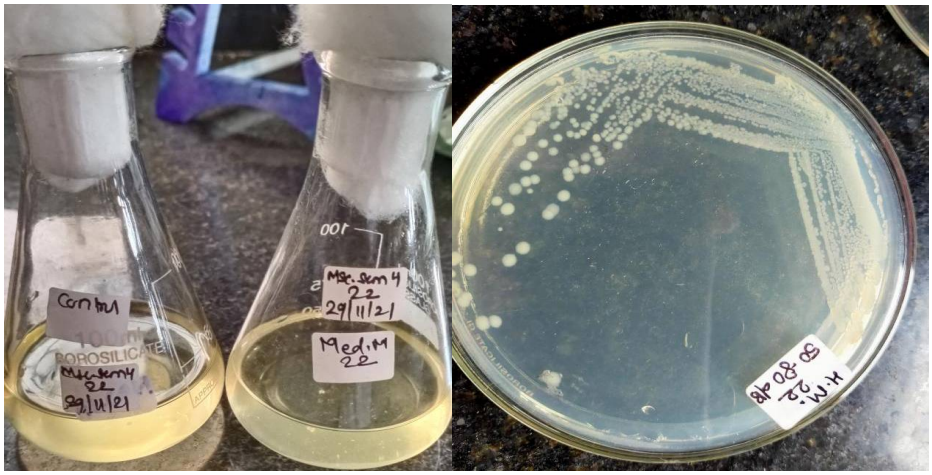

Figure 1: Growth obtained after sound exposure of 4 hrs

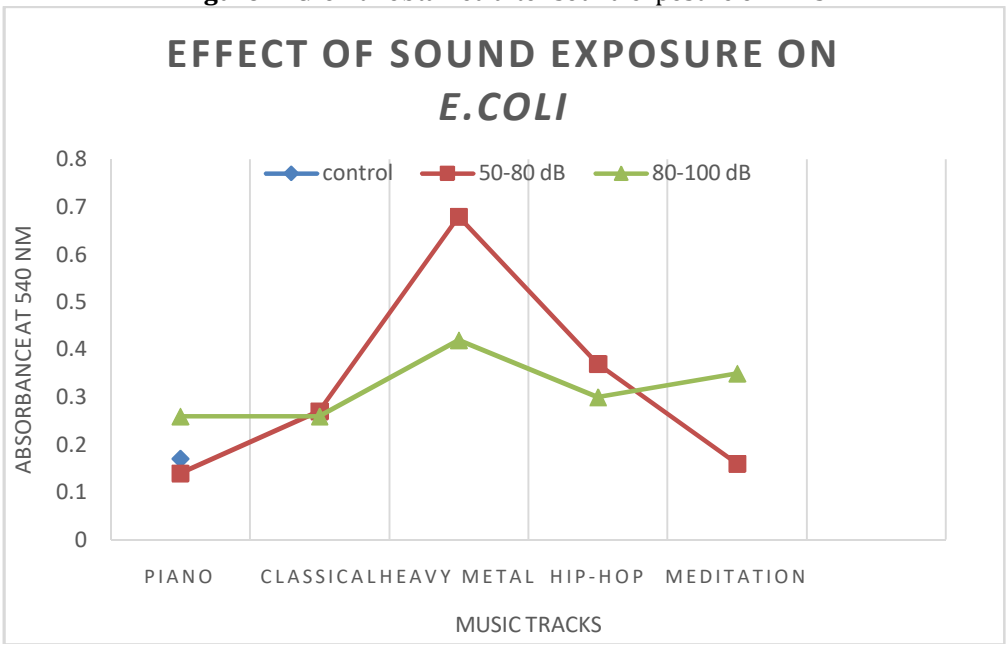

Figure 2: Graph of Absorbance at 540 nm Vs Music tracks

Table 1. Effect of different sound frequencies on microbial growth of *Escherichia coli.*

Experimental setup	Growth (OD_{540}) At 50-80 dB	Growth (OD_{540}) At Above 80-100 dB
Control (without sound exposure)	0.17	
Classical	0.27	0.26
Heavy metal	0.68	0.42
Hip-Hop	0.37	0.30
Meditation	0.16	0.35
Piano	0.14	0.26

Table 2. Biochemical tests results obtained from sound treated culture of *Escherichia coli* after 4 hours.

Tests	Standard results	Tests results
Indole test	Positive	Positive
Methyl red test	Positive	Positive
Vogues-Proskauer test	Negative	Negative
Citrate utilization test	Negative	Negative
Oxidase test	Negative	Positive
Carbohydrate fermentation in TSI agar slant	Positive	Positive
Carbohydrate fermentation a) Lactose b) Mannitol c) Sucrose	 Positive (acid + gas) Positive (acid + gas) Positive (acid + gas)	 Acid + gas Acid + gas Acid + gas
Motility test	Positive	Positive
Catalase test	Positive	Positive
Gram nature	Gram negative Bacilli	Gram negative Bacilli

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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