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Research Article

THE EFFECT OF MUSIC ON THE GROWTH OF ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

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ABSTRACT

All organisms are surrounded by sound waves. It has been discovered that these sound waves can have an influence on organisms. Sound waves can enhance the process of metabolism and the permeability and selection of the plasma membrane in plants, thus influencing their growth (Zhao *et al*., 2002). Sound waves can affect animals as well. In 2007, cows at a farm in Spain were exposed to Mozart's music during milking time. It was observed that milk production increased in these cows to 30-35 liters of milk per day, as compared to 28 liters of milk in other farms, where cows were not exposed to the music (Makiello, 2012). This work has been focused on studying the effect of music on the growth of bacteria such as *Escherichia coli* and *Staphylococcus aureus*. The bacteria, when exposed to music of different genres, were seen to have various growth rates. The results of the experiment showed that not only did music enhance the growth of bacteria, but also, the genre, Heavy Metal, increased bacterial growth maximally in both strains as compared to other genres. This effect can be utilized on a larger scale in wastewater treatment industries where sewage-degrading bacteria are inoculated in the sewage. The tanks can be exposed to music and the efficiency of the bacteria can increase significantly, improving the efficiency of the wastewater treatment plant (Pornpongmetta and Thanuttamavong, 2010). This helps in reducing the amount of sludge being transported during wastewater treatment, thereby saving transport costs.

INTRODUCTION

Different types of mechanical waves have been shown to have an effect on microorganisms. A sound wave is a disturbance which is transported through a medium via the mechanism of particle interaction. Almost all living organisms in nature are exposed to sound and interact with them. The biological effect of ultrasound has been studied in Ultrasonic Biophysics and its mechanism can be applied for therapeutic purposes and diagnostic ultrasound imaging (O'Brien, 2007). Similar studies have been carried out for infrasound waves (Anastassiades and Petounis, 1976). As far as audible sound is concerned, there are many studies on how audible sound (20 Hertz-20KiloHertz) is produced, absorbed, transmitted and reflected. The biological effect of audible sound on plants has been studied by Zhao and his colleagues in two separate papers on *Dendranthema morifolium* (Chrysanthemum). They had seen that the physical state and metabolism of the plasma membrane in a Chrysanthemum callus is sensitive to sound stimulation (Zhao *et al*., 2002). A year later, Zhao and his colleagues had published another research wherein they discovered that sound can increase the soluble protein and sugar content in the plant callus, both of which are needed for cell division and growth (Zhao *et al*., 2003). In the case of animal studies, it becomes slightly difficult to understand the biological effect of audible sound due to the complexity of such organisms. In this study, we have attempted to use unicellular microorganisms that are easily available such as *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive), to investigate the biological effect of sound on the *growth* of the microorganisms. Different genres of music were selected for the study as a source of audible sound. Both of the test organisms were inoculated in nutrient broth and exposed to the music for a specific duration. After this duration, the growth was measured in terms of optical density. The results showed that the growth was higher in presence of music. This research may be used as an application in wastewater industries to increase the growth of sewage-degrading bacteria.

Experimental

Preparation of Bacterial Culture

Laboratory strains of *Escherichia coli* and *Staphylococcus aureus* were obtained and cultured in nutrient agar slants (0.5 g beef extract, 1 g peptone, 0.5 g NaCl, 2 g agar, 100 ml distilled water, pH 7.2) at 37°C for 18 hours.

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Stock Preparation: Sterile Nutrient Broth

Nutrient Broth was chosen as the medium for growth of the test organisms. Nutrient broth powder (10 g/L peptone, 10 g/L beef extract, 5 g/L NaCl, pH 7.2) was taken and 25 gm of it was suspended in 1000 ml of distilled water in a sterile flask. Two such 1000 ml flasks were prepared. 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 of *E.coli* was inoculated into 50 ml of sterile nutrient broth in a 100 ml Erlenmeyer flask. Similarly, 18 hours old culture of *S.aureus* was inoculated into 50 ml of sterile nutrient broth in another Erlenmeyer flask (100 ml). The absorbance of the flasks was adjusted to 0.2 at 540 nm using a calibrated colorimeter. 30 such flasks were prepared, 15 each of *E.coli* and *S.aureus*.

Incubation

Three flasks containing Nutrient Broth inoculated with *Escherichia coli* and three flasks containing Nutrient Broth inoculated with *Staphylococcus aureus*, each were placed in 5 sound-proof chambers. Four of these sound-proof chambers contained one mobile phone each, which played music of a particular genre continuously. The music genres chosen were: (1) Classical-"Mozart's Symphony No. 40", (2) Bollywood, (3) Indian Hymn- "Gayatri Mantra", (4) Heavy Metal-"Devil's Dance by *Metallica*" The fifth sound-proof chamber served as a "Control" in which no music was played. The flasks containing these bacterial cultures were incubated for 96 hours at room temperature.

Measurement of Growth

After a period of 96 hours, the absorbance of the solutions in all of the conical flasks was measured at 540 nm, using a colorimeter. A cuvette containing only the sterile nutrient broth was used as the blank. The results were recorded, tabulated and analyzed.

RESULTS

It was observed that music enhanced the growth of the bacterial cultures of *Escherichia coli* and *Staphylococcus aureus*. The absorbance readings of the experimental setups, in which different types of music were played to the bacterial cultures, had higher values when compared to the absorbance readings obtained from the control setup (wherein no music was played). The percentage increase in absorbance values for the music selections in *E. coli* were 430% (Metallica), 415% (Mozart), 405% (Gayatri Mantra), and 381.5% (Bollywood). The percentage increase in absorbance for the control setup in *E. coli* was only 250%. Similarly, the percentage increase in absorbance values for the music selections in *S. aureus* were 185% (Metallica), 101.5% (Mozart), 95% (Gayatri Mantra), and 93% (Bollywood). The percentage increase in absorbance for the control setup in *S. aureus* was only 25%. The values show that maximum growth occurred in both of the bacterial cultures when they were exposed to Heavy Metal (Metallica) genre of music. It was also seen that the growth of *Escherichia coli* was more than that of *Staphylococcus aureus* in all of the experimental setups (Fig. 1)

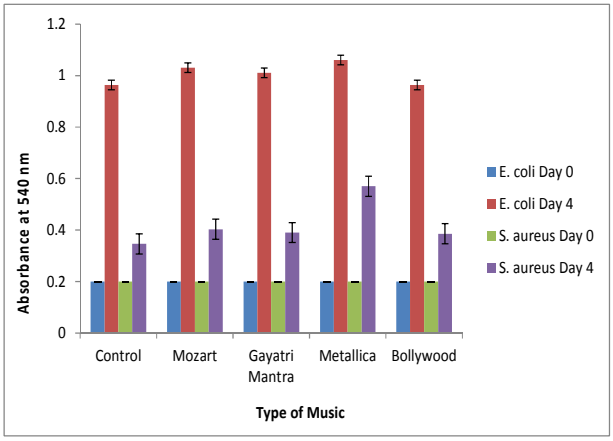

Fig 1 Bacterial Concentration after Exposure to Music

Figure Legend 1

Fig 1: Estimation of Absorbance of the two bacterial cultures after 96 hours of exposure to various types of music (of varying frequencies)

An average of 3 estimations were taken per sample (n=3) with error bars representing standard error of mean (SEM). (Control= No exposure to sound). This figure indicates that overall, Escherichia coli cells were affected by the vibrations of the music more than the cells of Staphylococcus aureus. As a result, the broth containing Escherichia coli had comparatively higher absorbance values than that of Staphylococcus aureus. Further, we observe that the genre of music that enhances the bacterial growth the most, in both E.coli and S.aureus, is Metallica followed by Mozart, Gayatri Mantra, and finally, Bollywood music.

DISCUSSION

Music inevitably affects the growth and metabolic behavior of organisms. Since music has sound waves which are mechanical in nature, it acts as a mechanical stress for the test organisms- *Escherichia coli* and *Staphylococcus aureus*. Sound at a proper frequency and intensity can promote cell division (Hongbo *et al*., 2008). It must have produced a periodic oscillation within the bacterial cells. This form of an alternative mechanical stress can cause motion within the internal fluid of the cell and possible deformation of the plasma membrane (Shaobin *et al*., 2010). Membrane fluidity in a cell can increase under sound stimulation and make changes in the protein structure of plasma membranes. The increase in membrane fluidity leads to substance transport and signal transduction across the membrane which promotes cell division, growth, and metabolic activity (Zhao *et al*., 2002). Mechanical stimulation can transfer energy into the cell, and this can benefit the cell by promoting absorption of nutrients and combination of DNA in the "S" phase, as well as synchronizing the cell cycle (Zhao *et al*., 2003). Mechanical stimuli such as sound can also modulate membrane traffic and accelerate metabolic activity. Transduction of mechanical stimuli into changes in exocytosis or endocytosis may involve the cytoskeleton, stretch-activated channels, integrins, phospholipases, tyrosine kinases, and

cyclic adenosine monophosphate (cAMP) (Apodaca, 2002). Zhao and his colleagues had studied the effect of sound stimulation on the growth of Chrysanthemum callus and found that it can increase the content of soluble protein and sugar in plant callus. The division and growth of plant tissues are related to the content of protein. Soluble proteins in the cell are necessary for the accumulation of materials needed for cell division, production of enzymes, and metabolic activity. Thus Zhao concluded that sound stimulation at a certain frequency and strength may induce an increase in soluble protein and sugar content which could provide a basis for cell division and growth (Zhao *et al*., 2003). Certain investigations show that cells can sense mechanical stress through their extracellular matrix (ECM) and can respond to altered patterns of protein expression (Chiquet, 1999). Sound stimulation promotes lipid anabolism in the membrane of the cells which causes membrane lipid fluidity (Zhao *et al.,* 2002). This might explain why *Escherichia coli* is affected by music more than *Staphylococcus aureus*. This is because Gram-negative bacteria have a higher content of lipopolysaccharide in their cell membranes (Pelczar, Chan, and Krieg, 1993). Heavy Metal (Metallica) genre of music enhanced the growth of *Escherichia coli* and *Staphylococcus aureus* maximally as compared to other genres of music. There was a study published recently involving the effects of music on the microbial substrate utilization of aerobic bacteria, which states that if the music's wave components (tempo) are more varied, then their effect on microbial substrate utilization would be stronger (Pornpongmetta and Thanuttamavong, 2010). Metallica's music has kick drums, electric guitars, cymbals whose sound vibration frequencies fall into the range of 2 to 12 kHz (White and Senior, 2001). Classical music (Mozart) had also affected the growth of both test organisms to a great extent, giving absorbance values very close to those obtained for Metallica. This may be due to the different tempos such as Allegro, Presto, Adante grazioso, and Adagio as well as the high frequencies of Mozart's composition (Bauman, 1991) as compared to the Indian Hymn and Bollywood music. Matsuhashi *et al*. demonstrated that sound waves between 6 kHz and 40 kHz can induce colony formation in *Bacillus carboniphilus* (Matsuhashi *et al.*, 1998). Ying's experiment of the effect of sound on bacteria showed that there is a significant increase in viable cell count at sound frequencies of 5 kHz. Ying's results show that sound treatment at 5 kHz gave a higher value of CFU (Colony Forming Units) as compared to frequency 15 kHz (Ying, 2009). In another study involving the effect of sound on the growth of *E. coli*, the relative colony forming efficiency reached 141.6%, 130.0% and 131.1% , 22 hours after stimulation by sound wave with the frequency of 1, 5 and 10 kHz, separately, which were significantly higher than that of the control (100%). According to the author and his colleagues, the frequency of 1000 Hz of sound wave seemed to be the best to promote the *E. coli* cells to grow (Shaobin *et al*., 2010).

CONCLUSION

In this study, we have presented the results of an experiment on the effect of music on the growth of two easily available unicellular microorganisms, *Escherichia coli* and *Staphylococcus aureus*. All four types of music genres had increased the absorbance values of the cells during growth. This implies that sound stimulation, to some extent, has a positive effect on bacteria which results in growth (Ying, 2009). However, the level of response was different for different genres of music due to the frequency and tempo. In this experiment, maximum growth in *E. coli* and *S. aureus* was seen when exposed to Heavy Metal (frequency of 2-12 kHz). It is known that cells respond to mechanical stimulation from the environment. But the mechanisms by which cells sense and transmit the sound waves into the intracellular environment are not exactly clear (Zhao *et al*., 2002). It is understood that mechanical stimuli can modulate endomembranous transport, but several aspects regarding how they occur still remain to be explored (Apodaca, 2002). Although this research involves the use of easily available test organisms, it can be further extended to sewage-degrading microorganisms. This will stimulate the activity of the microorganisms due to the positive effect of music on cell growth, substrate utilization, and metabolic activity (Pornpongmetta and Thanuttamavong, 2010). This will improve the efficiency of biological wastewater treatment in India. Some microbes that thrive in wastewater are autochthonous bacteria such as *Escherichia coli*, *Bacillus spp*., and *Pseudomonas aeruginosa* (Dhall *et al*., 2012). Another study had identified sludge-degrading microorganisms as *Lactobacillus spp.* and *Klebsiella spp*. (Nwambo and Kehinde, 2013). Hence, these bacteria may be used for further research. This effect can be utilized on a larger scale in sewage wastewater treatment industries where sewage-degrading bacteria are inoculated into sewage. The tanks can be exposed to music and the efficiency of the bacteria can increase significantly. This has been tested by a sewage treatment plant in Berlin, Germany. A German company, Mundus, made an affordable sound system and played Mozart's "The Magic Flute", to biomass-eating microbes. After a year, the sludge was reduced by 1000 cubic meters since the bacteria performed better along with the music. The wastewater treatment plant saved an estimated 10,000 Euros on the cost of transporting the sludge (Makiello, 2012).

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