HIGH SCHOOL EDITION
Journal of Student Research
Volume 11 Issue 1 (2022)

Stress Hormone Adrenaline Boosts the Antibacterial Properties of *L. plantarum* and *E. hallii*

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ABSTRACT

Amid intense exercise, the human body's quick adaptability to stressful conditions is vital to cellular function, initiating what we recognize as an "adrenaline rush". Despite findings on the in vitro interaction of adrenaline with the gut microbiome to regulate its virulence gene expression through AI-3 quorum sensing, studies conducted to examine the influence of the stress hormone adrenaline released through exercise on the function of the microbiome in our gut are yet insufficient. The human gut consists of a variety of microbes such as *L. plantarum* and *E. hallii* that hold vital roles in maintaining metabolic balance in the gut microbiome. Through our research, we envisioned investigating how adrenaline treatment at different time intervals or concentrations mirroring the various circumstances of exercise may affect bacterial growth, antibacterial substance production, and their antibacterial properties. While both bacteria were not affected in its growth for 48 hours, in *L. plantarum*, demonstrating decreased growth in the first 8 hours, its main functions — lactic acid production — were furthered with the hormone. In *E. hallii,* butyric acid production, with the hormone, significantly increased in its supernatant, and the intermediate treatment of adrenaline for 8 hours served to catalyze the rate at which the bacteria reached its stationary phase of growth. The study holds great promise for future investigations on how such a mechanism can be applied to therapies boosting our existing abilities of protein disposal directly correlated to the prevention of diseases. What remains is, nevertheless, the discussion regarding the true control center of these bacteria that interact with adrenaline to cause changes in the antibiotic properties, function, and growth. In other words, future studies can be oriented towards understanding the changes in the antibacterial properties of given bacteria not indirectly through chemicals with antibacterial traits but rather through the quantitative measurement of growth inhibition with other bacteria or organisms.

Introduction

Defined by the World Health Organization (WHO) as "any bodily movement produced by skeletal muscles that require energy expenditure", exercise has been recommended by various health-related bodies due to its proven benefits [2]. In recent reports, epinephrine, a stress hormone released through exercise from the adrenal gland to initiate the "fight or flight" response with the activation of the α_1 and α_2 receptors, has been found to induce virulence gene expression in the gut microbiota through potential means of AI-3 quorum sensing or through the gut-brain axis (GBA) [3]. Scientific understanding changes by the hour, it seems, and stress accumulating through exercise possibly posing an effect on the bacterial function and structure is not yet analyzed to a sufficient degree. However, with the increased significance of the mechanism and role of microbiomes, this study examines how such long-term, intense exercise may or may not affect the growth and function of various bacteria, triggering a secondary effect in the performance of the body. Given that bacteria located in the gut hold such a fundamental function and value in maintaining the balance of metabolic activity within the human body, testing how and to what extent the hormones are released affect them is thus of utmost importance.

To comprehensively examine the effects of exercise on the gut microbiome, various types of bacteria including *Lactiplantibacillus plantarum* and *Eubacterium hallii* were employed. *L. plantarum*, a gram-positive bacte-

rium, is a rising candidate for future probiotics. Located in the gastrointestinal tract is this bacterium that conducts fermentation and is found to have a correlation in increasing muscle mass, producing gas and acid as a byproduct as an antibiotic. Next is the bacteria *E. hallii* that utilizes glucose, lactate, and acetate to create butyrate and hydrogen as an antibiotic for other microbes. Recently, in the preceding research, *E. hallii* was found to maintain the metabolic balance of the gut by preventing several intestinal diseases and malabsorption [4]. If the presence or interaction with hormones such as epinephrine may enhance or hinder the function of such bacteria, there may be a possibility in which the human body may be much more susceptible to the effects of such diseases or an imbalance in the metabolic activity.

Methods

1. Culture of *L. plantarum* and *E. hallii*

Utilize the appropriate media for each respective bacteria— De Man, Rogosa and Sharpe Broth agar for *L. plantarum* and chopped meat agar for *E. hallii*. In the petri dish with the solidified agar, add the bacteria with the use of the streaking plate method, and incubate the dishes at 36° C for 48 days for L. plantarum or in an anaerobic container at room temperature for *E. hallii*. After such time, employ a dissecting microscope to examine the margin, elevation, and color of the bacteria.

2. Chemotaxis of *L. plantarum*

Pour 100 ml of distilled water and 0.5g of agar into a flask. Dissolve the solution by heating it with a microwave for 3 to 5 minutes. Pour approximately 10 ml of the solution into 4 petri dishes, and cool down at room temperature for approximately 15 minutes. Afterward, place a disk paper at the left end for the 4 petri dishes. For 2 of the petri dishes, pour 20 µl of adrenaline on the disk, and for the other 2 petri dishes, pour 20 µl of distilled water onto the disk. On the right end of all 4 petri dishes, put lactobacillus with a needle. Last but not least, store the petri dishes in the incubator for 48 hours at 36° C.

3. Bacterial Growth

3.1. 48 hours

Prepare De Man, Rogosa and Sharpe Broth (MRSB) for *L. plantarum* and Chopped Meat Broth (CMB) for *E. hallii*, and add 0.1 ml of 2 ppm adrenaline to each of the 3 conical tubes. Afterward, add 100 µl of *L. plantarum* to the 20 ml of MRSB and CMB into the conical tube, and incubate the tubes in the incubator at 36° C for 48 hours. Employ the spectrophotometer with the wavelength of 600 nm to measure the concentration of the bacteria within the conical tube with the standard being MRSB and CMB respectively and record the data, calculating the average.

3.2 8 Hour with Varying Concentration

Prepare the adrenaline solutions with the appropriate concentration that will be inserted into the conical tubes — 2 ppm and 20 ppm — and label the conical tubes with the bacteria name and epinephrine concentration. Furthermore, prepare the media for the bacterium, which is MRSB and CMB respectively. Pour 100 ml of each broth each into 3 flasks, and pour 1 ml of each adrenaline concentration into the respective flasks. Last but not least, place 1 µl of the cultured *L. plantarum* or *E. hallii* into all flasks. Record the growth of the bacterium with a spectrophotometer with the optical density being 1 for 0 hours, and measure the growth over the necessary intervals being 0, 0.5, 1, 2, 4, 6, and 8 hours.

3.3 Delayed Adrenaline Treatment with Varying Concentration

First and foremost, prepare 3 disinfected flasks and 3 conical tubes for the experiment, and pour 50 ml of MRS broth and 2000 µl of *L. plantarum* into each flask. For *E. hallii*, add 20 ml of CMB to each conical tube, and add 2000 µl of *E. hallii* with a micropipette. Utilizing the spectrophotometer at 600 nm, evaluate the amount of bacteria at 0 hours with the standard being simple MRSB or CMB. With regards to *E. hallii*, place the conical tubes into an anaerobic container that removes oxygen.

At 30 minutes for *L. plantarum* and 1 hour for *E. hallii*, measure the amount of bacteria in each flask with the spectrophotometer at 600 nm and place the adrenaline solution of 0, 2, and 20 ppm in each respective flask. Continue measuring the amount of bacteria through the spectrophotometer at the indicated intervals with the same standard being the MRSB or CMB.

4. Antibacterial Substance Production

4.1 Lactic Acid (L. plantarum)

First, create a 0.2% $FeCl_3$ solution by taking 1 g of the $FeCl_3$ and 50 ml of distilled water. Afterward, dilute the solution, adding 1 ml of the solution and 9 ml of distilled water. Next, utilize a sample of 20 µl of MRS, the supernatant of L. plantarum, and the supernatant of L. plantarum with adrenaline and add 2 ml of the 0.2% $FeCl_3$ solution. To examine the color change from the reaction, employ the spectrophotometer at 390 nm with the standard being pure lactic acid. Record the data, and take note of qualitative observations such as color change.

4.2 Butyric Acid (E. hallii)

Utilize the centrifuge at 2000 RPM for 10 minutes to thoroughly separate the supernatant and the bacteria for *E. hallii* with and without adrenaline. Remove the upper layer of the solution with a syringe and filter with a pore size of 0.5 µm to extract around 5 ml of filtered while pouring the rest of the supernatant into the original conical tube. Next, employ the pH meter to measure the pH or acidity.

After this process, create 0.1 M NaOH by mixing 1 L of distilled water and 4 g of NaOH, and from each sample, pour 3 ml of the respective solution and 100 µl of 0.2% phenolphthalein into a flask. Employing titration, slowly add droplets of NaOH with a pipette to the created solution until the substance turns to a pink shade, and measure the amount of NaOH required for each sample to result in the color change.

5. Protein Degradation (*L. plantarum*)

Place 100 ml of distilled water, 1g of agar, and 1g of skim milk into a flask, and place a wrap on the top with holes poked. Afterward, heat the flask for 5 minutes in the microwave, and pour approximately 10 ml of the solution into the petri dishes. After approximately 15 minutes at room temperature, place a disk paper at the center of each disk, and pour 20 µl of adrenaline and distilled water on the disk paper in each respective group and *L. plantarum* with a needle. Next, place the petri dishes into the incubator at 36° C for 48 hours, and record the diameter of the clear boundary around the center of the disk with a ruler.

6. Antibacterial Properties

For *L. plantarum* and *E. hallii,* employ the centrifuge at 2000 RPM for 10 minutes to thoroughly separate the supernatant and the bacterium for the 2 conical tubes of 25 ml — one containing solely the bacterium and the broth with the other containing the bacterium that had been previously exposed to epinephrine which had been removed— and remove the upper layer of the solution with a syringe and filter with a pore size of 0.5 µm to extract around 5 ml. Afterwards, for *L. plantarum*, take 3 conical tubes for each group — nutrient broth (NB), *L. plantarum*, *L. planta-*

rum with adrenaline — place 10 ml of the solution each. On the other hand, for E. hallii, take 3 conical tubes for each group — control, *E. hallii*, *E. hallii* with adrenaline, and CMB — and add 10 ml of each solution. For both bacterium, insert 100 µl of *E. coli* into each conical tube, and store the tubes of *L. plantarum* in the incubator at 36° C and place the tubes of *E. hallii* in an anaerobic container at room temperature. After 24 hours, remove the tubes, and measure the growth of *E. coli* through the spectrophotometer at 600 nm with the comparing standard being NB.

3. RESULT

1. Culture of *L. plantarum* and *E. hallii*

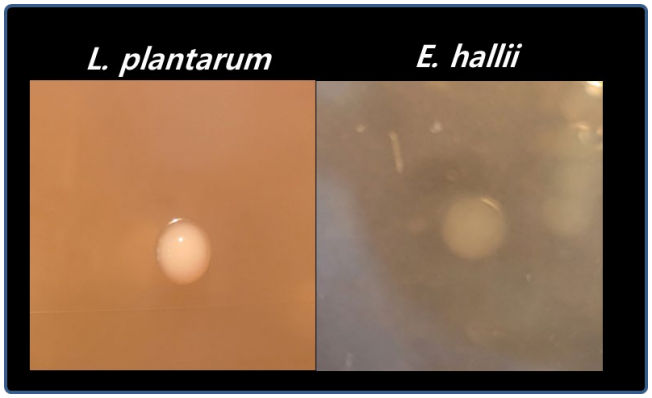

Figure 1. Morphology of *L. plantarum* and *E. hallii*. Filmed from a petri dish on a microscope.

In terms of bacterial morphology, the colonies of *L. plantarum*, and *E. hallii* can be categorized by their margin, color, elevation, and shape. In fact, both forms of bacteria share the same shape being round as shown through the extracted colony. However, from *L. plantarum* to *E. hallii,* the elevation turns increasingly from a pulvinate, or cushion-shaped, to a raised structure. In terms of color, all 3 types possess a milky shade for their colonies although being influenced by the shade of the plate to a certain degree.

2. Chemotaxis (*L. plantarum*)

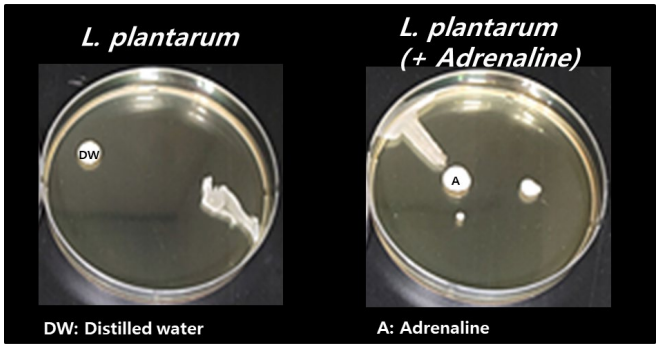

Figure 2. The Effect of Adrenaline on the Chemotaxis of *L. Plantarum*. Left disk paper, distilled water. Right disk paper, adrenaline.

The purpose of this experiment is to identify the possible chemotaxis of adrenaline and *L. plantarum*, thus monitoring the movement of the bacteria in both the presence and absence of the hormone. It was discovered that adrenaline had an attractive and positive chemical relationship with *L. plantarum*, pulling the bacteria towards the hormone.

First and foremost, with the case of distilled water or the control, the bacteria in both plates grew and expanded their colony in the opposite direction of the disk paper that contained distilled water, suggesting that the *L. plantarum* clearly did not possess a chemical preference for the distilled water. However, in the plate with adrenaline, fig. 2. clearly represents the growth of *L. plantarum* near the disk paper with adrenaline; the plate on the right, for instance, not only contains long colonies extended towards the disk paper but also multiple circular colonies formed. Comparing such pieces side by side, such data brings high validity and evidence to the conclusion that suggests that adrenaline possesses traits of chemotaxis to influence the movement of biological organisms such as *L. plantarum* in its presence.

3. Bacterial Growth

3.1. 48 Hours

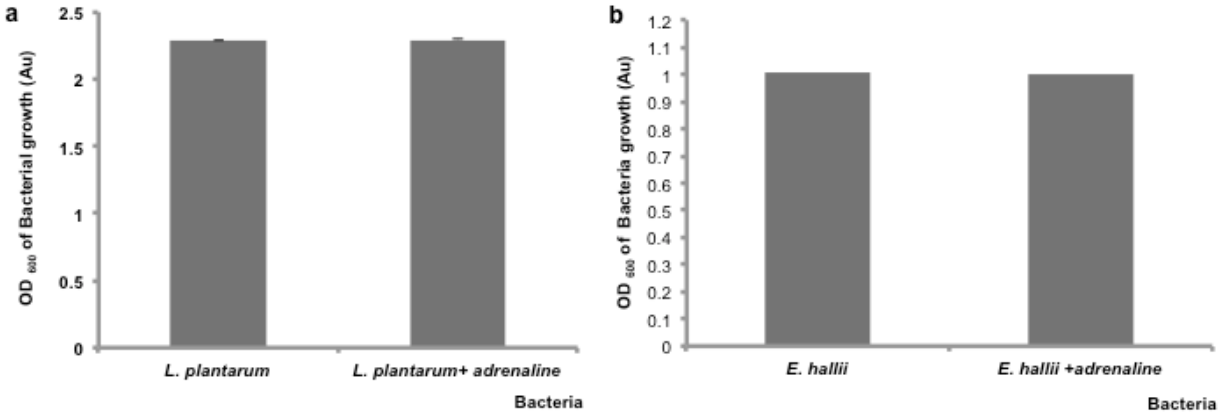

Figure 3. a. The Growth (+ SD) of *L. plantarum* (Au) with and without adrenaline for 48 hours. Incubated at 36° C in MRSB. Data collected through the spectrophotometer at OD_{600}. b. The Growth of *E. hallii* (Au) with and without adrenaline for 48 hours. Incubated at room temperature in CMB. Data collected through the spectrophotometer at OD_{600}.

For *L. plantarum*, the addition of adrenaline essentially had no impact on the long-term growth as the recorded bacterial growth for both the control and experimental group were nearly identical with the error bars overlapping. The overlapping error bars demonstrate that the measured difference, if any, has no statistical significance. For solely *L. plantarum*, the bacterial growth was recorded to be 2.29 ± 0.00057 Au while the bacteria incubated with adrenaline was recorded as 2.294 ± 0.0072 Au. Even if there were or were not drastic changes in the pattern of growth in the early hours of the incubation, at the end of the 48th hour, the growth was nearly equivalent, thus hinting that the adrenaline itself does not have a significant long term effect on the growth of *L. plantarum.*

Similarly for *E. hallii*, the presence of adrenaline essentially had no influence on the growth of *E. hallii* as the conical tubes with and without adrenaline were 1.001 and 1 Au respectively. Even if the presence of adrenaline may have had an antibiotic or rather a positive effect on the growth of *E. hallii* during the initial hours which were not reflected through the measurements, the marked difference between the two groups at the 48th hour was extremely minor, thus emphasizing that an single, initial adrenaline treatment had no impact on the overall growth in an extended period of time for *E. hallii.*

3.2. 8 Hour with Varying Adrenaline Concentration

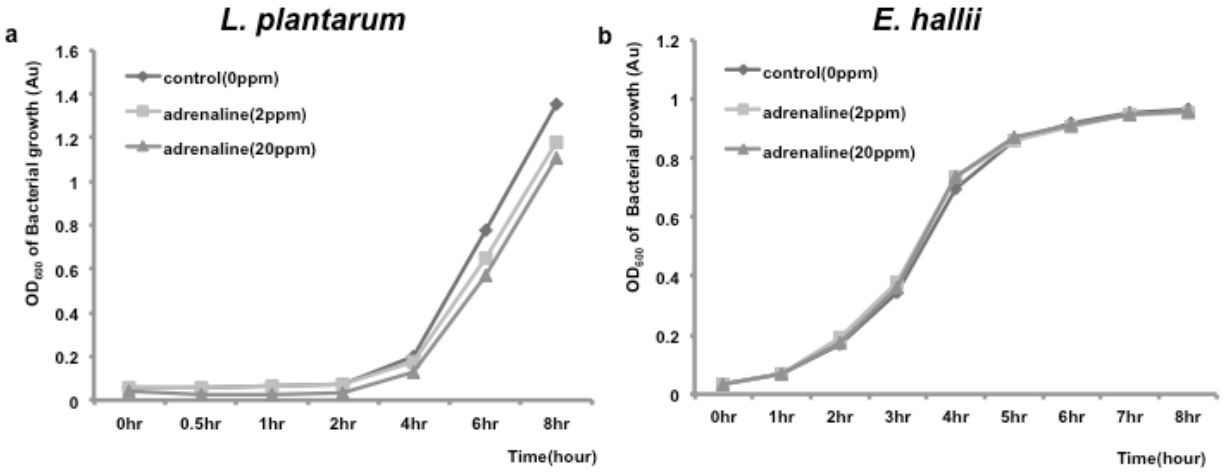

Figure 4. a. The Effect of Varying Adrenaline Concentration (ppm) on the Growth (Au) of *L. plantarum*. Utilized adrenaline concentrations of 0, 2, and 20 ppm. Data collected through the spectrophotometer at OD_{600}. b. The Effect of Varying Adrenaline Concentration (ppm) on the Growth (Au) of *E. hallii.* Utilized adrenaline concentrations of 0, 2, and 20 ppm. Data collected through the spectrophotometer at OD_{600}.

In the previous experiment, the presence of adrenaline seemed to have very little to no effect on the growth of *L. plantarum* when measured at the end of the 48 hour period of incubation as the respective data were nearly identical. However, to reflect the varied level of exercise, this experiment consisted of different adrenaline concentrations (ppm) ranging from 0, 2, to 20 ppm and more frequent measurements by the hour. As a result of such a procedure, it was measured that the hormone concentration nor presence had no effect on the growth of *L. plantarum* in the first 2 hours as demonstrated by the graph. According to the data, starting at 0 hours with 0.057, 0.058, and 0.04 Au of *L. plantarum* in the order of the concentration, by the second hour, the bacterial growth was recorded as 0.077, 0.075, and 0.034 Au. However, after the second hour, the differences between the plates of 0, 2, and 20 ppm adrenaline began to steadily increase to reach 1.356, 1.182, and 1.112 Au respectively. In short, the plates with the highest concentration of adrenaline resulted in the least bacterial growth, thus implying that adrenaline itself has antibiotic characteristics and therefore has a negative impact on the growth. In other words, such investigation demonstrates that not only does an increase in the concentration of adrenaline decreases the bacterial growth but also that such change in the pattern of growth is apparent from the second hour. However, other factors may also have played an influence such as the humidity, temperature, or availability of resources.

Investigating the pattern of growth in *E. hallii* over a shorter period of 8 hours, different concentrations (ppm) of adrenaline were employed to analyze the impact on the rate of growth of *E. hallii*. Beginning with similar amounts of *E. hallii* being 0.032, 0.031, 0.031 Au respectively, the three lines — control, 2, and 20 ppm — maintained similar slopes throughout its development over the span of 8 hours as evident through the single line expressed in the figure. With a subtle diversion of data at 4 hours, the groups reach the stationary phase of growth of 0.964, 0.952, 0.96 Au by the end of the 8th hour. Thus, the data suggest that, unlike *L. plantarum*, both the presence and the concentration of the initial adrenaline treatment do not affect the magnitude nor pattern of bacterial growth in *E. hallii* both in its short and long term.

3.3 Delayed Adrenaline Treatment with Varying Adrenaline Concentration

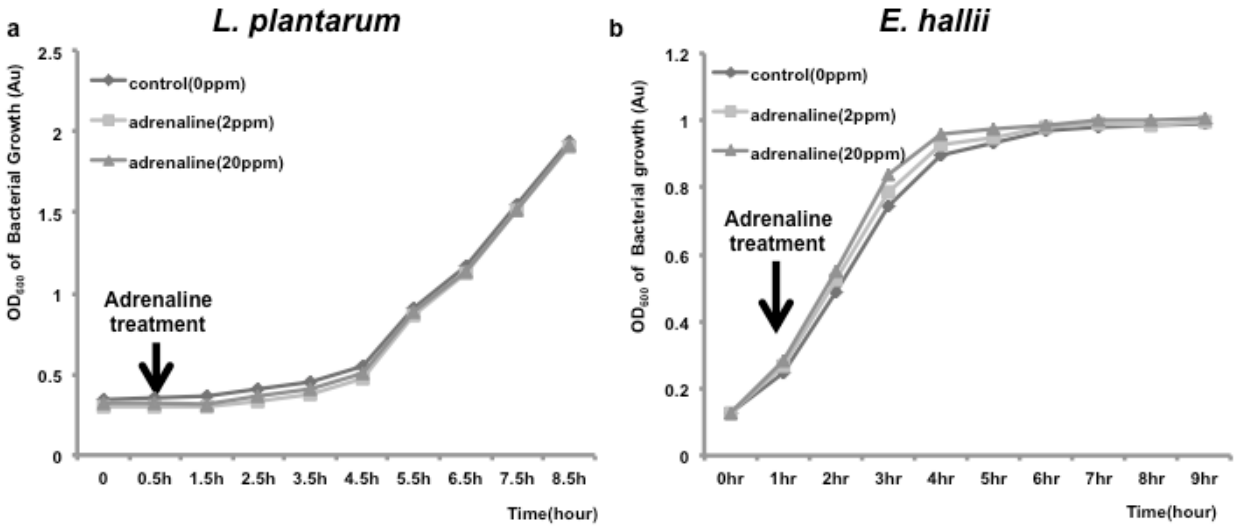

Figure 5. a. The Growth of *L. plantarum* (Au) for delayed adrenaline treatment of different concentrations (ppm). Adrenaline concentrations of 0, 2, and 20 ppm inserted after 30 minutes of incubation. Incubated at 36° C in MRSB. Data collected through the spectrophotometer at OD_{600}. b. The Growth of *E. hallii* (Au) for delayed adrenaline treatment of different concentrations (ppm). Adrenaline concentrations of 0, 2, and 20 ppm inserted after an hour of incubation. Incubated at room temperature in CMB. Data gathered through the spectrophotometer at OD_{600}.

While previous experiments were performed in a manner in which adrenaline was inserted from the start to measure growth, in order to more closely mirror the similar circumstances of the human body during exercise, the investigation employed 3 relatively high adrenaline concentrations from 0, 2, and 20 ppm given that the basal plasma adrenaline level increases nearly 10 times from approximately 0.28 ± 0.04 nmol/l to 2.19 ± 0.29 nmol/l even during moderate exercise and also utilized intermediate instead of initial adrenaline treatment [5]. Juxtaposing to the results of the initial adrenaline treatment where lower concentrations of adrenaline led to greater bacterial growth in the order of 0, 2, and 20 ppm, delayed treatment during mid-development did not demonstrate any variation in its level of bacterial growth by the concentration of adrenaline, thus forming a single line especially near 7 and 8 hours. In fact, the differences among the experimental and control groups rather lessened as the time of incubation increased, resulting in the similar final growth of 1.939, 1.912, 1.921 Au respectively.

Similar to the results obtained through the trial with *E. hallii* in which adrenaline was treated at the beginning of the incubation, all 3 groups fairly maintained steady patterns of growth, meeting its final capacity at the 4th hour with the stationary phase. However, in comparison to the initial experiment with a different time interval of adrenaline treatment, this experiment found that the later treatment resulted in a more dramatic and rapid increase towards its maximum capacity of growth, or stationary phase; whereas it nearly took *E. hallii* 4 to 5 hours for the initial experiment, the results for this design rather took 3 to 4 hours with similar final amplification of nearly 1 Au, thus hinting that the treatment of adrenaline during the period of bacterial growth further increases the rate of development but not the final value.

4. Antibacterial Substance Production

4.1. Lactic Acid (L. plantarum)

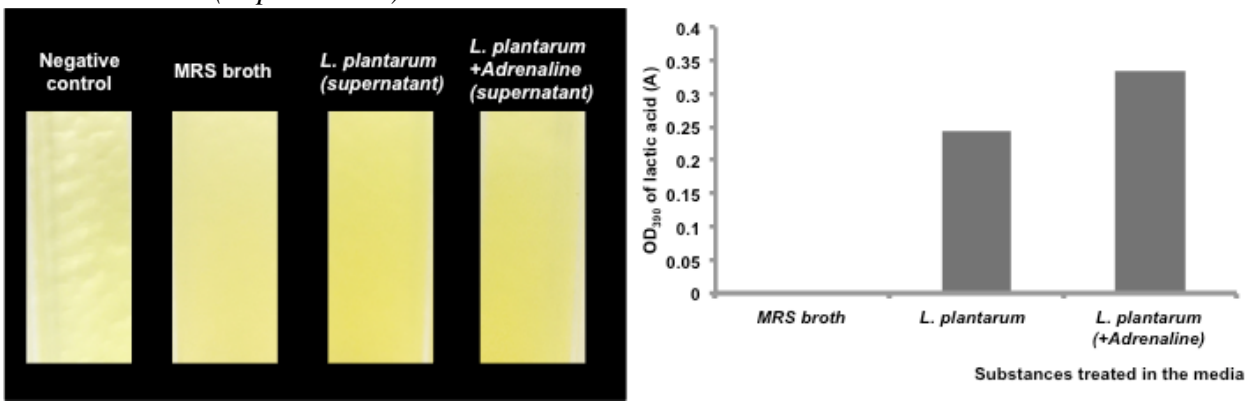

Figure 6. The Effect of Adrenaline on the production of lactic acid of *L. plantarum*. $FeCl_3$ was added to react with the lactic acid to measure the production. MRSB is the control. Samples were filtered supernatants. Data collected through the spectrophotometer at OD_{390}.

The purpose of conducting this experiment was to evaluate to what extent the hormone adrenaline affects the main function of creating an acidic environment of *L. plantarum*. Predominantly, implied through its name, *L. plantarum* not only produces lactic acid but also utilizes the produced substances to initiate fermentation in various food products while being located in the gastrointestinal tract of animals.

Employing the spectrophotometer to measure the concentration of lactic acid in each substance, it was found that not only did *L. plantarum* possess the function to produce lactic acid for fermentation but adrenaline also certainly had a positive effect on increasing the amount of lactic acid production of *L. plantarum*. Simple MRSB, the control group, had 0 Au lactic acid production because no *L. plantarum* had been inserted, thus resulting in the lack of fermentation. In comparison to MRSB, the latter groups — *L. plantarum* and *L. plantarum* with adrenaline — had significantly higher lactic acid production. In fact, the group with the highest recorded lactic acid production was the supernatant of *L. plantarum* with adrenaline possessing 0.335 Au while the *L. plantarum* without adrenaline recorded a lower production of lactic acid being 0.244 Au.

In fact, preceding studies highlight lactic acid's antimicrobial effect on important pathogens; disrupting the cytoplasmic membrane and causing leakage in bacterial proteins, lactic acid was found to completely suppress the growth of *E. coli*, *Salmonella*, and *Listeria* cells with a concentration of 0.5% [6]. Thus, given such preceding information, the results showing a positive correlation between the presence of adrenaline and the lactic acid production point towards the potential indirect influence adrenaline may pose on the antimicrobial effect of *L. plantarum*.

4.2. Butyric Acid (E. hallii)

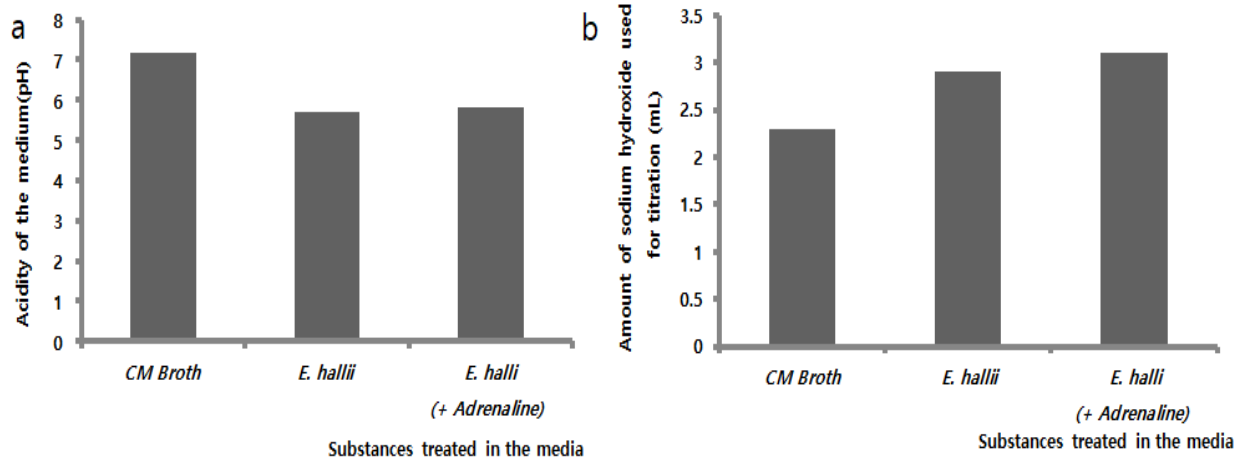

Figure 7. a. The Effect of Adrenaline on the acidity of *E. hallii*. CM broth is the control. (F) refers to the filtered supernatant. Data collected through the pH meter. .b. The Effect of Adrenaline on the production of butyric acid of *E. hallii*. CMB is the control. (F) refers to the filtered supernatant. Sodium Hydroxide (NaOH) was employed to react with the butyric acid produced by the bacteria and was measured to compare the amount (ml) of butyric acid. Data collected through the amount of sodium hydroxide (NaOH) required for the titration of butyric acid with pink representing a more basic pH while the clear shade represented acidity.

Utilizing glucose, acetate, or lactate as substrates for the production of butyric acid is *E. hallii* that also conducts fermentation, thus maintaining the metabolic activity within the body. In other words, with butyric acid affecting the ability of the body to sustain metabolic balance, it would be of utmost importance to measure the extent to which adrenaline affects such a role of the bacteria with the indicator set as butyric acid.

Among all groups, CMB, as the control group, possessed the highest pH, or the most basic solution, due to the lack of *E. hallii* to compose an acidic environment. In terms of the pH, however, adrenaline did not seem to have a dramatic positive nor negative effect on the acidity as the filtered supernatant with and without adrenaline both had a pH 5.8 and 5.71 respectively. Although the pH measurements cannot engender a comprehensive explanation on the butyrate content within the environment as there may be other factors that may influence the pH, it was found through the data that the hormone adrenaline does not have a major effect on the acidity of the environment around *E. hallii*.

Utilizing sodium hydroxide for the titration of butyric acid, we measured the amount of NaOH to complete the reaction; in that sense, higher levels of acidity or butyric acid production thus corresponded to the larger amount of NaOH for titration. In comparison to the CMB, the control group, E. halli both with and without adrenaline had visibly larger use of NaOH for titration, proving that *E. hallii* was capable of producing butyrate acid to react with the sodium hydroxide. In fact, adrenaline served to increase the production of butyric acid as indicated by the figure with the amount of NaOH for the bacteria with and without adrenaline being 3.1 and 2.9 ml respectively.

5. Protein Degradation (*L. plantarum*)

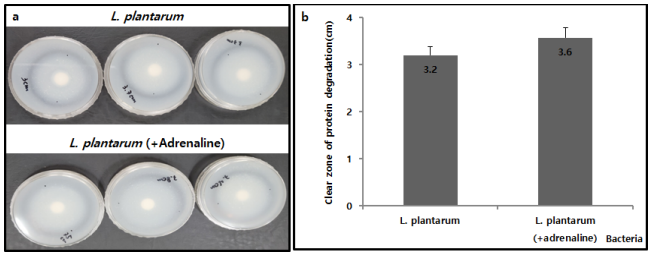

Figure 8. a. The Diameter (cm) of the clear zone formed in *L. plantarum* with the breakdown of protein. Left group, *L. plantarum*. Right group, *L. plantarum* and adrenaline. The bacterium was incubated for 48 hours in an incubator at 36° C. b. The Mean Diameter (+ 1 SD) of the Clear Zone in *L. plantarum* with and without adrenaline. The clear zone is the area in which protein was broken down through protease turning from white to transparent.

Utilizing its protease to break down protein in the plate, the agar that consists of the protein turns into a transparent shade after protein degradation [7]. In other words, the inner cloudy portion of the ring represents *L. plantarum* growth beginning from the disk paper where it was inserted; on the other hand, the radius of the transparent ring itself represents the extent of the activity of the protease. As a matter of fact, the clear zone is of utmost significance in this investigation not only because it engenders a distinct measurement of the effectiveness of the protease but also a statistical comparison in its function with and without adrenaline. Thus, the motive of this experiment is to compare and examine how the presence of adrenaline may enhance or inhibit the bacteria's function in proteolysis with the size of the clear zone formed in the plate after approximately 48 hours.

It was found that adrenaline increased protein degradation in *L. plantarum*; the diameter of the clear zone in the media with the hormone witnessed a larger measurement being 3.567 cm than that of *L. plantarum* alone being 3.2 cm. Although the 0.367 cm difference may be regarded as negligible, given that we utilized solely 20 µl of *L. plantarum*, such difference is statistically significant along.

In fact, also referred to as proteolysis, molecules of protein are divided into its monomers, or smaller segments, such as amino acids through hydrolysis, adding water to the peptide bond to separate it. What is significant about this study, however, is that proteasomes — the protein complexes in proteolysis — degrade the unneeded external layer of protein, allowing the body to protect itself from toxic diseases within such material [8].

6. Antibacterial Properties

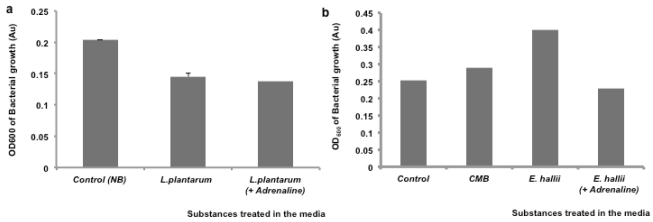

Figure 9. a. The Effect of Adrenaline on the antibiotic activity of *L. plantarum*. *E. coli* was inserted into all tubes, and its growth (Au) was measured in all groups to evaluate the rate of the antibiotic activity of *L. plantarum*. The NB was the control group. Data collected through the spectrophotometer at OD_{600}. b. The Effect of Adrenaline on the antibiotic activity of *E. hallii*. *E. coli* was inserted into all tubes, and its growth (Au) was measured in all groups to evaluate the rate of the antibiotic activity of *E. hallii*. All samples aside from the control and CMB are filtered supernatants. The control group is the NB. Data collected through the spectrophotometer at OD_{600}.

In the experiment to analyze the direct effect of adrenaline on the antibiotic qualities of *L. plantarum* on the growth of *E. coli*, the groups of filtered supernatant both with and without adrenaline were utilized for comparison with the control group being NB receiving the same initial treatment of *E. coli*. As depicted through part a of figure 9, the growth of *E. coli* was suppressed with the presence of *L. plantarum* and further lowered with the addition of adrenaline. Thus, based on the representation of the data set, it was concluded that adrenaline essentially does play a role in inhibiting the antibiotic traits of *L. plantarum*, which is consistent with the prior findings of the increase in the lactic acid production in connection to its antimicrobial properties.

While *E. coli* was equally inserted into all groups, the nutrient broth served as a control group recording a growth of 0.252 ± 0.035 Au, allowing for comparison on whether *E. hallii* alone or with adrenaline had an antibacterial effect on the growth of *E. coli*. In terms of supernatants, both solutions with and without the hormone recorded higher levels of bacterial growth being 0.534 ± 0.091 and 0.607 ± 0.1497 Au respectively. In other words, under the same conditions except for the presence of adrenaline, the hormone rather reduced the bacterial growth from the original supernatant with *E. hallii* and *E. coli*. In fact, this phenomenon also occurred for the filtered solutions; *E. coli* in an environment with and without adrenaline grew to the measurement of 0.2287 ± 0.0032 and 0.4017 ± 0.236 Au respectively. The rather astounding factor of the results was that the filtered solution with adrenaline resulted in even lower growth than the control containing solely *E. coli*, thus highlighting the extent to which both *E. hallii* and adrenaline combined together have an antibiotic effect on the original growth rate.

Discussion

With increased attention towards stress and its physiological impact on the body, the gut microbiome has now arisen to be a subject of extensive discussion especially in its correlation to infectious diseases. Yet, despite such growing interest, research and studies on the effect of hormones on bacteria are insufficient. Thus, the principle of paramount significance in this research was to evaluate the degree to which hormones released during intense exercise affect gut microbiomes such as *E. coli*, *L. plantarum*, and *E. hallii* in their antibacterial properties, metabolism, and growth.

One of the most predominant stress hormones produced through exercise is epinephrine, also more commonly referred to as adrenaline. Adrenaline has been found to possess an important role in the gut through the suppression of tonic contractions in the digestive tract, thus suggesting that the hormone itself has an effect on the gut [8]. With the utilization of the 3 main bacteria within the gut structure, our research oriented its focus on uncovering the role of adrenaline on the bacteria and the balance of metabolic activity.

Conclusion

With *L. plantarum* and *E. hallii*, adrenaline directly increases the production of lactic acid and butyric acid respectively whereas, for *E.coli*, the opposite occurs. Moreover, demonstrating chemotaxis for adrenaline, *L. plantarum* witnessed increases in its rate of protein degradation or proteolysis with increase activity of the protease with adrenaline. In other words, the magnitude and extent of proteolysis were found to be increased with the clear zone indicating the activity of protein degradation. Moreover, in the environment without adrenaline, *L. plantarum* with initial exposure to adrenaline displayed increased protein degradation with a higher area of a clear zone.

In terms of antibacterial properties, however, adrenaline did not have a direct effect on *L. plantarum* and its inhibition of the growth of E. coli; rather, regardless of whether the bacteria were treated with adrenaline or not, the antibacterial activity remained the same. On the other hand, similar to the increase of the production of butyric acid that also consists of antibiotic characteristics, *E. hallii* witnessed increases in its antibiotic properties, lowering the growth of *E. coli* below that of the control.

Evaluating the effect of adrenaline in terms of growth and its potential antibiotic traits, the presence of the hormone had varied effects on each respective bacteria. For one, in the given 48 hour period, the presence of adrenaline essentially had no impact on the growth of all 3 groups of bacteria as the experimental group and the control group without the hormone had very little to no visible changes in its bacterial growth and colony formation. Under a shorter period being 8 hours with varying hormone concentrations — 0%, 0.0002%, and 0.002% — adrenaline reflected an antibiotic effect in the growth of *E. coli* and *L. plantarum* from the fourth hour leading to decreased bacterial growth by the order of increasing concentration while *E. hallii* remained unaffected. Intermediate treatment, furthermore, proved to have no relation to bacterial growth in *L. plantarum* and *E. hallii*; on the other hand, the intermediate treatment contributed to the decrease of bacterial growth in *E. coli,* not by high concentration but instead by its mere presence in the order of 0.0002%, 0.002%, and 0%.

While previous studies investigated how bacteria affect the human body, future researches should be oriented towards analyzing the opposite, that is to say, the signal transduction pathway between hormones and microbes on a molecular level with a wide range of hormones with varying degrees of intensity and duration. Moreover, with scientists now looking toward samples of the gut microbiome as indicators of conditions such as inflammatory bowel disease (IBD), colorectal cancer, and type 2 diabetes, analyzing and comparing the effects of stress hormones released through exercise must hold precedence [9]. Future researches could build upon such a connection to further analyze not only the clear zone formed through proteolysis from *L. plantarum* with and without adrenaline but also the mechanism in which the addition of adrenaline may induce or reduce muscle development. Moreover, future studies could also identify whether the increased antibiotic effect witnessed in *E. hallii* was in correlation with the increased production of butyric acid along with the mechanism in which the antibacterial properties remained consistent in *L. plantarum* even with increased production of lactic acid.

Acknowledgements

I would like to thank all the people who have endlessly supported me through this journey in crafting this research paper. From my caring family members to the encouraging staff members at my current school, I would like to show my utmost gratitude to my advisor Ms. Song, who helped me to view my work in the most objective point of view and to Dr. Rose Tyvand who provided me with the constructive and insightful feedback throughout my research process.

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