The Effect of Paromomycin on the Population Heath of *Tetrahymena thermophila*

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Abstract. *Tetrahymena thermophila*, a fresh water ciliate, is a unicellular eukaryote that feeds by phagocytosis. *Tetrahymena* reproduce by asexual fission (Asia and Forney 2000). The mutant (TTHERM_00442300) is resistant to paromomycin, an aminoglycoside antibiotic. In this experiment, we observed the effects of paromomycin on the population health of both wild type and mutant *Tetrahymena thermophila*. Both types were placed in a growth medium to which paromomycin had been added and observed following immediate exposure to different concentrations of paromomycin. Population health was measured by counting motile cells in the sample taken immediately after cells were placed in a paromomycin medium and after an overnight incubation. Dividing cells were counted after an overnight incubation in paromomycin medium. Proportions of motile cells were calculated relative to the control for each treatment. Peptone growth medium with a 0µg/ml paromomycin concentration yielded about 70 dividing cells per slide throughout the observation time; a 10 µg/ml concentration reduced this number to 50, and later on to 30, after 15 minutes of observation. Thus, on average a reduction of 100 cells per slide at the end of 30 minutes' observation was noted for three replicates in 10, 100 and 200 paromomycin concentrations. The wild-type cells did not undergo any cell division after prolonged exposure to paromomycin and the number of motile cells was reduced to 0-5 cells. Exposure to paromomycin had a dramatic effect on wild type *T.thermophila* and resulted in no dividing cells after 24 hours. Even though immediate exposure to paromomycin had no apparent effects on the mutant, it did negatively affect the population health when grown in paromomycin medium for 24 hours. Paromomycin can result in the inhibition of protein synthesis (Eustice and Wilhelm 1984) as well as the mistranslation of the polypeptide chain (Wilhelm *et.al.* 1978). Thus, the population health of both the wild type and the mutant was reduced as the concentration of paromomycin increased.

Introduction. *Tetrahymena thermophila* is a ciliated protozoan with a very complex cellular structure and function, which is somewhat comparable to that of human and other metazoan cells. *T. thermophila* has two nuclei the macronucleus (larger) is somatic and transcriptionally active while the micronucleus (smaller) is a germline nucleus and transcriptionally inactive (Asia and Forney 2000). During the sexual phase of the life cycle micronuclei undergo meiosis and fertilization (Allis and Dennison 1982). Gene expression for the vegetative (asexual) phase occurs in the macronucleus, where cells divide by binary fission (Allis and Dennison 1982). The macronucleus elongates, constricts, and then divides and is distributed into daughter cells (Asia

and Forney 2000). The mutant used in this experiment (TTHERM_00442300) is resistant to paromomycin. Paromomycin is an antibiotic which inhibits protein synthesis in both eukaryotes and prokaryotes (Bruns *et al.* 1985), and is used for treating intestinal infections in humans. According to Spangler and Blackburn (1985), the resistance to paromomycin in *T.thermophila* results from a single base change mutation near the 3'end of the 17 S rRNA that is involved in protein synthesis. *Tetrahymena thermophila* has high susceptibility to a wide range of antibiotics; as a result, this organism may be an ideal system through which to study antibiotic action in eukaryotes (Eustice and Wilhelm 1984). The sequence of the *Tetrahymena* small subunit rRNA is homologous to that of other eukaryotes (Spangler and Blackburn 1985); thus the paromomycin mutants can aide in the development of high quality antibiotics. The objective of this experiment is to study the effect of paromomycin on the population growth of wild type and mutant cells of *Tetrahymena thermophila.* Therefore, different concentrations of paromomycin were added to both mutant and wild type cell cultures, and the effects were observed over 24 hours. Studying the effect of paromomycin on the population growth of mutant *T. thermophila* will indicate the efficiency of this antibiotic. According to Wilhelm *et.al* (1978), both wild type and mutant cells of *Tetrahymena thermophila* show a decline in population growth as the concentration of paromomycin increases, and this decline is more evident among wild type cells. The null hypothesis is that an increase in paromomycin concentration has no effect or increases the population growth of wild type and mutant cells of *Tetrahymena thermophila,* while the alternate hypothesis is that an increase in paromomycin concentration decreases the population growth of wild type and mutant cells of *Tetrahymena thermophila.*

Methods. To observe the effect of paromomycin on the population growth of *T. thermophila*, the proportion of motile cells was calculated once after the addition of different concentrations of this antibiotic until 30 minutes after, and once after an overnight incubation of cell cultures with paromomycin for 24 hours. Both mutant and wild type cells were treated with 200 µg/ml, 100 µg/ml, and 10 µg/ml of paromomycin. The control was no added paromomycin to both mutant and wild-type. Three replicates were tested for each treatment. The number of motile cells was counted, as an indicator of healthy cells based on their motility, once after each treatment within three time intervals of 0, 15 and 30 minutes. In order to observe the population growth, the treated cultures were left at room temperature (25°C) for 24 hours. The numbers of motile cells were again counted over three time intervals of 24 hrs, 24 hrs 15 mins, and 24 hrs 30 mins. The total number of motile cells was then calculated for each replicate and averaged for each treatment. The proportion of motile cells for each replicate was calculated by dividing the total number of motile cells by the number of motile cells from the control.

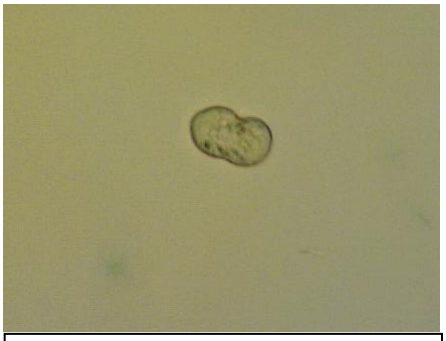

Figure1. Dividing Tetrahymena thermophila under 100x magnification.

The principle method of this experiment involved counting the number of motile cells observed in a 20µl sample which was placed on a slide and viewed under the 100x magnification of a compound microscope. Cells were defined as motile if they were rapidly swimming on the slide, and they were defined as dividing if they were as shown in *T. thermophila* were originally grown in Neff growth medium provided in the lab with a composition of 0.25% proteose peptone, 0.25% yeast extract, 0.55% glucose and 33µM ferric chloride. Paromomycin was diluted from the stock concentration of 50 mg/ml to 10 µg/ml, 100µg/ml and 200 µg/ml in the growth medium. The range used was based on previous studies regarding the paromomycin treatment of wild type *Tetrahymena* (Presscott 2000).

All culture manipulations were performed at a room temperature of 25˚C. We took care to cover test tubes that contained cells overnight to ensure that they were not exposed to direct light from the lamps in the laboratory. Our previous observations showed that the overexposure of cells to lamp or microscope light has a negative effect on the movement of *T.thermophila.* To ensure the uniform distribution of cells on each slide of samples, tubes were shaken prior to pipetting a sample. Also, the top of each tube was flamed to avoid any cell contamination. The experiment was undertaken in one single trial within a 24 hour period.

Basic statistics, such as the average count of cells in the replicates for each treatment, standard deviation, and 95% confidence intervals, were used to analyze the results. To assist with comparisons, absolute numbers were converted to proportions relative to the original number of motile cells in a 0 µg/ml paromomycin concentration.

Results. Bar graphs were constructed to represent percentages of motile cells relative to the control, which was taken as 100%. Averaged absolute values were divided by the average absolute value of the control for each treatment within each replicate in order to obtain relative percentages (e.g. at 200 µg/ml Day 1, MUT1, 242/291*100= 83.16%). Figures 2 and 3 represent the proportion of motile cells from right after the addition of the antibiotic until 30 minutes later.

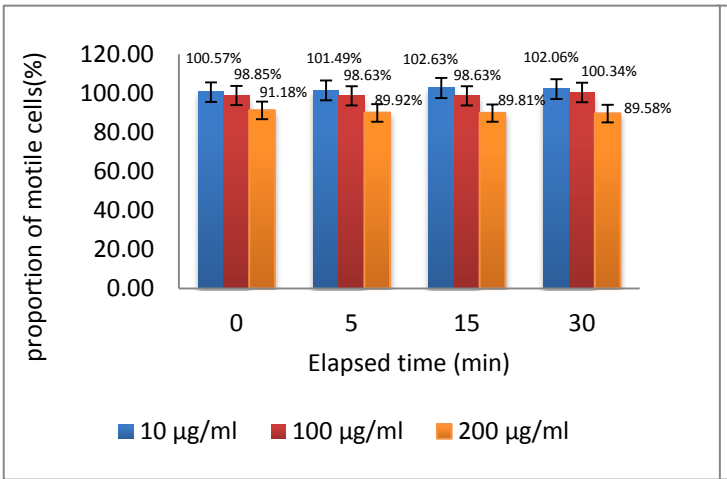

Figure 2. Effect of Paromomycin on wild type *Tetrahymena thermophila*

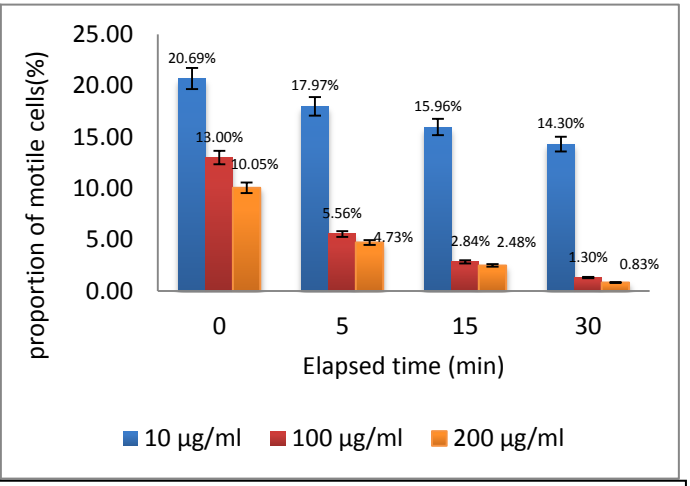

Figure 3. Effect of Paromomycin on mutant *Tetrahymena thermophila*

As shown in Figure 2, the proportion of wild-type motile cells decreased dramatically as the concentration of paromomycin increased; moreover, these proportions decreased further over time. For example, the average number of motile cells was reduced to 3 at the end of 30 min. when a maximum concentration of 200 µg/ml was applied. In the case of mutant cells, a steady decline in the proportion of motile cells was observed as the concentration of paromomycin increased.

Confidence intervals for wild type cultures do not overlap, showing significant statistical differences. However, as expected, no significant difference was observed in the mutant after exposure to paromomycin. The average number of motile cells remained around 291. Cells neither appeared circular nor did they lose motility after exposure to paromomycin.

Figures 4 and 5 represent the proportion of motile cells after treatment with different concentrations of paromomycin after 24 hours. As is indicated in Figure 4, the proportion of motile wild-type cells is very low; thus wild type cells are not healthy. However, according to Figure 5, a significant result is observed and, the population motility decreased as the concentration of paromomycin increased.

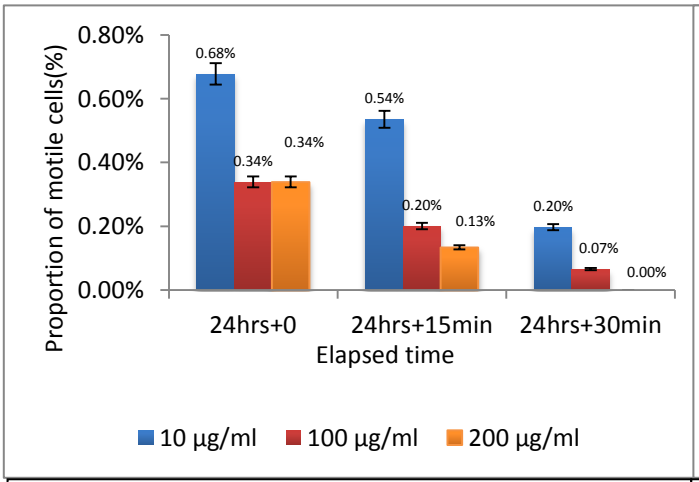

Figure 4. Effect of paromomycin on wild type *Tetrahymena thermophila* after overnight incubation

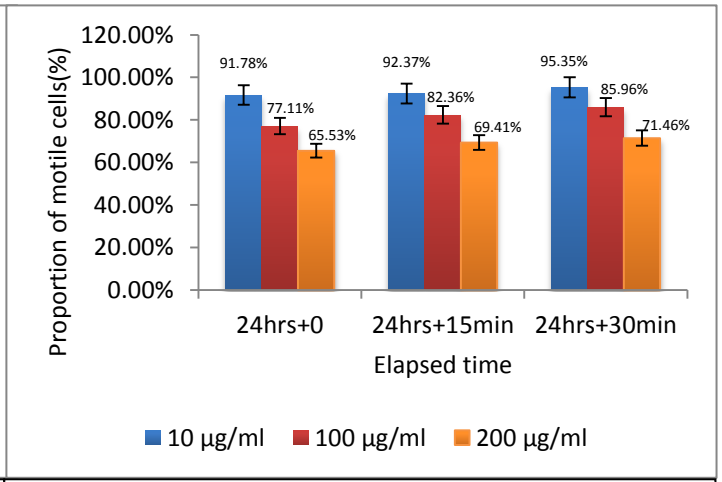

Figure 5. Effect of paromomycin on mutant *Tetrahymena thermophila* after overnight incubation

Furthermore, the proportion of dividing mutant cells was obtained (the wild type culture contained no dividing cells). Figure 6 illustrates the number of dividing cells as a proportion relative to the control. This figure emphasizes the decrease in the proportion of dividing cells as the concentration of paromomycin increased. For instance, the highest proportion of dividing mutant cells (57.87%) occurred right after the overnight incubation (for 24 hours) with 10µg/ml of paromomycin. In contrast, the lowest proportion of dividing cells (18.52%) was obtained after 24 hours of exposure to 200 µg/ml of paromomycin. It is important to note that as time passed, these proportions decreased further.

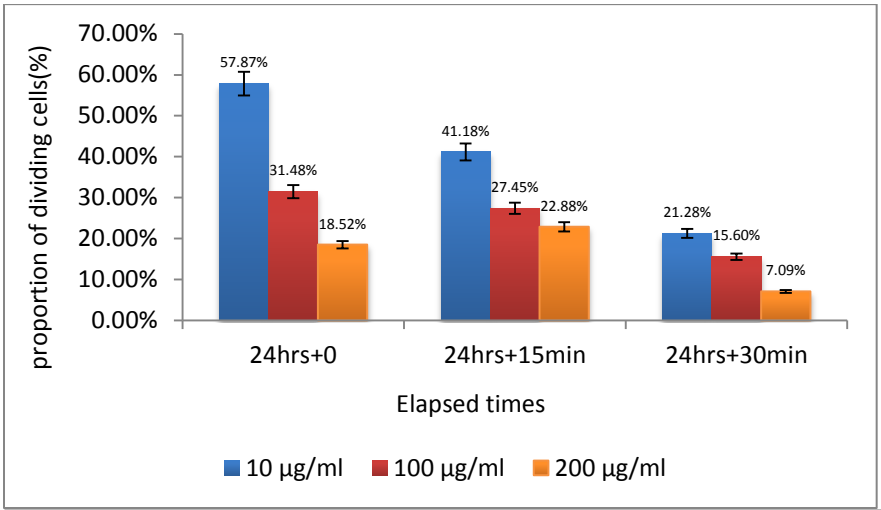

Figure 6. Effect of paromomycin on dividing cells (mutants) of *Tetrahymena thermophila* after overnight incubation

Discussion. Through treatment with a variety of concentrations of paromomycin on the wild-type and mutant cell cultures of *Tetrahymena thermophila*, it was possible to distinguish the extent of the sensitivity of mutant and wild type cells to different dosages of paromomycin. Paromomycin is an aminoglycoside antibiotic that inhibits the growth of eukaryotes and prokaryotes by inhibiting cytoplasmic protein synthesis (Eustice and Wilhelm 1984).

Long-term exposure was achieved by treating cell cultures with different concentrations of paromomycin for 24 hours. After this time interval, a decline was still found in the proportion of moving cells over time. Even though the proportion of moving cells was very low, there was

no statistically significant difference, and hence we can reject the null hypothesis and provide support for the alternate hypothesis. In other words, an increase in paromomycin concentration decreases the population motility of wild type *Tetrahymena thermophila.* Concentrations of paromomycin at 22 µM, 10 µM, and 17 µM have been shown to inhibit the growth of wild type *T.thermophila* by 50% (Eustice and Wilhelm 1984). In our experiment we can infer that the concentrations of 10, 100, and 200 µg/ml are potent inhibitors of motility in wild-type cells since the proportion of motile cells declined rapidly over time. According to Eustice and Wilhelm (1984), a paromomycin concentration of 300 µM results in a substantial loss of polysome content, leading to a decline in protein synthesis. Thus, it is relevant to note that only 0.83% of wild type cells continued moving over time after the addition of 200µg/ml paromomycin. Binding of paromomycin to small ribosomal subunit prevents protein synthesis (Recht *et.al* 1999). Eventually all of the existing rRNAs will become inhibited, reducing the number of motile cells to zero.

The short-term exposure of mutant cells to different concentrations of paromomycin was shown to have no significant effect on the motility of mutant cells since all the confidence intervals overlapped. However, we observed that higher concentrations of paromomycin caused a steady decline in the proportion of moving cells. This resistance clearly confirmed the expected drug-resistant phenotype of mutated *T. thermophila* cells. After long term exposure of mutants to different concentrations of this antibiotic, the decline in the proportion of moving cells was again apparent, however there was also a significant difference observed. According to Figure 5, none of the confidence intervals overlapped, thus we can reject the null hypothesis and provide support for the alternate hypothesis; that is, an increase in paromomycin concentration decreases the motility of mutant cells of *Tetrahymena thermophila.* Both wild-type and mutant cells were reduced in number when high concentrations of paromomycin were added you can't say this unless you have data i.e., counts of the number of cells. Eustice and (1984) found that

paromomycin exhibited the same effect on the growth of mutant *T. thermophila* cells as it did on the wild-type cells. They suggest that inhibition of protein synthesis is not the primary function of paromomycin rather it must also affect other aspects of protein translation. According to Wilhelm *et.al* (1978), aminoglycosides such as paromomycin also stimulate the misreading of the polypeptide chain in addition to the direct inhibition of protein synthesis by binding to rRNA. Misreading leading to mistranslation of the polypeptide chain can result in the production of functional and non-functional proteins (Wilhelm *et.al* 1978). As a result, mutant cells of *Tetrahymena thermophila* can also be affected by paromomycin, but not to the extent that wild type cells are affected. In other words, wild-type cells undergo both mistranslation of proteins and inhibition of protein synthesis, while mutant cells only undergo mistranslation of proteins.

A graph representing the proportion of dividing cells was constructed to further demonstrate the decline in population growth of mutant cells. Figure 6 represents the proportion of dividing cells and it indicates the decline in number of dividing cells. We can provide support for the alternate hypothesis and reject the null hypothesis which states that an increase in paromomycin concentration has no effect or increases the population health of wild-type and mutant cells of *Tetrahymena thermophila.* Although the proportion of dividing cells observed at 24 hrs 15 min. was greater than at 24 hours, we concluded that increase in paromomycin concentration decreased the potential population growth based on the pattern observed in all the other graphs (Figure 3, 4, and 5). This inconsistency may have been due to the errors associated with this experiment.

One major source of error that most likely impacted our experiment is the error associated with assessing motility in *T. thermophila*. Live cells move relatively fast, and as a result, our counts may not have been accurate. Since the cells move in all direction we might have counted some cells twice. The microscope's light might have also impacted the movement of cells leading to miss counting of motile cells. Biological variation among the *T. thermophila*

cells such as their maturity also contributes to errors associated with this experiment. For example, not all cells of *T .thermophila* undergo division at the same time. The cells must reach a certain level of maturity prior to division (Rogers and Karrer 1985). This may be another explanation for the inconsistency in the results obtained from the division of mutant cells after long time exposure to paromomycin.

Conclusion. We have rejected the null hypothesis and provided support for the alternate hypothesis. In other words, an increase in paromomycin concentration decreases the motility of wild-type and mutant cells of *Tetrahymena thermophila.* It is critical to note that only mutant cells were observed to undergo cell division after exposure to paromomycin for greater than 24 hours. The proportion of wild type cells moving after 24 hours of treatment in paromomycin was very low.

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