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The Effects of varying pH on Plasmid Transfer from Escherichia coli to Enterobacter aerogenes.

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ABSTRACT

Antibiotic resistance in bacteria is turning into a major cause for concern in the medical profession. With increased resistance of illness causing bacteria for modern drugs, the need for new drugs is becoming increasingly important. This was a study to determine if pH effects conjugation; the means by which genetic information is transferred from bacterium to bacterium.

Keywords: *Escherichia coli, Enterobacter aerogenes, conjugation, plasmid transfer, and pH*

INTRODUCTION

Escherichia coli uses the process of conjugation to transfer plasmids from donor to recipient cells. Conjugation is a process that involves direct cell-to-cell contact. The structure used for the cell-to-cell contact is called a sex pilus. The genetic material that codes for the pilus is located at the *tra* region of the F plasmid. Not all strains of E. coli, or all strains of any bacteria for that matter, have the genes on the plasmids to code for the pilus. These strains are forced to use other means to receive or donate plasmids. Strains of bacteria that have the ability to form a sex pilus are not capable of receiving plasmids because the plasmid causes the alteration of certain surface receptors. However, once a cell receives the pilus forming plasmid from a donor, the recipient can now act as a donor.

Rolling circle replication is how DNA is transferred from the donor, through the sex pilus, to the recipient. Rolling circle replication is first initiated by the direct cell-to-cell contact that occurs during conjugation. Then, one strand of the plasmid DNA is nicked and the 3'-end of the nick is used to prime synthesis of a new plasmid DNA strand. Finally, before the two ends of the strand connect, the strand is passed through the pilus. Rolling circle replication best describes this process because if the original plasmid is labeled, some labeled DNA is transferred to the recipient but only a single labeled strand is transferred. The result of this process, if uninterrupted, is that both the donor and recipient possess completely formed plasmids.

The entire process of conjugation is highly efficient. Virtually every recipient that comes into contact with a donor receives the donor's plasmid. This is what makes conjugation an important topic to study. As stated earlier, once a recipient has received a donated plasmid, it can now act as a donor. This is how antibiotic resistance is spread so rapidly in a colony of bacteria (since antibiotic resistance is mainly transferred on plasmids and not within the host chromosome).

It is possible for a transferred plasmid to become integrated into the recipient's chromosome; such a plasmid is referred to as an episome. Plasmid integration is a very simple way to mobilize other genetic material. The F plasmid of Escherichia coli is an example of an episome. The F plasmid is not only conjugative but it can also integrate itself into the recipient's chromosome. Strains of bacteria that possess an integrated F plasmid are called Hfr, which stands for high frequency of recombination. This allows the recipient's chromosome to be conjugative as well. Most chromosomes contain too much DNA to completely transfer so it is common for only part of the chromosome to be transferred from one cell to another. This can lead to many different forms of recombinant DNA; which means that any number of traits can be transferred from donor to recipient. The transfer of antibiotic resistance or the ability to ferment lactose are perfect examples of this.

Scott and Flint (1995) studied plasmid transfer under rumen conditions. The measured transfer rates varied under aerobic and anaerobic conditions. Fernandez-Astorga et. al (1992) investigated various abiotic factors affecting plasmid transfer in Escherichia coli. They discovered that the transfer frequencies did not vary with any significant difference as pH was adjusted. Khalil and Gealt (1987) came to a different conclusion when they studied the effects of pH, temperature, and cations on conjugation. They discovered that the alteration of pH and temperature did effect conjugation. However, Khalil and Gealt were looking mostly at the number of transconjugates present. It is unknown which one of these studies is more accurate

The purpose of this study is to determine whether or not pH effects the conjugation of Escherichia coli and Enterobacter aerogenes. In this study three pH's will be investigated; 6.0, 7.0, and 8,0. With the conclusion of this study, there will be further evidence as to whether pH effects conjugation or not.

MATERIALS AND METHODS

Escherichia coli pGFPuv and Enterobacter aerogenes were the two bacteria used in this study. The pGFPuv plasmid of E. coli codes for a green, fluorescent protein and ampicillan resistance. They were first inoculated into lactose broth (LB) individually and left to incubate for 24 hours. Each species was then inoculated onto three different types of agar as a control. The three

agars used were LB+KCN, LB+Ampicllan (AMP), and LB+KCN+AMP; pH's were not adjusted for the control groups, but the experimental groups were exposed to various pH levels. These agars were chosen because E. coli should not grow on agar containing KCN. Likewise, E. aerogenes should not grow on agars contains ampicillan. 4.50 ml of an aqueous 3.0 percent KCN solution was added to 300 ml of LB agar to make the LB+KCN agar and 600 microliters of ampicillan was added to 300 ml of LB agar to make the LB+AMP agar.

E. coli and Ent. aerogenes were then inoculated into 18 test tubes containing LB broth and left to incubate for 24 hours. Tubes 2, 4, 8, 9, 11, 14, and 15 were randomly chosen. These tubes were then streaked onto the previously mentioned agars with varying pH levels and left to incubate for 24 hours.

RESULTS

EXPECTED RESULTS

	E. coli	Ent. aerogenes
LB+KCN	NO GROWTH	GROWTH
LB+AMP	GROWTH	NO GROWTH
LB+KCN+AMP	NO GROWTH	NO GROWTH

CONTROL

LB+KCN	GROWTH	GROWTH
LB+AMP	GROWTH	GROWTH
LB+KCN+AMP	GROWTH	GROWTH

EXPERIMENTAL

pH 6.0			
	LB+KCN	GROWTH	GROWTH
	LB+AMP	GROWTH	GROWTH
	LB+KCN +AMP	GROWTH	GROWTH
pH 7.0			
	LB+KCN	GROWTH	GROWTH
	LB+AMP	GROWTH	GROWTH
	LB+KCN +AMP	GROWTH	GROWTH
pH 8.0			
	LB+KCN	GROWTH	GROWTH
	LB+AMP	GROWTH	GROWTH
	LB+KCN +AMP	GROWTH	GROWTH

From the control study, E. coli grew on the agar containing KCN and KCN+AMP. Likewise, Ent. aerogenes grew on agars containing AMP and KCN+AMP. From the experimental study, growth was observed on all agars. The only difference is that the agars with a pH of 7.0 had more growth and larger colony sizes than that of pH 6.0 and 8.0.

DISCUSSION

The results of this experiment were inconclusive as to the effects of pH on the conjugation of E. coli and Ent. aerogenes. One possible explanation for such results is that not enough KCN and ampicillan were added to the agar solutions. Though KCN hinders E. coli growth, when not enough is present, growth may still occur. The same is true with Ent. aerogenes and ampicillan. If KCN and AMP were too dilute in their respective solution, it is possible the growth occurred in regions of the agar plates that did not contain any of these additives.

LITERATURE CITED

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