

# **Dissertation Research: The Ecology of Colicins**

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**NSF Unit of Consideration: DEB**

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## Project Summary

Colicins are narrow spectrum antibacterial proteins produced by and active against *Escherichia coli* and related enterics. The potential of colicins to structure the microbial population of enteric bacteria in the mammalian large intestine has been debated for several decades. In nature they are ubiquitous and costly resistance factors are common. In culture, colicins are highly effective. However, early attempts (1969-71) to provide evidence of this effect *in vivo* were unsuccessful, in part because experiments that could have demonstrated a fitness increase due to colicin production were invalidated by extraneous factors.

Advances in molecular biology allow the creation of isogenic strains varying only in the presence of colicin resistance or production. These will be introduced into mice which have been cleared of gram-negative organisms but which still possess the bulk of their native bacterial symbionts. The population kinetics will be studied via fecal sampling. Additionally, local metapopulations will be studied directly by destructive sampling of the mice. The bacteria will be labeled with autofluorescent proteins so that they can be differentiated under a microscope. These studies together are expected to confirm the important role of colicins within the large intestine and reveal the conditions under which colicins are effective.

# Project Description

## Introduction

### 1.1 Introduction to Colicins

Bacteriocins are narrow spectrum antibacterial proteins, active against species closely related to the producing strain (Braun 1993). An immunity protein protects cells carrying the bacteriocin (Sidikaro and Nomura 1974). Bacteriocins are produced in large numbers (10,000 and 500,000 colicin molecules/lysis event; Gordon and Riley, 1998) and released through the lysis of the cells that produce them.

The first bacteriocins discovered were produced by *Escherichia coli* and were designated colicins (alt. spelling “colicines,” first observed by Gratia 1925). Studies of colicin function have revealed that colicins partition their functions into three domains (target recognition, cell membrane translocation and killing; Griko *et al.* 2000). Diversity in each domain allows colicins to vary the target cell surface receptors (OmpF, FepA, *etc.*), target translocation mechanisms (TolQ, TonB, *etc.*), and method of killing (pore formation, RNase, DNase, *etc.*; Pugsley 1984).

Colicin gene clusters are normally plasmid borne, on both small multicopy plasmids (A, the E family, and others) and large plasmids (B, Ia, Ib, M). The clusters generally contain three kinds of genes which encode for the following three proteins: Colicin (Col), the toxin itself; Immunity (Imm), which binds to the active site of the killing domain, and lysis (Lys), which assists in export and cell death.

### 1.2 The Population Genetics of Colicins

The frequencies of colicin production from enterics in medical specimens (Riley and Gordon 1992) and in fecal specimens from several animal hosts (including pigs, Craven *et al.* 1971; Dealwis and Tomlins, 1973, and Willinge and Trcka, 1973; chickens, Blanco *et al.* 1997; mice, Gordon *et al.* 1998, and various Australian mammals, Gordon and Lee 1999, Gordon and Fitzgibbon 1999) have shown no obvious differences among the host species. Five to fifty percent of enteric populations surveyed are colicinogenic (Riley 1996). In addition to sensitive cells and colicinogenic cells, there are “resistant” and “tolerant” cells. If a cell’s receptor has been altered in such a way that a colicin cannot attack the cell, then the cell is “resistant” to the colicin. If the cell’s translocation machinery has been altered, the result is “tolerance” (Feldgarden and Riley 1999). Resistance reduces the performance of the receptor. Under laboratory conditions, 24% of resistant strains were less fit than otherwise isogenic strains (Feldgarden and Riley 1999), and tolerance has an even higher fitness cost (Riley 1998). Resistance to colicins is common, with 96% of the population resistant to cosegregating bacteriocins (Feldgarden 1999). These two frequencies bear testimony to the importance of colicins, but more surprising is the fact that sensitive strains also persist in the intestines in which colicins are present (Gordon *et al.* 1998).

### 1.3 Colicin Ecology *in vitro*

Culture systems for studies of colicin ecology include serial transfer (Tan and Riley 1996), chemostats (Adams 1979; De Jong *et al.* 1986), and plate surface competitions (Chao and Levin 1981; Kim, dissertation; Kerr, unpublished data). In a well-mixed system, like a chemostat, the colicinogenic strain quickly eliminates the

sensitive cells or goes extinct due to lysis (Adams 1979). No mixed population including colicinogenic strains is robustly stable in such a homogenous system.

On plates, however, spatial structure allows initially rare colicinogenic strains to invade an established population (Chao and Levin 1981). A structured environment permits the concentration of colicin in the space adjacent to the colicinogenic strain, which effectively reserves nutrients for the colony and allows the colony to spread. When the plate is replicated, an increased amount of space is devoted to the initially rare invader. During this invasion dynamic, both sensitive and colicinogenic strains coexist for a relatively long period. This can result in the selection of resistant strains.

#### **1.4 Colicin Ecology *in silico***

Results from mathematical models generally correspond closely to results from *in vitro* experiments (Dykhuisen and Hartl 1983; Gordon and Riley 1999). Modeling studies confirm that the suite of known interactions is sufficient to explain the *in vitro* results. More sophisticated models have included spatial structure using standard differential equations (Iwasa *et al* 1998), discrete mathematics, and even game theory (Durrett 1998). Mathematical studies have also suggested that certain parameters, such as the number of colicin molecules produced per cell or the fitness cost to particular resistance mutations, are particularly important in determining the outcome of the experimental competitions (Gordon and Riley 1999).

Certain models also seek to explain the findings of the population survey studies and to reconcile them with *in vitro* experiments. Bacteriocin-producing, resistant, and sensitive strains are all commonly found by phenotypic surveys (Gordon *et al.*, 1998). As noted above, stable retention of both sensitive and bacteriocin producing strains is not observed in a well-mixed container. This has been repeatedly confirmed *in vitro* and through mathematical modeling (Frank, 1994). Mathematical modeling has demonstrated that diversity can be maintained by imposing spatial structure on the environment (Chao and Levin, 1981; Nakamaru and Iwasa, 2000; Kim, thesis). Additionally, modeling has tied survival of sensitive strains to high nutrient concentrations and survival of colicinogenic strains to low nutrient environments (Frank, 1994, Gordon and Riley, 1999).

#### **1.5 Colicin Ecology in the Host**

The discovery of antagonistic mechanisms *in vitro* (Gatia 1925) led authors to consider antagonistic interactions *in vivo* (Ikari *et al*, 1969). Unexpectedly, studies using gnotobiotic hosts showed little benefit that could be attributed to bacteriocinogenesis. Ikari *et al* (1969) showed that bacteriocin producers were easily out-competed by a laboratory strain (K-12) of *E. coli* in gnotobiotic mice. Even when the colicin-bearing strain equilibrated in the mouse first, the sensitive strain still drove it out. Further studies (reviewed in Hardy 1975) were inconclusive due to failed controls. Kelstrup and Gibbons (1969) fed gnotobiotic mice with a laboratory strain carrying colicin E-2 and found that the colicinogenic strain was successful in displacing even colicin resistant strains, thus the results were inconclusive regarding the benefit of colicin mediated antagonism. Craven *et al* (1971) used natural isolates in gnotobiotic pigs. Once again the colicinogenic strain dominated regardless of resistance (Craven *et al*, 1971).

These unsuccessful attempts to demonstrate that strongly antagonistic strains have an advantage in displacing conspecifics *in vivo* cast doubt on the effectiveness of bacteriocin-mediated intraspecific antagonism (Hardy, 1975; Freter, 1983). The discrepancies between *in vivo* and *in vitro* findings regarding the effectiveness of bacteriocins have spawned considerable debate (Kelstrup and Gibbons, 1969; Freter 1983; Wilson, 1997; Feldgarden *et al.*, 1995). This conflict can best be resolved by observing controlled competitions among colicinogenic, sensitive, and resistant strains in an intestinal habitat.

## 2. Introduction to Experimental Approach

Within the past decade, a system for controlled competition among murine enterics in a nearly natural habitat has been developed and applied to the analysis of several colonization factors (Wadolowski *et al* 1988a, Wadolowski *et al* 1988b, Que and Hentges, 1985). Mice are raised normally until a couple days before the experiment, when a narrow spectrum antibiotic is introduced to their water. This selectively eliminates the native enteric strains. The engineered competitor strains, which are resistant to the antibiotic by design, are then inoculated orally into the mouse (Wadolowski *et al* 1988a). The enteric strains in the intestine shed into the lumen and can be counted in the feces. While some strains may engineer their own retention more successfully and thus shed less frequently at first, the fecal sampling does provide an accurate picture of which strains are being forced out over time (Cohen pers. comm.).

Practically, there are two concerns about using this system. One is that the antibiotic resistance marker might alter the colonization and competition among the bacteria. However, many enteric bacteria pick up identical resistance elements in nature (Mullany 2000). As both competitors have identical resistance markers, the markers should not create a fitness difference unless they interact with the colicin genes. The second concern is that the antibiotic could eliminate species of bacteria that are not enteric. No commonly used antibiotics are specific for the enteric clade alone. The antibiotic currently in use is streptomycin, which eliminates all gram-negative bacteria. The majority of the bacteria in the lumen of the intestine is gram-positive and remains undisturbed (pers. comm. Cohen), but prominent genera (i.e. *Bacteriodes*) are directly harmed by the streptomycin treatment. When strains are introduced into the mouse, they grow to occupy some areas normally populated by other gram-negative strains (Wadolowski 1988a). Even so, the fidelity of this model to nature exceeds that of previously constructed systems for competing enteric bacteria.

Only once have colicinogenic organisms been competed within this model system. One strain produced colicin F-18, carried on an 86 kb plasmid. The other strain had been cured of that plasmid but retained resistance to the colicin (Wadolowski 1988a). The colicinogenic strain displaced the resistant strain due to other colonization factors on the large plasmid. Thus this experiment also fails to address the question of colicin function in the large intestine.

In culture and mathematical models, spatial structure is critical to the ecological function of colicins (and other allelopathic agents, Shimoda 1997, Iwasa *et al* 1998, Nakamura and Iwasa 2000). Thus, an investigation into the dynamics of the competitions would be best conducted in tandem with an investigation of the spatial structure assumed by the competitors. The direct assay for spatial structure in microbial populations is

performed through microscopy. However, microscopy does not allow for the execution of common phenotypic assays that distinguish related strains. Recently, researchers have gained the ability to insert genes for several green fluorescent protein derivatives (autofluorescent proteins, AFP) into enteric bacteria (Qbiogene Inc.). Expression can be linked to other genes or to leaky promoters for constitutive expression. In this fashion, the organism can be identified under fluorescence microscopy without any staining.

In the mouse model, FISH has been applied to localize the enteric competitors introduced into the large intestine (Poulson *et al* 1994, Favre-Bonte *et al* 1999). In one study, FISH revealed that the enteric strains were clustered in the mucous but not adjacent to the epithelium. Instead, the bacteria seemed to be adhering to the mucous (Poulson *et al* 1994). In a second study the enteric bacteria that were successful in colonizing the intestine were scattered and in the deeper fascia of the mucous, while the cells of the unsuccessful strain were clustered tightly and less well entrenched in the mucous (Favre-Bonte *et al* 1999). AFP constructs have not yet been used to investigate competition among enteric bacteria. Multicolor AFPs are a recent development (Qbiogene Inc.), as is the evidence that AFPs both fold correctly in the *E. coli* periplasm and do not interfere with enteric bacteria cellular function (Casey *et al* 2000).

## **Aims and Approaches**

### **3.1 Aim 1. Investigate colicin-mediated competition kinetics in a natural environment.**

**Approach:** Pairwise competitions between strains that differ in their colicin status: resistant, colicinogenic, and sensitive. A variety of colicins will be tested to compare the effectiveness of various killing methods and translocation mechanisms. The competitions will be accompanied by *in vitro* and *in silico* experiments.

### **3.2 Aim 2: Study the mechanism by which colicin-mediated antagonism influences enteric ecology.**

**Approach:** An examination of fine spatial structure established by competitors in their host environment. The competitors examined under **Aim 1** will be labeled with AFP, and cross sections of mouse intestine will be examined microscopically to determine the spatial structure present under different competitive regimes.

## **Methods**

### **4.1 Initial Bacterial Strain Construction**

For each colicin under investigation, a colicinogenic, sensitive and resistant strain will be derived from the plasmid free, colicin sensitive BZB1011 laboratory strain and a strain that is the source for characterized colicin plasmid. This will allow the same sensitive strain to be used for all the colicins. The colicin sensitive strain has been isolated by selecting for resistance on streptomycin LB plates (Cohen, pers. comm.). The resistant strains are derived from the sensitive strain by selecting for colicin resistance on LB plates spread with lysate from the original colicin producing strain. Colicinogenic strains are derived from the sensitive strain by electroporating the strain in the presence of purified plasmid from the original colicin bearing strain.

This protocol has already yielded the desired strains for colicin E2, but for each colicin the resulting strains need to be tested. Two controls are required:

1. Confirm long term (3 week) strain stability in LB broth serial transfer
2. Confirm that strains conform to the expected competition outcomes in LB serial transfer experiments

#### **4.2 Fluorescent Bacterial Strain Construction**

A second group of strains will be constructed for the purpose of microscopy. These strains differ from the strains discussed above in that they are labeled with AFP genes. Because all competitions are pairwise, only two different AFP genes are needed. Particularly bright and easily distinguished AFPs currently available are sgGFP and sgBFP from Qbiogene. These genes differ by only a few substitutions and can be expected to have similar fitness costs. They are commercially available behind bacterial promoters, as well as on reporter and cloning vectors. The colicin will be removed from its native plasmid and placed into a common vector plasmid that already bears an AFP and resistance markers appropriate to the individual competitions.

These strains will be tested for stability and general growth rate in the same way the first set of competing strains was. They can be expected to have a lower maximum growth rate because of the AFP. If this creates a problem in colonizing the mouse, the constructs can be altered to use a weaker promoter, for instance, replacing the T7 promoter with a T7/lac repressed promoter (Stratagene).

#### **4.3 Mouse Model**

Mice (CD-1, obtained from Charles River) with unmodified bacterial floras and normal eating habits will be maintained on lab food and water containing 5 g/l streptomycin sulfate (roughly 15 mg/day; normal prophylaxis is 5 mg/day, LD<sub>50</sub> for mouse is 5 mg/10 g). This treatment will begin 24-48 hours prior to an inoculation. The mice will be processed three to a treatment (Wadolowski *et al.* 1988), with each treatment housed in a separate cage. Cages will be rid of pellets daily.

Mice will be euthanized by CO<sub>2</sub> unless they can be transferred to another researcher alive for further use. Because the protocol causes no known discomfort (pers. comm. Cohen), there is no plan for anaesthetics or analgesics. Standard proactive veterinary services are part of the *per diem* allocated for care of the mice in the protocol submitted to the appropriate review boards.

#### **4.4 Protocol for Competition Experiments**

There are two kinds of competition experiments: *in vitro* and *in vivo*. *In vitro* competition experiments are performed as serial transfer experiments within 10 ml streptomycin LB flasks, each with a culture period of 24 hours. At each period, 100 µl of the culture is moved into a fresh flask, which is incubated at 37°C, shaking, for the next 24 hours. Samples are removed on selected days and frozen at -80°C for plating. These methods were chosen to correspond to earlier methods (Feldgarten and Riley 1999).

To initiate an *in vivo* competition, bacteria (10<sup>5</sup> cell/ml in bicarbonate buffer, Freter *et al* 1983, roughly 10<sup>4</sup> cell total inoculum per mouse, Wadolowski *et al.* 1988) will be administered by intragastric lavage after an overnight fast. In most competitions, this will be followed by an equilibration period before a second inoculation, but in some,

the second will follow closely after the first. Fresh stool samples will be collected from the mice themselves at the intervals of 2-72 hours, more frequently early in the experiments. The samples will be placed immediately in 1% peptone (Maximum Recovery Diluent) for up to 2 hrs at 4°C to keep the cells moist and avoid osmotic shock before plating. Cells will be plated on MacConkey media with streptomycin and then tested for the relevant phenotypes: oxidase, colicin resistance, and colicin production. When the cells are AFP labeled, other options to streamline their enumeration may include observing colonies for fluorescence directly, or filtering the diluent and performing a direct count.

During the experiments to achieve **Aim 2**, there is a greater risk that incidental contamination by streptomycin resistant enterics could upset the competition because the competitors will possibly be hindered by the AFP production. In this case, sampling will be monitored regularly during the equilibration period by plating on MacConkey with ampicillin to confirm the total counts on MacConkey with streptomycin.

#### **4.5 Strain Colonization Controls**

After the construction, it is necessary to demonstrate that the strains behave similarly within a mouse. Inoculating each strain into a separate mouse and then tracing the population dynamics by sampling the feces allows investigation of both the initial colonization dynamics and the resource limited carrying capacity. It is expected that both colicin production and resistance will bear a significant cost, as will each AFP. At the same time, it is expected that the loads will be low enough that each strain will be able to colonize mice. If this assumption is invalid, it will be necessary to alter the constructs to reduce the load of the marker, resistance mutation, or colicin plasmid.

#### **4.6 Aim 1 Experiments and Hypotheses**

The experiments used to achieve **Aim 1** are pairwise competitions among the basic constructs relating to each separate colicin. Because there are three strains per colicin, there will be three pairwise competitions. *In vitro*, these are carried out by serial transfer as described above. *In silico* models (see Appendix; Gordon and Riley, 1998) can be constructed to correspond to these spatially homogenous *in vitro* competitions. These three pairwise competitions are also to be carried out *in vivo*. It is the comparison of the kinetics among these three different classes of model that lends particular power to the analysis.

*In vivo*, the order of inoculation is also of potential interest. Cells behave differently during colonization and at equilibrium, so it is necessary to give each strain the opportunity to equilibrate in the mouse before its competitor is introduced. Because of the need to study the pairs in both inoculation orders as well as when they are simultaneously introduced into the host, nine experiments are required to cover all the possible combinations of the three strains.

#	Sensitive	Resistant	Colicinogenic
1	Offense	Defense	
2	Defense	Offense	
3	Simultaneous	Simultaneous	
4	Offense		Defense
5	Defense		Offense
6	Simultaneous		Simultaneous
7		Offense	Defense
8		Defense	Offense
9		Simultaneous	Simultaneous

**Table 1: The Competitions**

Equilibration in similar experiments takes approximately a week (Wadolowski *et al.* 1988). Competitions themselves may vary in duration, from a few days to weeks, depending on the strength of the selection. Sampling will occur at intervals as short as 2 hours initially to capture the dynamics of colonization and estimate the bowel mobility of the mice. Subsequently, the sampling rate will diminish over time.

In assembling a coherent hypothesis about the outcome of these competitions, several assumptions based on the literature were formulated. These are:

1. Colicin is not effective against resistant strains
2. Colicin is effective against the sensitive strain
3. Sensitive strains grow more quickly than resistant strains
4. Eventual fixation of one phenotype
5. Colicin is produced in the intestine

The strains are constructed such that the first two assumptions are met *in vitro*. The third assumption has been shown to be reasonable *in vitro* (Feldgarden and Riley 1999) and will be tested *in vitro* in both LB and minimal media before the *in vivo* competitions. The fourth assumption is drawn from the need to posit that each competition is a simple choice. Stable associations of two direct competitors are unstable in a homogenous environment. Because these strains are almost isogenic, it is doubted that they would populate different microhabitats. Therefore, it is expected that the competitions will be resolved. If the fifth assumption is violated, no killing will take place. The sensitive strain would displace all other competitors. This seems unlikely, but would be evident during the experiment.

The idea that colicin production is primarily a trait for retaining space already colonized has motivated several previous experiments (Kelstrup and Gibbons 1969, Ikari 1969). This hypothesis is tested in the planned experiments. If, in fact, colicinogenic strains displace sensitive strains regardless of order of inoculation, then there would be a transitive ordering: colicinogenic beats sensitive, sensitive beats resistant, resistant beats colicinogenic. If true, it would be difficult to explain the presence of sensitive strains and cosegregating colicins in the same host (Gordon *et al.* 1998). If, as hypothesized, inoculation order matters, then there is asymmetry in the competition, and the entire table of outcomes will be as below.

Defense ↓ Offense →	Sensitive	Colicin Producing	Resistant
Sensitive	-	Sensitive	Sensitive
Colicin Producing	Colicin Producing	-	Resistant
Resistant	Sensitive	Resistant	-

## Table 2: Strain Hypothesized to Survive *in vivo* Competition

The experiments that involve simultaneous inoculation of both strains will be interpreted less on the basis of the final outcome than on the detailed kinetics. It is currently thought that enteric cells enter the host in logarithmic growth phase and that a molecular switch (pers. comm. Cohen) puts them in a slower metabolic state when colonization is complete. Neither strain will have obtained an equilibrium state, so the strains will be competing directly for the empty niches throughout the intestine. By comparing the dynamics of these competitions to those of the competitions with a single established culture, the relative success in establishment and particularly the activity of colicins prior to establishment can be determined.

These experiments would be less profitable were the colicinogenic strain to demonstrate no apparent killing phenotype in the mouse. That would be recognized by identical kinetics when colicinogenic cells interact with sensitive and resistant cells. Another colicin could be tested, but in essence this would have demonstrated replication of the results from gnotobiotic animals (Ikari 1969). Those results led to hypotheses that colicins are active anticompetitive agents in the water, soil or feces, rather than the intestine, or that colicin immunity conferred phage immunity and that colicins themselves are incidental. One further option, should we obtain results identical to Ikari, is that we have not sufficiently eliminated the refuge provided by the lumen. It has been noted that the addition of the streptomycin elevates the number of enteric organisms by two orders of magnitude because the various gram-negative anaerobes and microaerophiles are removed as collateral damage. Some of these gram-negative genera are particularly notable, like *Bacteriodes*. The selection of streptomycin resistant strains would allow them to survive in the intestine, increasing the fidelity of the model. If the population of enterics were reduced to its natural density, then repetition of key competitions would allow this model to function as intended.

### 4.7 Aim 2 Experiments

The competitions that are described for **Aim 1** in detail provide the format, preliminary data, and underlying motivation for the experiments that approach **Aim 2**. The major alteration is that during the competitions, the intestines are dissected out, sectioned, and visualized. The most important control that will be performed is the comparison between the gross kinetics observed in the previous experiments and the same gross kinetics observed in the corresponding AFP<sup>+</sup> strains. If the kinetics are nearly identical, then it is very unlikely that the mechanism of the interaction has changed.

Normally, FISH requires tissue fixation and a number of other washes. However, in this case because the cells are autofluorescent, preparation is minimized. The mouse will be euthanized (using CO<sub>2</sub> inhalation) and the large intestine dissected out. The intact large intestine is bathed in 30% glucose and then OCT to harden the epithelial cells for sectioning. Approximately 7 mm long sections of the gut (each a future fecal pellet) are then frozen in dry ice/ethanol slurry within an OCT block. It can be cross-sectioned to ten microns or less. Every effort will be made to obtain successive sections, approximately 5 groups of 5 from each OCT embedded block. The sections will then be placed on a poly-L-lysine-coated slide and observed directly.

The microscopy will be performed on a Zeiss Axiovert 135 with a Photometrix PXL camera. The fluor will be excited by a 28 $\mu$ J visible band tunable dye laser (Laser Science Inc DYE-120). Emission will be controlled by narrow band emission filters (Chroma Technologies). Normally, even with narrow band excitation filters, there is intense autofluorescence in all bands, but by exciting at precisely one frequency and limiting the range of emission tightly, it is expected that autofluorescence can be limited.

The observations taken by microscope are expected to reveal 100-1000 cells per section. The enteric cells will be arrayed around the intestine in the mucous layer that surrounds the lumen, apart from the epithelium and from the lumen contents. The strain that is most successful in establishing itself is hypothesized to occupy a position on average closer to the epithelium (Wadolowski *et al* 1988). It may be that unsuccessful strains will be clustered together while successful strains will be widely distributed, as with *Klebsiella* (Favre-Bonte *et al* 1999). Recording the coordinates of each cell (NIH Image) and performing hierarchical cluster analysis on the numerical data (Mathematica) will lead to a quantitative measure of structure. This will reveal whether the cells form colonies, as well as the relative size of the colonies and whether the colonies tend to associate with each other around the intestinal wall. Finally, it is expected that colicinogenic strains will produce zones of clearance around the areas that they populate. However, these may not be obvious without making the colicins themselves visible.

# Timeline

	May	June	July	Aug.	Sept.	Oct	Nov.	Dec.
Colicin E2	Construct Strains	Testing Strains	<i>In vivo</i> Competitions					Construct AFP strains
Colicin Ia						Construct Strains	Testing Strains	
Colicin 3						Construct Strains	Testing Strains	
Colicin 4						Construct Strains	Testing Strains	
Colicin 5						Construct Strains	Testing Strains	

Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	2002	2003
		Testing AFP Strains	<i>In vivo</i> AFP Competitions										
<i>In vivo</i> Competitions				Construct AFP strains	Testing AFP Strains	<i>In vivo</i> AFP Competitions							
<i>In vivo</i> Competitions				Construct AFP strains	Testing AFP Strains	<i>In vivo</i> AFP Competitions							
<i>In vivo</i> Competitions				Construct AFP strains	Testing AFP Strains	<i>In vivo</i> AFP Competitions							
<i>In vivo</i> Competitions				Construct AFP strains	Testing AFP Strains	<i>In vivo</i> AFP Competitions							

## Appendix: Mathematical Model

This model is derived from Gordon and Riley, 1999.

There are 5 differential equations, one for each type of cell (Sensitive, Producing/colicinogenic, Resistant), one for the limiting resource, and one for the colicins themselves.

$$S' = \Psi_{S(r)}S - \alpha Sc + \Psi_{R(r)}R(\mu_S/2) - \Psi_{S(r)}S(\mu_R/2)$$

$$P' = \Psi_{P(r)}P - \lambda_r P$$

$$R' = \Psi_{R(r)}R - \Psi_{R(r)}R(\mu_S/2) + \Psi_{S(r)}S(\mu_R/2)$$

$$c' = \beta \lambda_r P - \alpha Sc$$

$$r' = -\varepsilon(\Psi_{S(r)}S + \Psi_{P(r)}P + \Psi_{R(r)}R)$$

Some of these equations contain a bit of shorthand, expanded below:

$$\begin{aligned}\Psi_{Q(r)} &= \Psi_{Q_{\max}} (r/(\Omega + r)) \\ \lambda_r &= \lambda_e + (1 - (r/(r+a))((r_{\text{ini}} + a)/r_{\text{ini}}))(\lambda_s - \lambda_e)\end{aligned}$$

The variables (other than S, P, R, c and r) are:

$\alpha$  is the absorption constant

$\beta$  is the colicin released per lysis event

$\lambda$  is a lysis rate (rate at which producing cells lyse)

$\varepsilon$  is the amount of resource taken to create a single cell

$\mu$  is a mutation rate (from some phenotype to the subscripted phenotype)

$\Psi$  is a growth rate

$\Omega$  is the half saturation constant for growth on the limiting nutrient

$a$  is an experimentally determined constant that controls response of lysis to starvation

## Budget

This budget specifically does not include salary, consumables common to the PI's facilities, or capital equipment.

Item	Quantity	Unit Cost	Total
Mouse acquisition	500	\$3.36	\$1700
Per diem care	3500	\$0.57	\$2000
Filters	6	\$250	\$1500
Dye for laser	3	\$500	\$1500
AFP gene kits	3	\$250	\$750
Microtome blades	1 case	\$300	\$300
Slides	10 gross	\$40	\$400
Lysine solution	200 ml	\$.50	\$100
Travel			\$250
		Total:	\$6500

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