Title: **Origin of Directed Mutations**

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ABSTRACT

This research provides experimental proof for directed mutation and the reasons why past and current research demonstrate apparent support for random mutation.

Escherichia coli demonstrates concurrency in number of streptomycin-resistant colonies across multiple plates when plated simultaneously, indicating a pattern in occurrence, rather than randomness.

INTRODUCTION

Mutations are conventionally considered to be random.

Classical experiments (1-4) have undertaken to prove that random mutation causes resistance to phage rather than an ability to adaptively mutate or mutate based on direction by a causative agent or factor. They have been corroborated by recent experiments (5-8).

Unsuccessful attempts have been made to demonstrate the possibility of directed mutation using beneficial interactions (9-16), which have been shown as mechanistically Darwinian and are instances where mutation is actually random by others (17-20).

Hypothesis

If mutation in a clonal population of bacteria is non-random and directed (assuming no naturally occurring genetic variation), then a state of cell division presents the only other genetic variation that can define whether an organism can mutate or not.

To test this, assume that a bacterial culture grown from a single cell would have all cells dividing at the same time. For example, if the first cell division is at 0 minutes and the time interval between cell division is 20 minutes, then the cells will divide synchronously, across the culture at 20 minutes, 40 minutes, 60 minutes etc.,

Assuming the bacterial cells can mutate if streptomycin is introduced at the time of cell division, then the colony should demonstrate jackpot number of resistant

bacteria at these cell division times. Initial experiments indicated increasing numbers of jackpots between the time intervals.

This was assumed to be caused by a time lapse between the formation of each daughter cell from the two poles of their parent cells. The time lapse, in turn, resulted in non-synchronous cell division times across the culture.

The assumption has been confirmed by prior research that has established that cell division in bacteria is asymmetrical (21).

Therefore, cell division being a factor in mutability, and thereby directed mutation cannot be proven using this method.

However, if jackpots are a result of coincidence of cell division for a majority of cells rather than synchronous cell division, or presence of offspring of a pre-adapted cell in an earlier generation, then parallel plates that have also been treated with streptomycin simultaneously (No gap in time between the treatment of the parallel plates) should exhibit presence or absence of Jackpot colonies in parallel.

With a high degree of precision in plating, the number of mutant colonies should be similar in both jackpot and non-jackpot colonies.

Materials and Methods

Wild type E.coli was isolated from fecal matter using FL002 flexi plates manufactured by Hi media laboratories were used for coliform isolation.

The isolated E.coli was cultured in nutrient beef broth for 24 hours.

40 test tubes (4 ml) containing 0.5 ml of streptomycin solution (1mg streptomycin and 0.2 µl nutrient broth in 500ml of distilled water) were divided into ten sets of 4test tubes each. The test tubes were inoculated in sets of four each. Each test tube in a set of four, received 0.1 µl of the innocula simultaneously (Time gap between first and last test tube inoculated in a concurrent set was lesser than 2 seconds).

The time interval between the inoculations of two consecutive sets was 5 minutes.

After 8 hours, 40 disposable MP001 nutrient agar plates manufactured by Hi media laboratories were plated with innocula from the test tubes (one test tube per plate).

The plates were examined after 36 hours and 48 hours and a colony count was taken.

DISCUSSION

The experimental results are consistent with the hypothesis of directed mutation occurring during cell division. They show parallels in the number of mutant colonies across each set of 4 parallel plates, when plated simultaneously without a time gap.

Both classical and modern experiments that sought the answer for the ‘Random’ versus ‘Directed’ mutation question, did not consider the state of cell division to be a factor in mutation.

Even if it had been considered, some of the methods employed would have still proved a case for random mutation as non synchronous cell division causes fluctuating numbers of dividing cells at different points of time, which mimics random mutation.

An argument for occurrence of pre-adapted mutants in the source culture before the parallel plates were plated cannot be made as it would have resulted in increasing number of mutants in the source culture, and consequently all sets of parallel plates subsequent to a set of jackpot colonies would have all demonstrated jackpot colonies, which was not the case.

The experiment only determines the condition required for directed mutation to occur and does not suggest a mechanism of the mutation. The actual mechanism of mutation may vary based on the selection mechanism, and the taxa of the species.

Summary

In summation, the experiment presents a case for the following:

1) Mutation is directed in bacterial cells, and occurs when a selective mechanism is applied at the time of cell division.

2) Occurrence of mutants may seem random, as cell division is asynchronous and cell division timing is not same, even in clonal cells.

3) Mutatability may vary based on specific selection mechanism, genetic makeup of target organism, and other factors.

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RESULTS

Table 1: Results of the Synchronicity Test

Set No.	Time of Plating HH:MM (AM)	Series (A)	Series (B)	Series (C)	Series (D)
1.	2:05	400+	400+	400+	400+
2.	2:10	48	400+	98	27
3.	2:15	15	35	11	4
4.	2:20	65	3	18	4
5.	2:25	3	9	18	14
6.	2:30	2	1	0	1
7.	2:35	16	4	12	18
8.	2:40	24	25	NR	5
9.	2:45	23	3	1	19
10.	2:50	9	17	10	5