

14-12-21

EXPERIMENT NO. 7

AIM OF THE EXPERIMENT - Linkage mapping based on data from conjugation.

THEORY -

In bacteria, in conjugation, transformation and transduction are critical phenomena for linkage based gene mapping. We know that the F-plasmid may serve as an episome that is it incorporates with the chromosomal DNA. Such F^+ -cells are called 'Hfr'. With different strains of bacteria having metabolic defects and antibiotic sensitivities which are F^- and having them conjugated with Hfr cells which have the above mentioned genes, linkage maps may be prepared.

When F-plasmid from an Hfr is transferred to an F^- -cell, depending on time taken for conjugation genes adjoining the F-plasmid may also get transferred making a F' -plasmid in F^- -cell. The linkage frequency, where F^- -cells become F^+ as well as containing other genes derived from Hfr cell indicates the distance between the F-plasmid and other transferred genes.

In transduction, a similar phenomena where a defective phage transfers bacterial genes may be used to determine linkage

map. In transformation, the frequency of transformed cells having derived different genes from the environment indicates distance between the genes.

MATERIALS -

Supplied data, pencil, Graph paper etc.

PROCEDURE -

(i) The supplied data is a graph showing frequency of conjugative transfer of different genes with time.

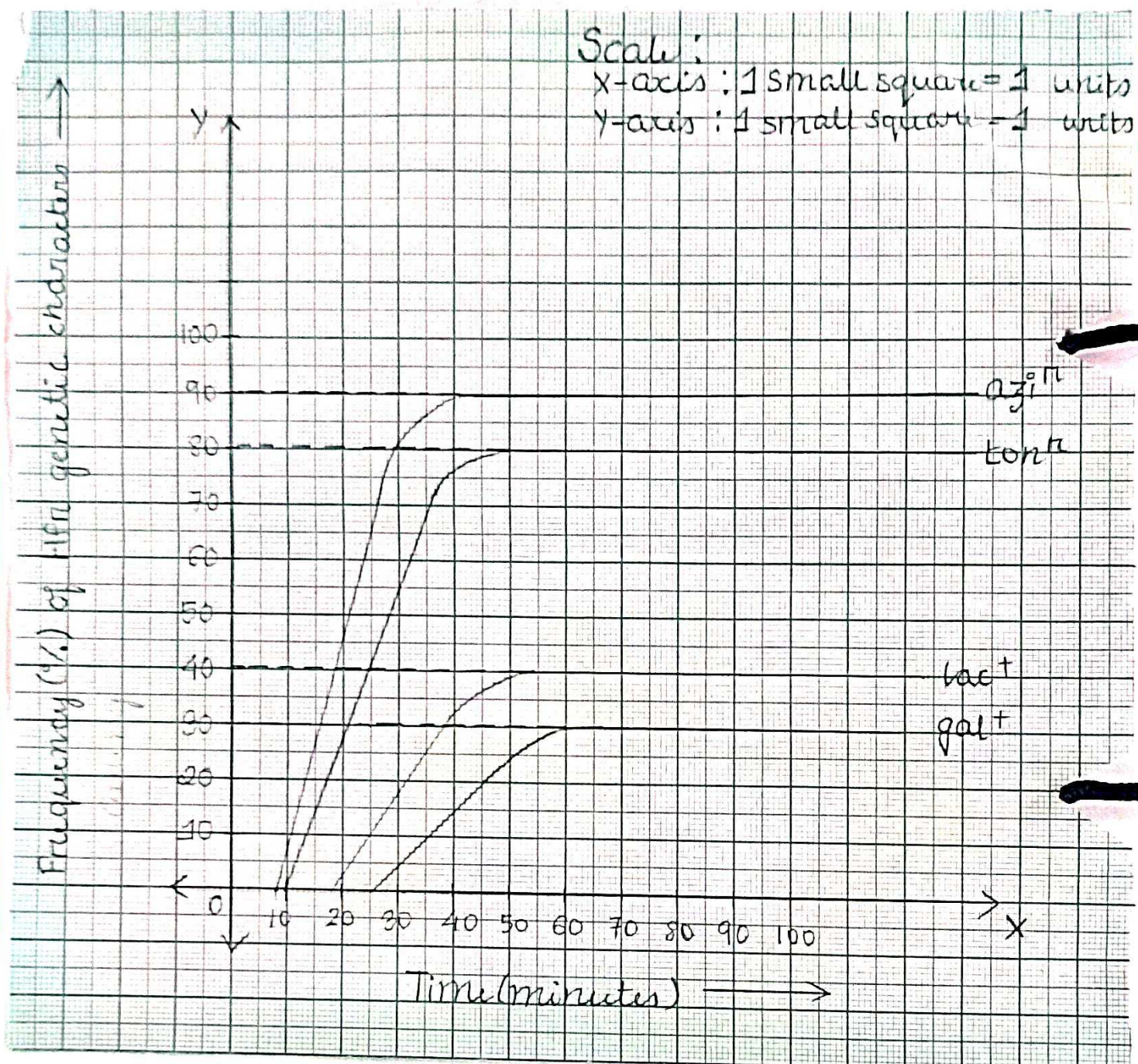
(ii) The graph indicates that the first recombinant that appears is Azithromycin resistant starting at 8 minutes. The next recombinant that appears is for ton^r at about 10 minutes. After 18 minutes and 25 minutes respectively the F^- cells become lac^+ and gal^+ .

(iii) The percentages of exconjugate colonies were 90% for Azi, 80% for ton^r , 40% lac^+ , and 20% gal^+ .

(iv) Since conjugative transfer is a linear phenomena, it is the time which determines the sequence of the genes. Further, the distance between the genes is indicated by the frequency of recombination between these two genes or with respect to F-plasmid.

(v) Accordingly, the linkage map is prepared as follows.

Scale:
 X-axis: 1 small square = 1 units
 Y-axis: 1 small square = 1 units



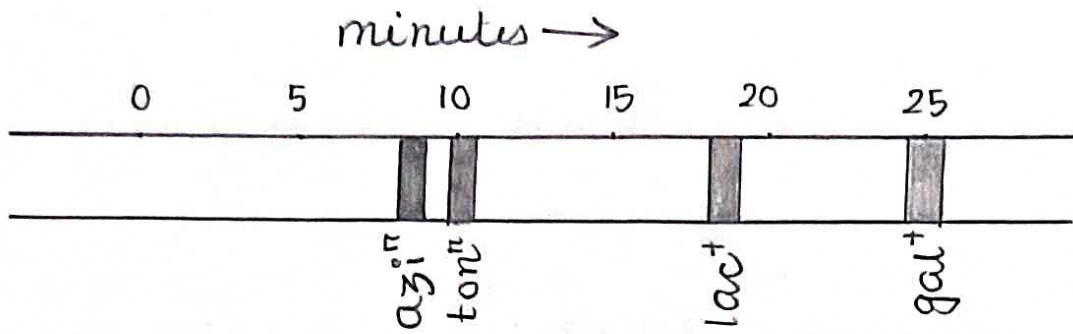
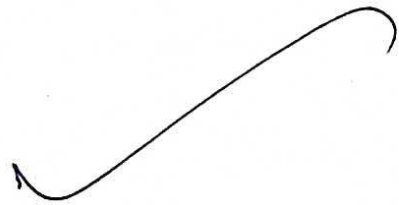


Fig. Linkage map representing azi^r , ton^r , lac^+ and gal^+ genes and their conjugative transfer with time.

Question



~~Mad 19/12/21~~