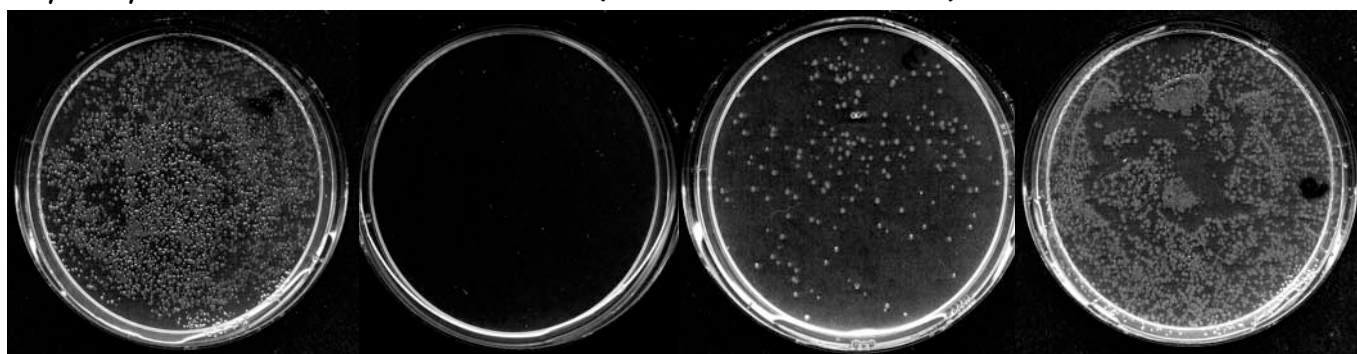


Ames Test - Section

The Ames test is commonly used as an efficient and inexpensive test for the mutagenic property of substances. Bacteria that are deficient in histidine biosynthesis (His^-) are spread on solid agar containing inorganic salts and sugars (minimal medium). Minimal medium does not contain the amino acid histidine or any other amino acid supplements. On minimal medium, the his^- bacteria will not grow unless they undergo a reversion mutation. In the Ames test, His^- bacteria are mixed with a substance and spread on minimal medium (plated). If the substance is mutagenic, some of the bacteria will undergo a reversion mutation and be able to grow in the absence of histidine. If the chemical is not mutagenic then the bacteria will not grow.

When a bacterial strain deficient in histidine biosynthesis (His^-), is plated for confluent growth (about 1×10^6) on minimal medium (containing only inorganic salts and sugar), it does not grow (See below). However, the bacteria grow when plated coated with a mystery substance on minimal medium (substances #1 and #2.)



RICH MEDIUM

MINIMAL MEDIUM

MIN + ? #1

MIN +? #2

a) Why did we plate on rich medium and minimal medium with no additions? What are these called?

Controls

b) What are two possibilities for the identities of the mystery substances?

Indicate which type of mystery substance corresponds with the growth on each plate.

The mystery substance could either be histidine itself (or something containing histidine), or a mutagen. If you add histidine itself, then all of the bacteria plated should grow (#2). If a mutagen is added, then only a subset of the bacteria, those in which a reversion mutation has occurred, will grow (#1).

c) You perform a series of experiments in which you plate proteins purified from soybeans along with the His^- bacteria on minimal medium. You find that the bacteria confluently grow on almost all of the plates. Does this mean that soybean proteins are mutagens, or could there be another explanation?

Most proteins contain histidine. When these proteins are broken down, they can be a source of histidine, thereby enabling the bacteria to grow.

d) If the mystery substance #1 is a mutagen, how would that enable the bacteria to grow in the absence of histidine?

The mutagen could cause a new mutation in the already mutant allele of the gene encoding the enzyme involved in histidine biosynthesis. This mutation could either restore wildtype sequence or make a compensating change (addition of the right number of basepairs to restore frame, or change a second basepair to restore the amino acid encoded). By "fixing" the gene, the mutation will also fix the protein product, and thus restore the phenotype to wildtype.

e) In this next experiment you have two bacterial strains that are deficient in histidine biosynthesis. One has a single base-pair substitution (Strain A) and the other has a frameshift (deletion) mutation (Strain B). You isolate the mutant histidine biosynthesis enzyme from Strain A and find that it differs from wildtype at only one amino acid. You isolate the mutant histidine biosynthesis enzyme from strain B and find that it is nearly full length, but only the first 50 amino acids are wildtype. You plate each strain either alone, with the addition of histidine, or with the addition of either drug X or drug Z. You then examine plates 1-8. The results are summarized below.

There are two mutagenic drugs used.

One drug causes the replacement of a G-C pair with an A-T pair.

The other drug causes the insertion or deletion of a single base-pair.

Examples of mutagenic drugs:

Ethylmethanesulfonate (EMS), GC→AT transitions
 Nitrosoguanidine (NG), GC→AT transitions
 Proflavin - intercalating agent → frameshift
 Acridine orange - intercalating agent → frameshift
 ICR-191 - intercalating agent → frameshift

Plate #	1	2	3	4	5	6	7	8
Strain #	A	A	A	A	B	B	B	B
Medium	Minimal	Minimal + histidine	Minimal + drug X	Minimal + drug Z	Minimal	Minimal + histidine	Minimal + drug X	Minimal + drug Z
Growth?	-	++++	+	-	-	++++	-	+

Explain why drug X enables strain A to grow on minimal medium, but does not have the same effect on strain B? Explain why drug Z enables strain B to grow on minimal medium, but does not have this effect on strain A?

Strain A has a point mutation (single base-pair substitution) and strain B has a frameshift mutation (either deletion or insertion of base-pair(s)). Therefore drug X is likely to cause single base-pair substitution mutations and restore the wildtype sequence in strain A, thus allowing it to synthesize histidine. Drug Z causes the insertion or deletion of base-pairs and therefore will restore the reading frame in strain B (but not necessarily make the whole protein wildtype), thus allowing it to synthesize histidine.

f) If you examine many control plates (lacking histidine and without the addition of a drug - minimal plates) you find the occasional colony with both strains A and B. Explain the presence of these colonies.

Spontaneous reversion mutations will occur at a very low frequency.