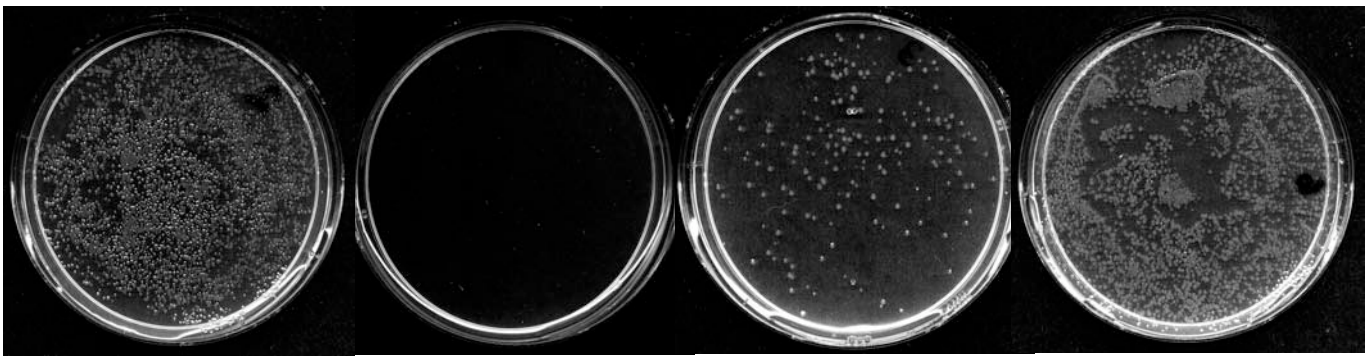
Ames Test - Section

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 X1 0 ^{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?

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.

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?

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

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?

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.