

## Hydrogen Sulfide Production & Motility

Many proteins are rich in sulfur-containing amino acids such as cysteine. When these proteins are hydrolyzed by some bacteria, the amino acids are released and taken up as nutrients. Cysteine, in the presence of the enzyme **cysteine desulfurase**, loses its sulfur atom through the addition of hydrogen from water to form hydrogen sulfide gas (Fig. 6-85 of the Atlas, p. 77).

Gaseous hydrogen sulfide may also be produced by the reduction of inorganic sulfur-containing compounds such as thiosulfate ( $S_2O_3^{2-}$ ), sulfate ( $SO_4^{2-}$ ) or sulfite ( $SO_3^{2-}$ ). For example, when certain bacteria take up sodium thiosulfate, they can reduce it to sulfite using the enzyme thiosulfate reductase with the release of hydrogen sulfide gas (Fig. 6-86 of the Atlas, p. 77). Such a reduction occurs during anaerobic respiration in which respiring cells use something other than oxygen (such as thiosulfate) as the final electron acceptor in the respiratory electron transport chain.

In this exercise, the SIM medium (named after J.S. Simmons in 1926) contains peptones and sodium thiosulfate as substrates, and ferrous ammonium sulfate,  $Fe(NH_4)SO_4$ , as the  $H_2S$  indicator. Cysteine is a component of the peptones used in SIM medium. Sufficient agar (about 0.4% instead of the usual 1.5%) is present to make the medium semisolid. Once  $H_2S$  is produced, it combines with the ferrous ammonium sulfate (ferrous sulfate will also work), forming an insoluble, black ferrous sulfide precipitate (Fig. 6-87 of the Atlas, p. 77) that can be seen along the line of the stab inoculation (Fig. 6-88 of the Atlas, p. 77). If the organism is also motile, the entire tube may turn black (Fig. 6-88 of the Atlas). This black line or tube indicates a positive  $H_2S$  reaction; absence of a black precipitate indicates a negative reaction.

SIM agar may also be used to detect the presence or absence of motility in bacteria as well as indole production. Motility is present when the growth of the culture is not restricted to the stab line of the inoculation. Growth of nonmotile bacteria is confined to the line of inoculation.

One can also use semisolid media (motility test medium deeps without 2,3,5-triphenyltetrazolium chloride) to determine whether a bacterial strain is motile. This medium also contains less than the normal 1.5% agar (e.g. 0.4% agar) to make it semisolid. During growth, motile bacterial will migrate from the line of inoculation to form a dense turbidity in the surrounding medium; nonmotile bacteria will grow only along the line of inoculation (Figure 6-63 of the Atlas, p. 68).

### Procedure:

To demonstrate the formation of  $H_2S$  and the use of motility agar, *Proteus vulgaris* and *Klebsiella oxytoca* will be used as the test organisms.

### First Period:

1. Label each of the SIM agar deep tubes and each of the Motility Agar tubes with the name of the bacterium to be inoculated, your name and date.
2. With an inoculating needle, and using aseptic technique, inoculate each tube with the appropriate bacterium by stabbing the medium  $\frac{3}{4}$  of the way to the bottom of the tube.

Be careful when inoculated the tubes to withdraw the needle from the agar in a line as closes as possible to the line used when entering the agar. Do not allow the inoculating needle to touch the bottom of the tube.

3. Incubate the cultures for 24 to 48 hours at 35 °C.

Second Period:

1. Examine the SIM cultures for the presence or absence of a black precipitate along the line of the stab inoculation. A black precipitate of FeS indicates the presence of H<sub>2</sub>S. Any blackening of the medium is considered a positive test for H<sub>2</sub>S production.
2. Based on your observations, determine and record whether or not each bacterium was capable of H<sub>2</sub>S production and the presence (+) or absence (-) of motility. Since the black precipitate may obscure motility results, also check for motility using the Motility Agar deeps that were inoculated along with the SIM tubes.

Note: an aid in visualizing motility is to slowly rotate questionable tubes containing small amounts of growth around the stab line. When this is done, if the growth appears much wider on the two opposite sides and narrower on the other two sides, the bacterium is **not** motile. If the growth is uniform all around the stab line and the growth does not have a “flattened appearance”, then the organism is likely to be motile. Make sure that the outside of your tubes are clean by wiping them with a Kimwipe.

3. If desired, one can also test for indole production by adding 5 drops of Kovacs reagent to the SIM tubes and looking for the development of a red color at the top of the deeps. The Indole Test will also be done as part of the IMViC (Indole, Methyl Red, Voges Proskauer, Citrate) series of tests to be conducted as a separate exercise.