BACTERIAL COLONIES

Models:

1. Petri dish top and bottom and a model representation of agar medium. The dish is the standard size (100 mm in diameter and 15 mm in height), and agar medium fills the dish approximately half-way. Models of three different colony types can be secured on the pegs

2. Steps in colony formation. Notice that after a cell divides, the daughter cells remain at the same location on agar medium.

3. The streaking out technique to isolate colonies arising from a single cell. The model is made up of two inserts, each representing agar medium, and can be placed in the Petri dish (**Model 1**). The label "S" indicates the location of the start of streaking, with the first phase just above.

4. A model representing swarming cells. This can be placed on the agar medium representation of **Model 1**, using the pegs for alignment. The original cell was deposited near the edge of the plate. As the cells divided, they moved out in swarms to form the wave-like pattern shown on the model.

While bacteria are frequently grown in liquid cultures, there are advantages to growing them on semi-solid medium in a Petri dish. This medium contains agar, a natural product from seaweed, in addition to nutrients. Agar powder is added to liquid medium and then heated in an autoclave, a vessel that sterilizes items by a combination of steam and high pressure. Under these conditions, the agar goes into solution. After autoclaving the medium is poured into a sterile Petri dish (**Model 1**) until it is filled about half-way. As the medium cools the long agar molecules begin to bind to each other, forming a molecular mesh that results in semi-solid medium. The medium is firm enough so that it retains its shape and does not move about in the dish. On the other hand, it can break apart easily if handled.

Most bacteria can form colonies on agar-containing medium. Colonies arise when a single cell is deposited on the medium. The cell uses the nutrients in the medium to grow and divide, but because the medium is semi -solid, cells formed during division remain in place and do not drift to different positions on the plate. **Model 2** is a magnified representation of the first steps in the formation of a colony. After many divisions, there are enough cells to result in a macroscopic aggregate called a colony.

Colonies are very useful to microbiologists. Different bacteria form colonies having characteristic shapes, textures and colors that are useful for identifying the kind of bacteria making up the colony. Part of **Model 1** is an insert representing the agar medium. This can be placed in the agar dish with the three pegs on the surface facing up. Several different colony types are available to place on the medium (the pegs will hold them in place). In this example, the morphology of each colony is very different, so each colony was formed by growth of a different bacterial species. This points out a very important property of colonies. Because each colony arose from a single cell (**Model 2**), all the cells in the colony are the same species. In other words, the colony represents a pure culture, that is, one consisting of the cells of only one kind of bacteria. Having pure cultures is very important to microbiologists. In the real world, bacterial habitats (our skin, colon, soil, lake water and so on) contain a mixture of many different bacterial species, often numbering in the hundreds or more. With such a mixture, studying the

properties of a single species would be virtually impossible. Growing bacteria from a single cell to form an isolated colony is a good way of getting a culture of one species.

The problem is: how do you isolate a single cell to start a colony? Bacteria are so small that a single cell can't be picked up directly and placed on agar medium. Fortunately, there is a simple way to obtain single cells on agar medium. **Model 3** consists of two representations of agar medium, each of which can be separately placed into the Petri dish that is part of **Model 1**. Orient each agar model so that the label "S" is on the left. Begin with the one having a star at the right of the label. We can imagine that this is sterile medium in a Petri dish.

We will use an inoculating needle to obtain single cells. This is a very thin, straight wire set in a handle so that it can be easily held. The wire end (but not the handle!) is heated to a very high temperature, often by the gas flame of a Bunsen burner. The needle is then removed from the heat source and allowed to cool. Because the wire is so thin, these steps occur in seconds. The result is a sterile wire, which is used to gently touch the source of the bacteria to be isolated. Although care is taken to avoid picking up a large sample, nevertheless the sample at the end of the wire will contain many cells. The tip of the needle is then dragged gently back and forth across part of the agar plate. This is illustrated on the agar plate model. Above the "S" label there are grooves representing the track of the needle as it is streaked back and forth. Notice what is happening here: the bacterial sample is being spread on the surface, effectively diluting it by placing the cells on a larger surface area than the needle tip. However, only one region of the agar surface is used. This step creates phase one of the isolation.

The needle is re-sterilized by heating, and then used to distribute further some of the cells deposited by the first streak. The needle is dragged back and forth from one region of phase one into a new area of the surface and at an angle from the original streak. Again, you can feel the streak marks extending from phase one to form phase two in **Model 3**. This procedure is repeated a few more times (phases three and four in **Model 3**). There are fewer and fewer cells in each phase until finally the needle is distributing single cells on the medium.

The plate containing the streaked cells is then incubated for the time required for the development of colonies from the single cells. The results are depicted with the second agar medium representation of M**odel 3**. This insert can be placed in the Petri dish (**Model 1**) and is oriented as before, with the label on the left. You can distinguish this insert from the first because there is no star next to the label. Moving upward from the label into phase one, there is a raised area along the track of the streaks. This represents confluent cell growth. There are so many colonies in the phase that no individual colonies were obtained. There is also confluent growth in phase two, again because too many cells were deposited. However, the number of cells in phase two is still less than the number in phase one, because only a small region of phase one was used to form phase two. By phase three the lines of confluent cells are broken up. This reflects regions on the streak where no cells were deposited because fewer cells were picked up by the needle. Finally, phase four contains colonies arising from individual cells, although there still might be occasional clumps of cells. By this point the sample on the end of the needle contains such a small number of cells that single cells are frequently distributed in forming the phase. Each of these individual cell forms a colony, a pure culture. These can then be picked from the plate with a sterile needle for subsequent study.

QUESTIONS TO CHECK YOUR UNDERSTANDING

1. Colony formation depends cells remaining at the same place after division. However, some species of bacteria have cells that can move on the agar medium. An example is *Proteus mirabilis*, which swarms outwards in waves from the inoculating site (**Model 4**). What problem does this present in using the streaking technique?

2. When a sample of the bacteria from a natural source is streaked on agar medium, why can the bacterial diversity in the sample be estimated by examining the resulting colonies?