Colony Morphology Protocol

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Information

History

Since bacteria were first cultured on solid media, describing the appearance of bacterial colonies has been an important tool for microbiologists. Observing colony morphology is a tool used by clinical microbiologists, in particular, and descriptions of colonies are often found in the primary literature. Distinguishing colony morphology is one of the first skills taught to microbiology students.

In an early text, *Principles of Microbiology*, Moore defined the practice as "The examination of plate cultures…determining the character of the different colonies, their action upon the medium, the rapidity of their development, and in case of quantitative analysis, the number and variety of colonies" (2). This practice remains consistent. Many different terms have been used to classify colonies themselves, however, and systems differ from simple to complex. Another early text suggested that colonies be described as conglomerate, rhizoid, curled, or myceloid (4). Later, additional morphological terms such as granular, arborescent, wavy interlaced, and filamentous" (1) and elevation, edge, color, and texture were used. In *Methods for General and Molecular Bacteriology* the terms were categorized by descriptions of color, form, elevation, margin, opacity, and texture (5).

Regardless of terminology, the practice of making observations of colony morphology remains a common exercise in introductory microbiology laboratory courses. Current laboratory manuals seem to be fairly universal in their use of the system proposed in *Methods for General and Molecular Bacteriology* (5). While different morphological characteristics are never comprehensively described or exemplified using photographic images of bacterial colonies, simple drawings are used to demonstrate just a few of the morphological characteristics instead (Fig. 1).

Purpose

Determining the morphology of a single colony growing on the surface of a plate culture can be an important tool in the description and identification of microorganisms.

Theory

On solid media, a colony is theoretically derived from a single cell. If well separated from other colonies, a colony will have a characteristic shape (both in elevation and margin), size, color, and consistency. Observation is often made with the naked eye, but dissecting microscopes are also used. The characteristics defined by a colony's morphology may be used at a superficial level to distinguish between types of microorganisms. For example, there are differences in morphologies when rough and smooth colonies of *Streptococcus pneumonieae* are examined. Another comparison can be made when describing pigmented colonies.

RECIPES AND PROTOCOL

Cultures of microorganisms can be grown on any medium that is appropriate for their isolation and cultivation. Since morphology is influenced by medium type and growth conditions, care should be taken to record these parameters. Good determination of colony morphology is predicated on good streak technique because it requires good separation of colonies.
Smibert and Krieg (5) proposed the following protocol:

1. Measure the colony diameter in millimeters.

2. Describe the pigmentation (distinguishing between pigmented colonies and those secreting diffusible pigments).

3. Describe the form, elevation, and margin as indicated in Fig. 1. Also indicate whether the colonies are smooth (shiny glistening surface), rough (dull, bumpy, granular, or matte surface), or mucoid (slimy or gummy appearance).

4. Record the opacity of the colonies (transparent, translucent, or opaque) and their texture when tested with a needle: butyrous (buttery texture), viscous (gummy), or dry (brittle or powdery).

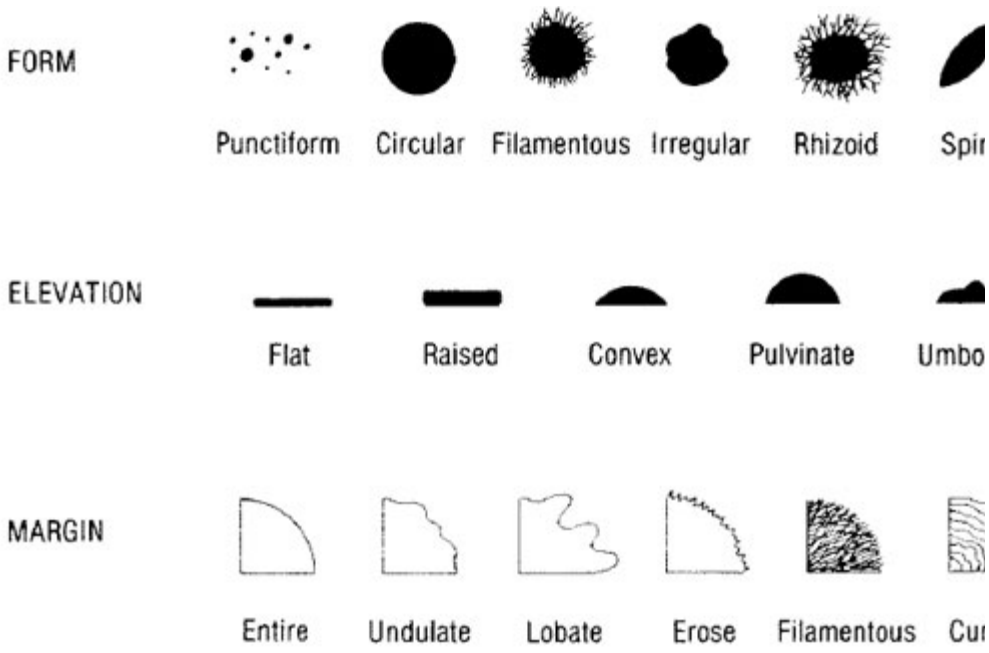

FIG. 1. Diagram illustrating the various forms, elevations, and margins of bacterial colonies (3).

Following the Protocol

A well-written description would include, therefore, each of the parts of their protocol. A description of *Sinorhizobium meliloti* is used as an example (Fig. 2).

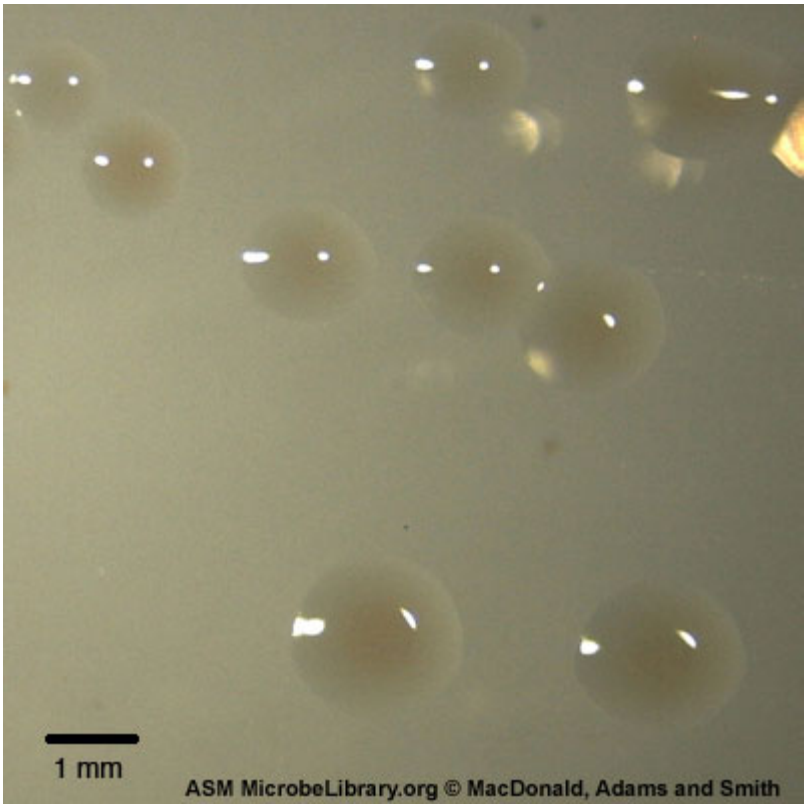

FIG. 2. Colonies of *Sinorhizobium meliloti* grown on trypticase soy agar are approximately 1 to 2 mm in diameter. They lack pigmentation and are translucent. Colonies are smooth and are circular in form with an entire margin. They have a convex elevation. When manipulated with a needle, the colonies are viscous.

SAFETY

Students working with live cultures of microbes must be able to explain and practice safe laboratory techniques. Good laboratory practice for microbiology students includes appropriate aseptic technique for protecting themselves and others. Physical barriers and cleanliness are simple measures against contamination of students and laboratory equipment. Working with gloves is a good idea when streaking with pathogens or suspected pathogens. Use of biological safety cabinets, splatter shields, and protective eye equipment is also recommended.

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

TIPS AND COMMENTS

Current availability of inexpensive digital cameras can enhance the teaching environment. In addition to having students make

representative drawings of what they observe, they can provide a digital image for the instructor to compare.

For many of the images originally submitted, a Leica EZ4 D 3.0 MPixel stereomicroscope with camera connected to a personal computer with Las EZ software was used for image collection. Images were cropped using Adobe Photoshop with the only manipulation being image and file sizes. Images were taken with culture dishes flat in the field of view or placed at varying angles up to about 80°. Beyond 80°, the edge of the dish obstructed the view.

It is difficult when using a dissecting scope to determine the exact magnification of colonies, hence this parameter is missing from legends in this Atlas collection. However, most colonies were between 1 and 3 mm in diameter. The purpose of this Atlas collection is to provide examples and descriptions of commonly seen colony morphologies, any of which might be similar to those found in the literature.

Colonies should be well isolated. This is particularly true when several colonies have coalesced and may appear to have an irregular margin. Please examine Figure 9 in the Atlas as an example of this.

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