presupposed a method for staining the minute bacteria in lesions so that they could be adequately visualized. Because of the fact that many bacteria exhibit the peculiar staining reaction which Gram describes here, it was possible to detect them much easier with his method.

For many years the main use of the Gram stain has been to differentiate species of bacteria. In the present paper, Gram describes several organisms that were not stained by his technique. We would call these Gram-negative, and the number of Gram-negative bacteria is probably larger than the number of Gram-positive bacteria. The Gram stain is one of the first procedures learned by beginning bacteriology students and is one of the first procedures carried out in any laboratory where bacteria are being identified. Its importance to bacterial taxonomy is therefore obvious.

The mechanism of the Gram stain is still a partial mystery. As Gram himself noted, the iodine-potassium iodide solution is essential in the reaction. We know that this solution must follow, and not precede, the gentian violet. We know that the iodine and the gentian violet form a complex inside the cell (Gram also noted this complex formation) which is insoluble in water but is soluble in alcohol. Apparently Gram-positive bacteria are those which are able in some way to keep the alcohol from reaching this insoluble complex. We know that the Gram stain is not an all-or-nothing phenomenon, but that quantitative variations in Gram-positivity exist between different species, and within the same species during different parts of the growth cycle or under different environmental conditions. We know that only intact cells are Gram-positive, so that cells which are even gently broken become Gram-negative. We know that bacterial protoplasts, devoid of cell wall, are still Gram-positive, indicating that it is probably the semipermeable membrane which is somehow involved in the reaction. Finally, we know that Gram-positivity is restricted almost exclusively to the bacteria, with only a few other groups, such as the yeasts, exhibiting this reaction. We can truly say that the implications of Gram’s discovery have been widespread.

A minor modification of the plating technique of Koch

1887 • R. J. Petri


In order to perform the gelatin plate technique of Koch, it is necessary to have a special horizontal pouring apparatus. The poured plates are then placed over one another in layers on small glass shelves in a large bell jar. In many cases it would be desirable to carry out the procedure with less
A minor modification of the plating technique of Koch

equipment, especially without the pouring apparatus. Since the first of the year I have been using flat double dishes of 10–11 cm. in diameter and 1–1.5 cm. high. The upper dish serves as a lid as usual and has a somewhat larger diameter. These dishes are sterilized by dry heat as usual and after cooling the nutrient gelatin containing the inoculum is poured in. The upper lid is lifted only slightly and used as a shield while the tube containing the gelatin, its edge previously flamed and cooled in the usual manner, is emptied into the bottom of the dish. Under these conditions contamination from airborne germs rarely occurs. The poured layer of gelatin soon hardens into a layer several millimeters thick which can be kept and observed for a long time because of the protecting upper lid. In studies of soil samples, sand, earth, and similar substances, it is advantageous to place the material in the dish and then pour the liquid gelatin over it. The material is well mixed with the gelatin by rotating the dish with short, intermittent movements. With the dimensions given, every spot on the gelatin surface is accessible with the low power microscope. Only when high power lenses are used is the area at the edge of the dish no longer accessible. The gelatin dries in these dishes quite slowly. They can be kept moist longer if 5–6 dishes are placed on top of one another on a disc of moist filter paper in a flat dish over which a bell jar is inverted. These dishes can be especially recommended for agar-agar plates, since agar-agar sticks poorly to simple glass plates unless special means are used. In addition, it is quite simple to count the colonies that have grown on the plates. The upper lid is replaced by a glass plate that has etched on it squares of known area. The colonies are then counted against a black background using a magnifier. The total area of the plate can be calculated from the diameter.

Comment

We have here the first description of the Petri dish, a simple yet effective device for culturing microorganisms on solid media. The original idea of Petri has not been improved upon to this day, and in bacteriology laboratories all over the world dishes are used of almost the identical features as those first described by Petri. The Petri dish is such a simple idea that if Petri had not thought of it, someone else probably would have conceived of it later. But because of its great usefulness and universality, its first description warrants inclusion in the present collection.

We also have an interesting sidelight in this article on the procedures used for viable counting. The methods did not differ much from those we use today, except that in the time of Koch and Petri, many more viable cells were put in a plate, resulting in a larger number of colonies and overcrowding. Because of statistical considerations, we do not use such large numbers of cells, but dilute our samples until there are between 30 and 300 viable colonies developing. In this way competition for nutrients is eliminated, and the apparent colony count is higher, being closer to the true number of viable cells.