

Cultures

Cultivation

Special spreading techniques should be used for the cultivation of individual colonies (= pure culture). The best spreading technique for a particular application can only be developed from extensive laboratory experience with the various types of cultures. Spreading should be performed either with an inoculation spatula, an inoculation needle or a loop. These are commercially available, also in calibrated form (e.g. 0.001 ml, 0.01 ml) as used for the semi-quantitative counting of bacteria.

A spatula should be used for the uniform inoculation of an agar surface as e.g. in the case of the determination of the antibiotic sensitivity of a particular organism. Using a glass spatula or sterile swab, the entire surface of the agar should be covered three times, the petri dish being rotated by 90° each time in order to achieve uniform distribution of the inoculum.

The classical form of inoculating solid media is the three loop smear, also known as a fractionated smear (Fig. 1a). In this process, the inoculum should be deposited at the edge of the dish. A sterile loop should then be used to remove a little material and to spread this over the surface. Once the inoculation loop has been flame-sterilised, it should be placed on the last inoculation spot, the dish rotated by 90° and again spread. This procedure should be repeated three times. In this way, it is possible to prepare single colonies which can then be used for further differentiation.

Another dilution technique is one in which, starting from the depot and using a sterile loop, a line is drawn over the surface. Using a freshly sterilised loop, a zig-zag line is then drawn transverse to the inoculation line. Depending on the organism count within the material to be investigated, the colonies will appear with various densities in the depot, on the inoculation line and on the inoculation lines transverse to them.

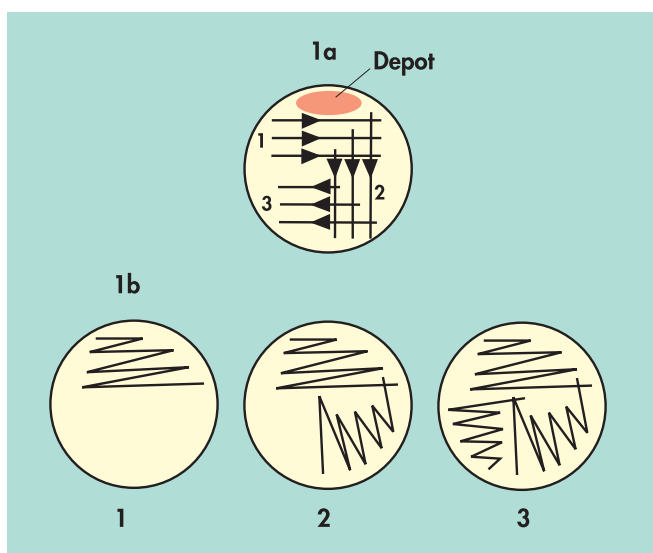


Fig. 1: Dilution smear; 2 methods

Growth and reproduction

Apart from the availability of nutrient, the growth of microorganisms is determined essentially by the pH, the redox potential, the osmotic pressure and the salt concentration of the medium. In addition other factors such as temperature and oxygen and CO₂ ratios are of considerable importance.

Human pathogenic organisms prefer temperatures around 37 °C, those of the saprophytic types on the other hand 20 to 25 °C. In general, one can also say that bacteria require higher temperatures and fungi lower temperatures for optimal growth. For this reason, within the framework of sterility testing, tests should be carried out for bacteria contamination at 30 to 35 °C, and at 20 to 25 °C in the case of contamination with yeasts and fungi. Apart from the anaerobic types, i.e. microorganisms which cannot tolerate oxygen and aerobic organisms, a degree of aero-tolerance exists in the case of many microorganisms. Most microorganisms, however, grow better under reduced oxygen conditions in a CO₂ atmosphere.

The pH optimum is frequently found in a slightly alkaline milieu around 7.2 to 7.6. Some organisms however, e.g. lactobacilli, grow even at pH values < 4.0. Bacteria reproduce only in a moist milieu; only few organisms can cope with drying out. In the case of longer incubation periods, constant humidity must therefore be guaranteed. This can be achieved by using swabs saturated with water, a dish filled with water or by automatic humidity regulation. Of course, care should be taken to avoid contamination; it is thus recommended that any water used should be renewed on a daily basis.

Reproduction takes place via separation or division. If the daughter cells remain attached to each other as e.g. in the case of streptococci, the formation of chains can be observed.

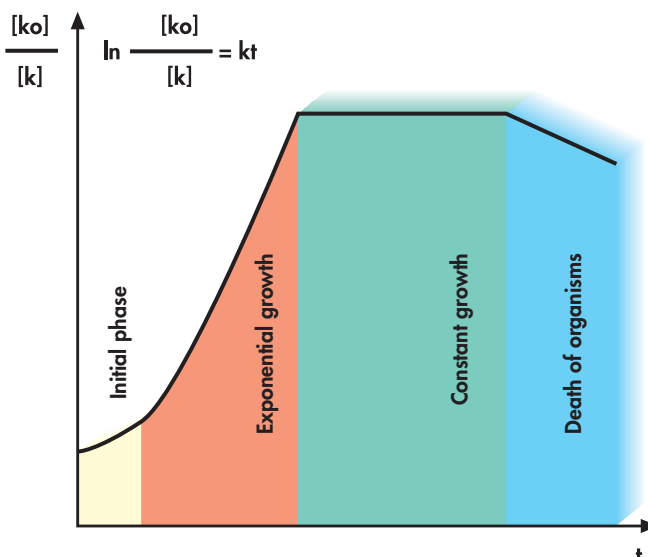


Fig. 2: Growth of a culture as a function of time

Growth in a culture begins with an initial phase (Fig. 2) during which the organisms adapt to their milieu. Subsequently, growth occurs exponentially, principally as first order kinetics. This then proceeds to a stationary phase. Here, the number of cells remains constant for a certain length of time as the metabolic substances produced and the reduced quantity of available nutrient decreases. Finally, lack of nutrient and the predominance of metabolic substances leads partially to death and hence to a decrease in the number of vital cells. This means that, for a particular cell culture, only a limited incubation period is meaningful.

Inoculation

Bacteriologically clean work is a precondition for obtaining pure colony material. In the case of Abimpfen, the bacteria colony under investigation is only just touched. In this way, microorganisms that may have accumulated on the surface are not carried onwards. For such microorganisms, growth conditions – selective agar, period of incubation, temperature, pH, etc. – are unfavourable.

Identification of bacteria

Many of the relationships between bacteria are still unclear. Thus, practical criteria are used in their classification. Bacteria can be classified by microscopic observation and gram staining into gram-positive and gram-negative cocci, rods and spirals (Table 1). The staining technique using carbolic gentian violet was developed by the Dane H.C.J. Gram. Further differentiation is possible using oxygen requirement in gram-positive aerobic, gram positive anaerobic cocci etc.

Gram-positive and gram-negative bacteria differ in the construction of their cell walls. However, in principle, the structure of the cell wall in the case of both bacteria types is similar. In both cases, a network structure comprising polysaccharide chains is present linked by short peptide chains. These peptides glycanes form the support skeleton which has the form of a sack (murein sacculus). The glycanes portion of the macromolecule consists of the two sugars N-acetylglucosamine and N-acetylmuraminic acid. In individual species, the type of peptide bridge, the number of muraminic acid units with their peptide chains and the composition of chains themselves vary.

Fig. 3: Classification of bacteria in the laboratory according to gram preparation and location

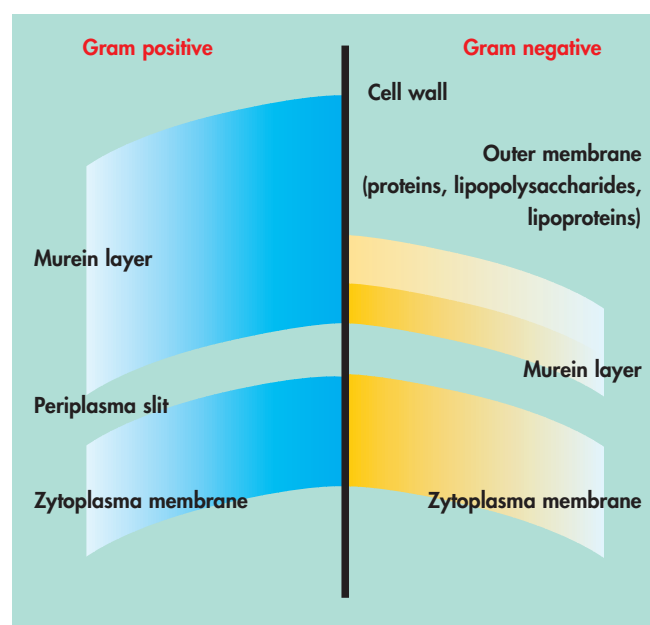
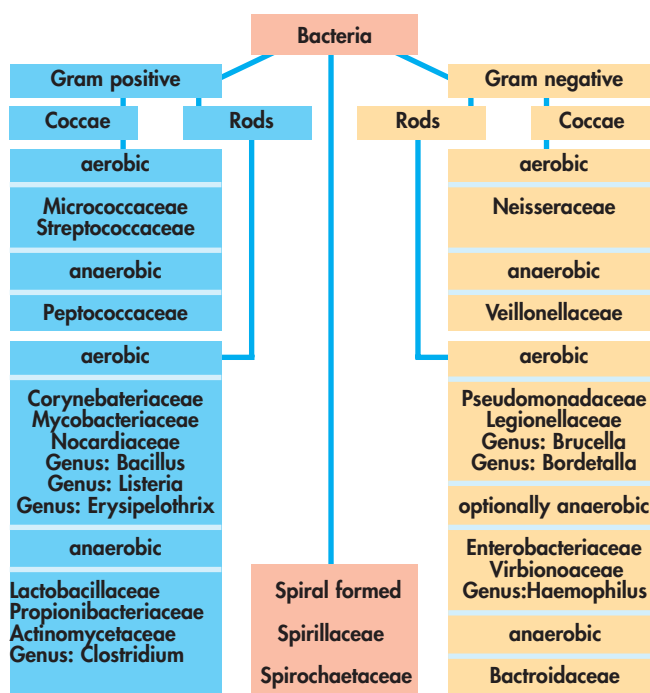


Fig. 4: Simplified schematic of bacteria cell wall.