**Protoplasts and spheroplasts**

Protoplasts and spheroplasts are altered forms of bacteria or yeast, in which the principal shape-maintaining structure of the bacteria is weakened. Each bacterium forms a sphere, which is the shape that allows the bacterium to withstand the rigours, particularly osmotic, of the fluid in which it resides.

The term protoplast refers to the spherical shape assumed by Gram-positive bacteria. Spheroplast refers to the spherical shape assumed by Gram-negative bacteria. The difference is essentially the presence of a single membrane, in the case of the protoplast, and the two membranes (inner and outer) of the Gram-negative spheroplasts. It is also possible to generate a gram-negative protoplast by the removal of the outer membrane.

Thus, in essence, protoplast refers to a bacterial sphere that is bounded by a single membrane and spheroplast refers to a sphere that is bounded by two membranes.

The peptidoglycan is the main stress-bearing layer of the bacterial cell wall and the peptidoglycan also gives the bacterium its shape. In the laboratory, weakening the peptidoglycan network in the cell wall generates both protoplasts and spheroplasts.



By exposing bacteria to an enzyme called lysozyme, the interconnecting strands of the two particular sugars that form the peptidoglycan can be cut. When this is done, the peptidoglycan loses the ability to serve as a mechanical means of support.

The situation in yeast is slightly different, as other components of the yeast cell wall are degraded to form the protoplast.

The process of creating protoplasts and spheroplasts must be done in a solution in which the ionic composition and concentration of the fluid outside of the bacteria is the same as that inside the bacteria. Once the structural support of the peptidoglycan is lost, the bacteria are unable to control their response to differences in the ionic composition between the bacterial interior and exterior. If the inner concentration is greater than the outer ionic concentration, water will flow into the bacterium in an attempt to achieve an ionic balance. The increased volume can be so severe that the bacteria will burst. Conversely, if the inner ionic concentration is less than the exterior, water will exit the bacterium, in an attempt to dilute the surroundings. The bacteria can shrivel to the point of death.

Bacteria are induced to form protoplasts or spheroplasts typically by laboratory manipulation. However, the formation of the structures can occur naturally. Such bacteria are referred to as L-forms. Examples of bacterial genera that can produce L-forms include *Bacillus*, *Clostridium*, *Haemophilus*, *Pseudomonas*, *Staphylococcus*, and *Vibrio*.

**Living protoplasts and spheroplasts are valuable research tools**

The membrane balls that are the protoplasts or spheroplasts can be induced to fuse more easily with similar structures as well as with eukaryotic cells. This facilitates the transfer of genetic material between the two cells.

As well, the sequential manufacture of spheroplasts and protoplasts in Gram-negative bacteria allows for the selective release of the contents of the periplasm. This approach has been popular in the identification of the components of the periplasm, and the localization of proteins to one or the other of the Gram-negative membranes. For example, if a certain protein is present in a spheroplast population—but is absent from a protoplast population—then the protein is located within the outer membrane.

Two types of L-forms are distinguished:

1. ***Unstable L-forms***, ***spheroplasts*** that are capable of dividing, but can revert to the original morphology,
2. ***Stable L-forms***, L-forms that are unable to revert to the original bacteria.

Some parasitic species of bacteria, such as ***mycoplasma***, also lack a cell wall, but these are not considered L-forms since they are not derived from bacteria that normally have cell walls.

L-forms may be created when cell wall-active antibiotics are used against *Listeria*, which can cause severe cases of food infection. The drugs cause the bacteria to shed their cell wall -- the attack point for the medication. The resulting vesicles are enclosed only by a cytoplasmic membrane, which renders those antibiotics ineffective.

L-forms occur not only in *Listeria* but have been described for many others bacteria. Moreover, *Mycoplasma,* *Rickettsiae* and *Chlamydiae*, all of which are human pathogens, also lack a stable cell wall. Yet, L-forms are viable only under certain conditions; if the osmotic conditions are not suitable or change rapidly, the cells become unstable and may burst.