Microbial Protoplasts, Spheroplasts and L-Forms

By-

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Introduction

- The cell wall usually is required to protect bacteria against destruction by osmotic pressure. Solutes are much more concentrated in bacterial cytoplasm than in most microbial habitats, which are hypotonic.
- During **osmosis, water moves across selectively** permeable membranes such as the plasma membrane from dilute solutions (higher water concentration) to more concentrated solutions (lower water concentration).
- Thus water normally enters bacterial cells and the osmotic pressure may reach 20 atmospheres or 300 pounds/square inch.
- The plasma membrane cannot withstand such pressures and the cell will swell and be physically disrupted and destroyed, a process called **lysis, without** the wall that resists cell swelling and protects it.
- Solutes are more concentrated in hypertonic habitats than in the cell.
- Thus water flows outward, and the cytoplasm shrivels up and pulls away from the cell wall. This phenomenon is known as **plasmolysis** and is useful in food preservation because many microorganisms cannot grow in dried foods and jellies as they cannot avoid plasmolysis.

Protoplasts

- The importance of the cell wall in protecting bacteria against osmotic lysis is demonstrated by treatment with lysozyme or penicillin.
- The enzyme **lysozyme attacks peptidoglycan by hydrolyzing** the bond that connects N-acetylmuramic acid with carbon four of N-acetylglucosamine.
- **Penicillin inhibits peptidoglycan** synthesis.
- If bacteria are incubated with penicillin in an isotonic solution, gram-positive bacteria are converted to **protoplasts** that continue to grow normally when isotonicity is maintained even though they completely lack a wall.

Spheroplasts

- Gram-negative cells retain their outer membrane after penicillin treatment and are classified as **spheroplasts because some of** their cell wall remains.
- Protoplasts and spheroplasts are osmotically sensitive.
- If they are transferred to a dilute solution, they will lyse due to uncontrolled water influx

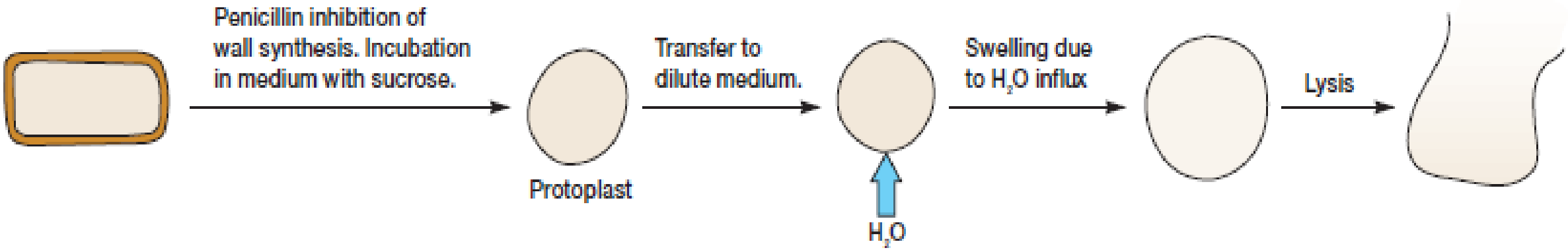
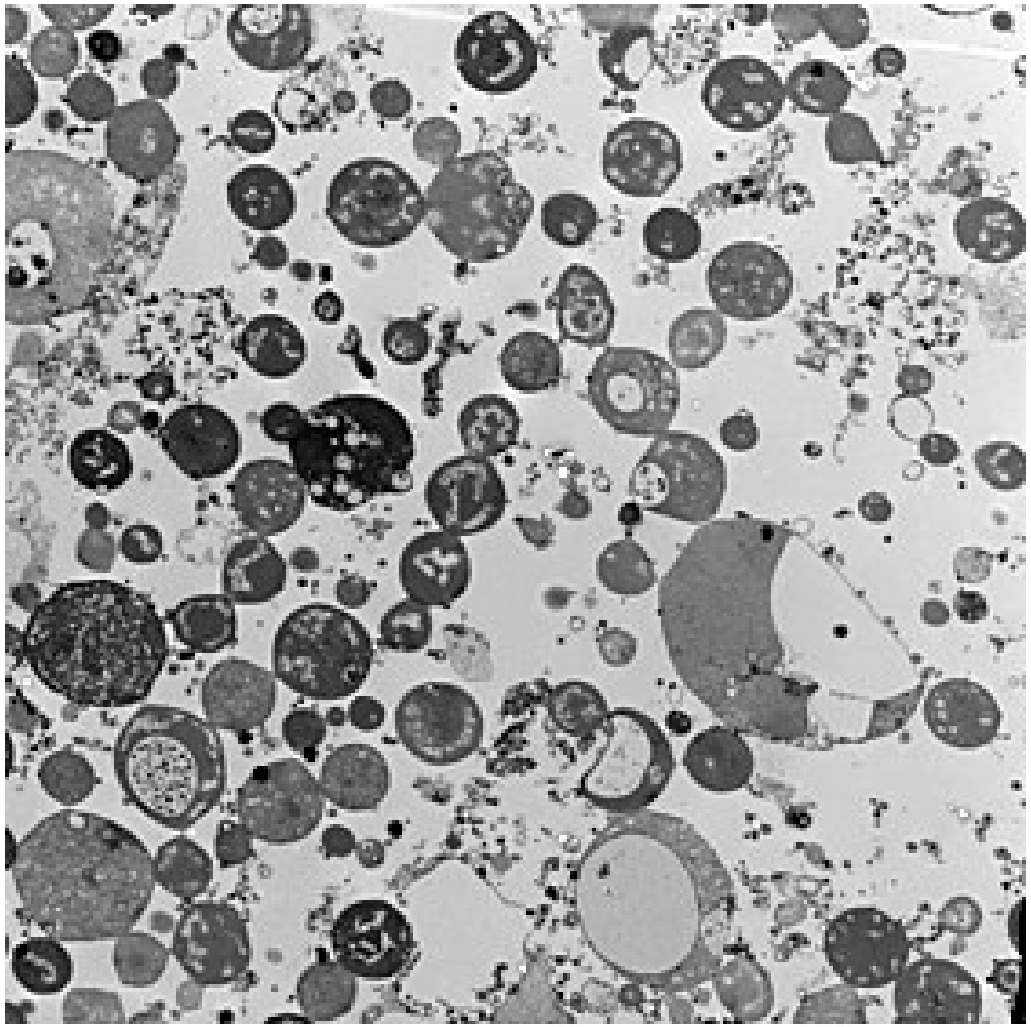
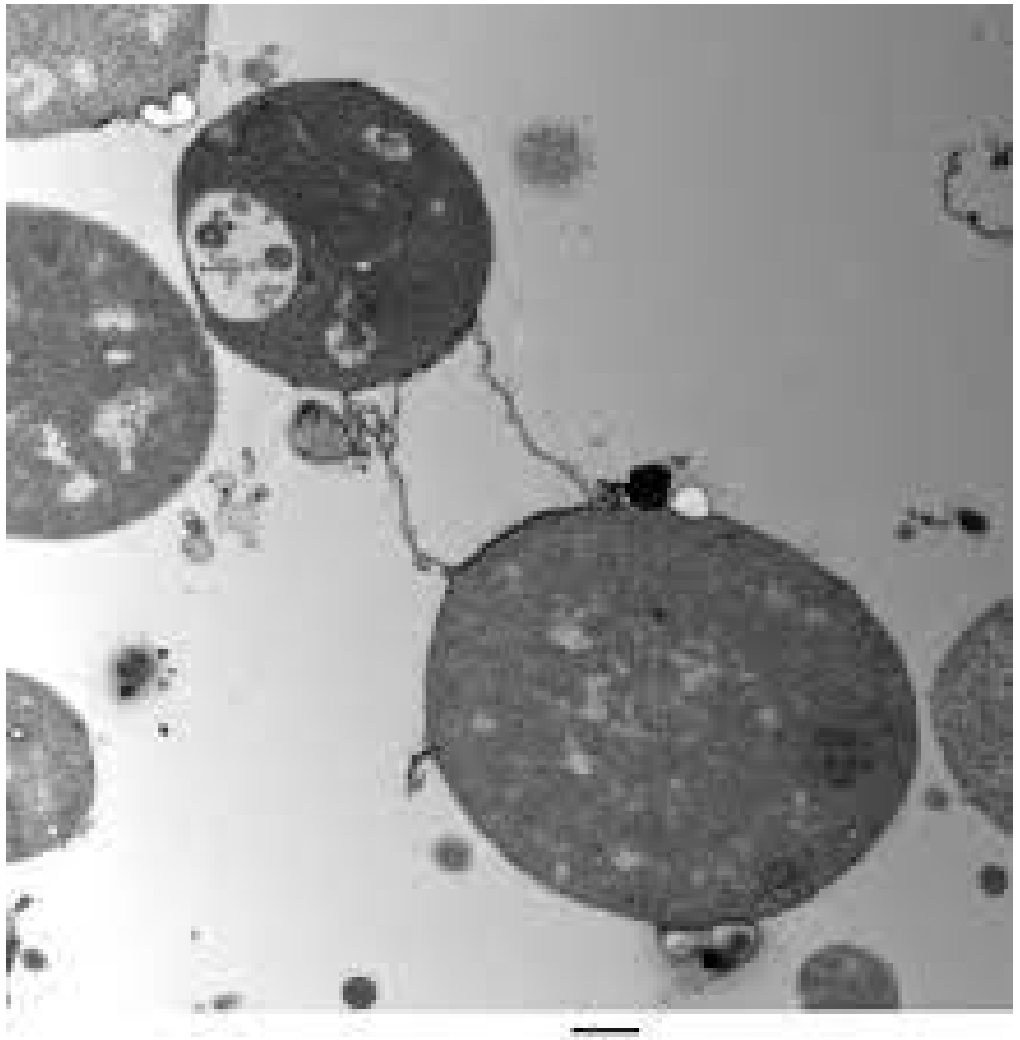


Figure 3.26 Protoplast Formation. Protoplast formation induced by incubation with penicillin in an isotonic medium. Transfer to dilute medium will result in lysis.

L-form bacteria

- **L-form bacteria**, also known as **L-phase bacteria**, **L-phase variants** or **cell wall-deficient** (**CWD**) **bacteria**, are growth forms derived from different bacteria.
- They lack cell walls. Peptidoglycan (murein) is absent.
- Two types of L-forms are distinguished:
  - *unstable L-forms*, spheroplasts that are capable of dividing, but can revert to the original morphology, and
  - *stable L-forms*, L-forms that are unable to revert to the original bacteria.
- L-form bacteria were first isolated in 1935 by Emmy Klieneberger-Nobel, who named them "**L-forms**" after the Lister Institute in London where she was working.


10 microns


500 nm

Appearance and cell division

- Bacterial morphology is determined by the cell wall.
- Since the L-form has no cell wall, its morphology is different from that of the strain of bacteria from which it is derived.
- Typical L-form cells are spheres or spheroids. For example, L-forms of the rod-shaped bacterium *Bacillus subtilis* appear round when viewed by phase contrast microscopy or by transmission electron microscopy.
- Although L-forms can develop from Gram-positive as well as from Gram-negative bacteria, in a Gram stain test, the L-forms always colour Gram-negative, due to the lack of a cell wall.
- The cell wall is important for cell division, which, in most bacteria, occurs by binary fission.
- The mode of division seems to involve the extension of thin protrusions from the cell's surface and these protrusions then pinching off to form new cells.
- The lack of cell wall in L-forms means that division is disorganised, giving rise to a variety of cell sizes, from very tiny to very big.

Generation in cultures

- L-forms can be generated in the laboratory from many bacterial species that usually have cell walls.
- This is done by inhibiting peptidoglycan synthesis with antibiotics or treating the cells with lysozyme, an enzyme that digests cell walls.
- The L-forms are generated in a culture medium that is the same osmolarity as the bacterial cytosol (an isotonic solution), which prevents cell lysis by osmotic shock.

Significance of L forms: L forms may produce chronic infections in the host. They may persist in protective regions of the body. Since L forms are relatively resistant to antibiotics, they are difficult to treat. Their reversion to normal form can result in relapse of infection.