

ESCHERICHIA COLI

SECTION I

Escherichia coli is a rod-shaped bacterium with a circular chromosome about 3 million base pairs (bp) long. It can grow rapidly on *minimal medium* that contains a carbon compound such as glucose (which serves both as a carbon source and an energy source) and salts which supply nitrogen, phosphorus, and trace metals. *E. coli* grows more rapidly, however, on a *rich medium* that provides the cells with amino acids, nucleotide precursors, vitamins, and other metabolites that the cell would otherwise have to synthesize. The purpose of this first section is to provide basic information necessary to grow *E. coli*. A more detailed introduction to certain aspects of *E. coli* biology may be found in UNIT 1.4.

When *E. coli* is grown in liquid culture, a small number of cells are first *inoculated* into a container of sterile medium. After a period of time, called the *lag period*, the bacteria begin to divide. In rich medium a culture of a typical strain will double in number every 20 or 30 min. This phase of *exponential growth* of the cells in the culture is called *log phase* (sometimes subdivided into *early-log*, *middle-log*, and *late-log phases*). Eventually the cell density increases to a point at which nutrients or oxygen become depleted from the medium, or at which waste products (such as acids) from the cells have built up to a concentration that inhibits rapid growth. At this point, which, under normal laboratory conditions, occurs when the culture reaches a density of $1-2 \times 10^9$ cells/ml, the cells stop dividing rapidly. This phase is called *saturation* and a culture that has just reached this density is said to be freshly saturated.

With very few exceptions, bacterial strains used in recombinant DNA work are derivatives of *E. coli* strain K-12. Most advances in molecular biology until the end of the 1960s came from studies of this organism and of bacteriophages and plasmids that use it as a host. Much of the cloning technology in current use exploits facts learned during this period.

Media Preparation and Bacteriological Tools

UNIT 1.1

Recipes are provided below for minimal liquid media, rich liquid media, solid media, top agar, and stab agar. Tryptone, yeast extract, agar (Bacto-agar), nutrient broth, and Casamino Acids are from Difco. NZ Amine A is from Hunko Sheffield (Kraft).

MINIMAL MEDIA

Ingredients for these media should be added to water in a 2-liter flask and heated with stirring until dissolved. The medium should then be poured into separate bottles with loosened caps and autoclaved at 15 lb/in² for 15 min. Do not add nutritional supplements or antibiotics to any medium until it has cooled to <50°C. After the bottles cool to below 40°C, the caps can be tightened and the concentrated medium stored indefinitely at room temperature. All recipes are on a per liter basis.

M9 medium, 5×

- 30 g Na₂HPO₄
- 15 g KH₂PO₄
- 5 g NH₄Cl
- 2.5 g NaCl
- 15 mg CaCl₂ (optional)

Escherichia coli,
Plasmids, and
Bacteriophages

M63 medium, 5×

10 g (NH₄)₂SO₄
68 g KH₂PO₄
2.5 mg FeSO₄·7H₂O
Adjust to pH 7 with KOH

A medium, 5×

5 g (NH₄)₂SO₄
22.5 g KH₂PO₄
52.5 g K₂HPO₄
2.5 g sodium citrate·2H₂O

Before they are used, concentrated media should be diluted to 1× with sterile water and the following sterile solutions, per liter:

1 ml 1 M MgSO₄·7H₂O
10 ml 20% carbon source (sugar or glycerol)
and, if required:
0.1 ml 0.5% vitamin B1 (thiamine)
5 ml 20% Casamino Acids *or*
L amino acids to 40 μg/ml *or*
DL amino acids to 80 μg/ml
Antibiotic (see Table 1.4.1)

RICH MEDIA

Unless otherwise specified, rich media should be autoclaved for 25 min. Antibiotics and nutritional supplements should be added only after the solution has cooled to 50°C or below. A flask containing liquid at 50°C feels hot but can be held continuously in one's bare hands. All recipes are on a per liter basis.

H medium

10 g tryptone
8 g NaCl

Lambda broth

10 g tryptone
2.5 g NaCl

LB medium

10 g tryptone
5 g yeast extract
5 g NaCl
1 ml 1 N NaOH

The original recipe for LB medium (sometimes referred to as Luria or Lenox broth), does not contain NaOH. There are many different recipes for LB that differ only in the amount of NaOH added. We use this formula in our own work. Even though the pH is adjusted to near 7 with NaOH, the medium is not very highly buffered, and the pH of a culture growing in it drops as it nears saturation.

NZC broth

10 g NZ Amine A
5 g NaCl
2 g MgCl₂·6H₂O
Autoclave 30 min
5 ml 20% Casamino Acids

Superbroth

32 g tryptone
20 g yeast extract
5 g NaCl
5 ml 1 N NaOH

TB (terrific broth)

12 g Bacto tryptone
24 g Bacto yeast extract
4 ml glycerol

Add H₂O to 900 ml and autoclave, then add to above sterile solution 100 ml of a sterile solution of 0.17 M KH₂PO₄ and 0.72 M K₂HPO₄.

Tryptone broth

10 g tryptone
5 g NaCl

2× TY medium

16 g tryptone
10 g yeast extract
5 g NaCl

TYGPN medium

20 g tryptone
10 g yeast extract
10 ml 80% glycerol
5 g Na₂HPO₄
10 g KNO₃

SOLID MEDIA

Liquid media can be solidified with agar. For minimal plates, dissolve the agar in water and autoclave separately from the minimal medium; autoclaving the two together will give rise to an insoluble precipitate. For rich plates, autoclave the agar together with the other ingredients of the medium. Cool the agar to about 50°C and add other ingredients if necessary. At this temperature, the medium will stay liquid indefinitely, but it will rapidly solidify if its temperature falls much below 45°C. Finally, pour the medium into sterile disposable petri dishes (*plates*) and allow to solidify.

Freshly poured plates are wet and unable to absorb liquid spread onto them. Moreover, plates that are even slightly wet tend to exude moisture underneath bacteria streaked on them, which can cause the freshly streaked bacteria to float away. So for most applications, dry the plates by leaving them out at room temperature for 2 or 3 days, or by leaving them with the lids off for 30 min in a 37°C incubator or in a laminar flow hood. Store dry plates at 4°C, wrapped in the original bags used to package the empty plates. Plates should be inverted when incubated or stored.

Minimal Plates

Autoclave 15 g agar in 800 ml water for 15 min. Add sterile concentrated minimal medium and carbon source. After medium has cooled to about 50°C, add supplements and antibiotics. Pouring 32 to 40 ml medium into each plate, expect about 25 to 30 plates per liter.

Rich Plates

To ingredients listed below, add water to 1 liter and autoclave 25 min. Pour LB and H plates with 32 to 40 ml medium, in order to get 25 to 30 plates per liter. Pour lambda plates with about 45 ml medium for about 20 plates per liter.

H plates

10 g tryptone
8 g NaCl
15 g agar

Lambda plates

10 g tryptone
2.5 g NaCl
10 g agar

LB plates

10 g tryptone
5 g yeast extract
5 g NaCl
1 ml 1 N NaOH
15 g agar or agarose

Additives

Antibiotics (if required):

Ampicillin to 50 µg/ml
Tetracycline to 12 µg/ml
Other antibiotics, see Table 1.4.1

Galactosides (if required):

Xgal to 20 µg/ml
IPTG to 0.1 mM
Other galactosides, see Table 1.4.2

TOP AGAR

Top agar is used to distribute phage or cells evenly in a thin layer over the surface of a plate. In a typical application, molten top agar is mixed with bacteria and the mixture poured onto a plate to make a thin layer that is allowed to solidify. This layer of cells then grows denser, forming the opaque *lawn* of cells. Top agar contains less agar than plates, and so stays molten for days when it is kept at 45° to 50°C. Top agarose is sometimes used when DNA is to be prepared directly from phage, and is also used when libraries are plated out to be screened by plaque lifting.

Prepare top agar in 1-liter batches, autoclave for 15 min to melt, cool to 50°C, swirl to mix, pour into separate 100-ml bottles, reautoclave, cool, and store at room temperature. Before use, melt the agar by heating in a water bath or microwave oven (see *UNIT 1.11*) then cool to and hold at 45° to 50°C.

H top agar

10 g tryptone
8 g NaCl
7 g agar

LB top agar

10 g tryptone
5 g yeast extract
5 g NaCl
7 g agar

Lambda top agar

10 g tryptone
2.5 g NaCl
7 g agar

Top agarose

10 g tryptone
8 g NaCl
6 g agarose

STAB AGAR

Stab agar is used for storing bacterial strains (see *UNIT 1.3*). The recipe below is for 1 liter.

Stab agar

10 g nutrient broth
5 g NaCl
6 g agar
10 mg cysteine·Cl
10 mg thymine

Thymine is included so that thy⁻ bacteria can grow. Cysteine is thought to increase the amount of time bacteria can survive in stabs.

TOOLS

Inoculating Loops

Inoculating loops are used to move small numbers of bacteria or phage to a plate or to a new container of liquid medium. Inoculating loops may be purchased from any general scientific supply company. However, most researchers prefer to use loops made in the laboratory. These are made by inserting both ends of a 10-in. piece of 28-G platinum wire into an inoculating loop holder (also widely available) and twirling the holder while tugging on the middle of the wire with the point of a pencil (see Fig. 1.1.1).

Sterilize the loop by holding it in a bunsen burner flame until it is red hot. Cool the loop by touching it to a sterile portion of the surface of an agar plate until it stops sizzling.

Sterile Toothpicks

The broad side of flat wooden toothpicks may also be used for streaking out bacteria. Round wooden toothpicks, or the pointed end of flat toothpicks, are sometimes used to pick individual colonies or phage plaques. To sterilize, place toothpicks in a small beaker, cover the beaker with foil, and autoclave. Alternatively, simply autoclave the whole box of toothpicks and hold them in the middle when picking them up out of the opened box. It is convenient to put used toothpicks into another smaller beaker which, when full, is covered with foil and autoclaved. Used toothpicks can be saved, reautoclaved, and used again (see Fig. 1.1.2).

Spreaders

Spreaders are used to distribute liquid containing bacterial cells evenly over a plate. They are made by heating and bending a piece of 4-mm glass tubing (see Fig. 1.1.3). Less durable spreaders can be made from a Pasteur pipet. Before each use, sterilize the spreader by dipping the triangular part into a container of ethanol, passing the spreader through a gas flame to ignite the ethanol, and letting the flame burn out. Be careful not to ignite the

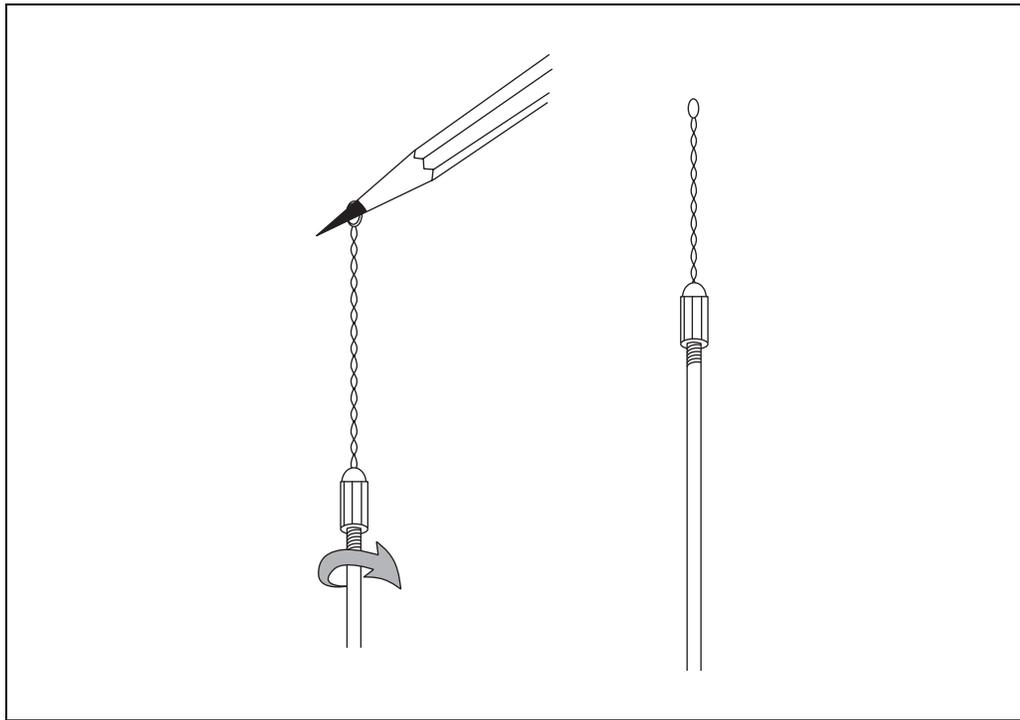


Figure 1.1.1 Making a loop.

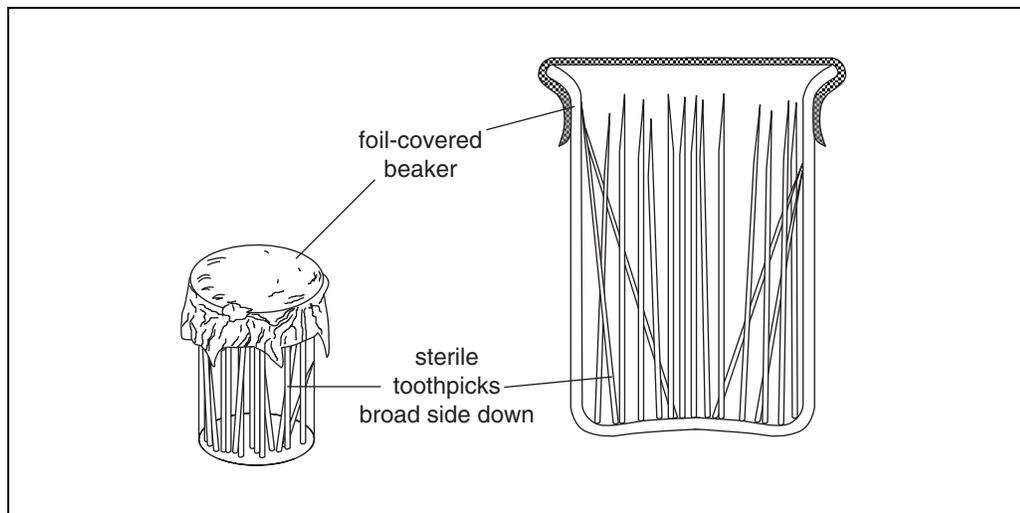


Figure 1.1.2 Preparing sterile toothpicks.

ethanol in the container. Cool the spreader by touching it to the surface of an agar plate that has not yet been spread with cells.

Glass Beads

Although spreaders are useful for many applications, when processing large numbers of plates it can become a time-consuming process. A popular practice is to use 4-mm glass beads that have been sterilized. A half dozen or more beads distribute the liquid culture on surface of the agar when the plate is shaken horizontally in all directions. The beads are then discarded and the plate inverted and placed in the incubator. Stacks of plates can be handled together when plating many culture samples.

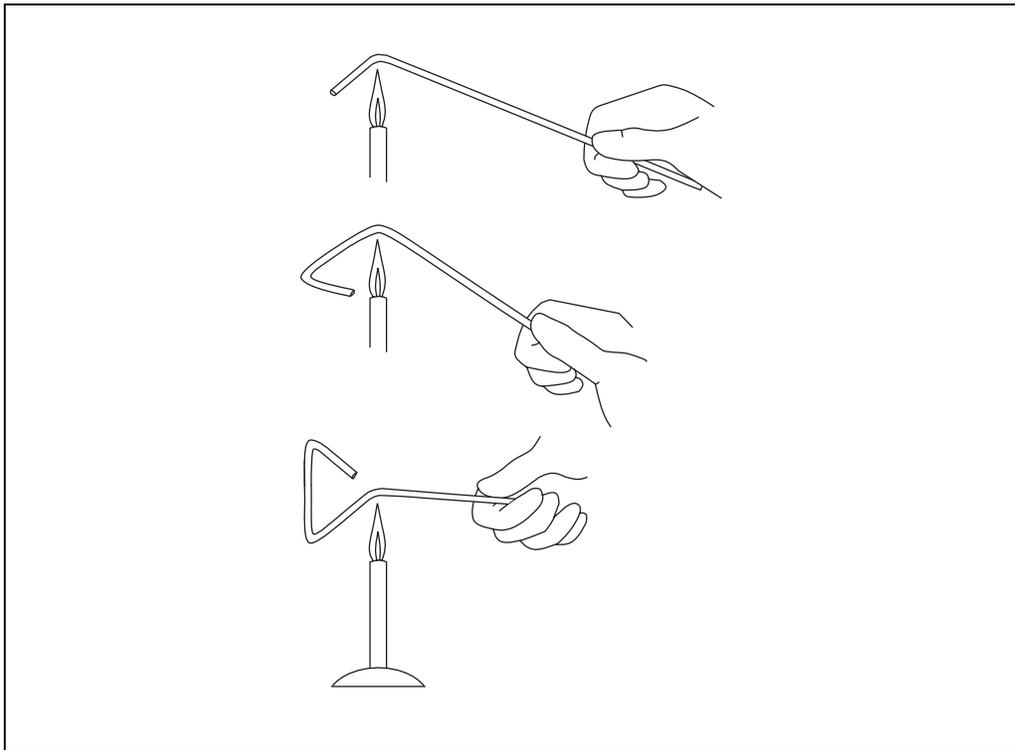


Figure 1.1.3 Making a spreader.

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