Luria Broth (LB) and Luria Agar (LA) Media and Their Uses Protocol

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Information

History

LB is a widely used bacterial culture medium today but it has its origins in the field of bacteriophagegenetics. Guiseppi Bertani created the LB recipe while trying to optimize plaque formation on a*Shigella* indicator strain (Bertani, 1952). According to Bertani, LB has been variously misconstrued to stand for "Luria Broth", "Luria-Bertani" medium, and "Lennox Broth"; however, the acronym originally stood for "Lysogeny Broth" (Bertani, 2004). The agar form of the medium should be designated LA but it is often referred to as LB. Although originally developed for bacteriophage studies and *Shigella*growth, LB subsequently became the medium of choice for growth of *Escherichia coli* and other related enteric species.

Purposes

LB medium is a rich medium that is commonly used to culture members of the *Enterobacteriaceae* as well as for coliphage plaque assays. LB and related media (SOC, Terrific Broth, 2xYT, etc) are used extensively in recombinant DNA work and other molecular biology procedures. Often an antibiotic is added to the sterilized medium to select for cells that contain a specific genetic element such as a plasmid, a transposon, or a gene disruption via an antibiotic resistance cassette. X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galactoside) may be added to sterile medium when using the blue-white screen for plasmids bearing the alpha fragment of the beta-galactosidase gene (a-complementation analysis). IPTG (isopropyl-beta-D-thiogalactopyranoside) is sometimes added to induce expression of genes controlled by the *lac* promoter. In addition to (or perhaps because of) its prominent position in the molecular biology field, LB has also been used as a general-purpose bacterial culture medium for a variety of facultative organisms. In the undergraduate microbiology teaching labs, LB is sometimes used as the growth surface when attempting to analyze bacterial colony morphology (See LB agar medium images).

RECIPE

Many, slightly different recipes for LB exist. LB (broth and agar formulation) is also commercially available in a premixed form.

Standard Recipe for 1 Liter of LB:

(Sambrook and Russell, 2001 Gerhardt, *et al.* 1994).

Tryptone	10 g
Yeast Extract	5 g
NaCl	10 g

Dissolve components in 1 liter of distilled or deionized water.
For LB agar* add agar to a final concentration of 1.5%.
Heat the mixture to boiling to dissolve agar and sterilize by autoclaving at 15 psi, from 121-124°C for 15 minutes.
* Strictly speaking, LB agar should be called LA.

LB Variations

Variations of the LB recipe can be found in the literature and lab manuals. One variation of LB ("Lennox Broth"), calls for the use of 5 grams per liter of NaCl (Lennox, 1955; Gerhardt, et al. 1994). In some research studies, a diluted version of LB (0.1% LB) has been used to culture environmental microbe isolates (Lee, 2000). To isolate marine microbes such as *Vibrio* spp., some researchers have used 30 grams per liter of NaCl (Nandi, et al., 2000). Glucose is sometimes added to a final concentration of 1 to 2 grams per liter of medium. Many recipes call for the use of a concentrated NaOH solution (~1-5 M) to adjust the pH to 7.0 prior to autoclaving. To maximize growth, especially when a carbon source such as glucose is added, phosphate buffer or Tris-HCl buffer may be added to maintain the pH. If the medium is to be used for bacteriophage growth, a sterile stock solution of $CaCl_2$ is often added to a final concentration of 2.5 $x10^{-3}$ M after autoclaving. Top agar (0.75 % agar plus the other LB components) is routinely used for plating of bacteriophage. The use of top agar facilitates diffusion of phage particles (For more information, see Plaque Assay Protocol entry).

Other additions and their purposes (Sambrook and Russell, 2001; Maloy 1990):

Chemical	Purpose	Stock Concentration	Workir Concer
$CaCl_2$	Bacteriophage lambda infection	1 M	2.5 mM
$MgCl_2$	Bacteriophage P1 infection	1 M	20 mM
X-gal	Chromogenic substrate for beta-galactosidase; screen for recombinant vectors	2% (w/v)	0.02-0
IPTG	Nonfermentable lactose analog; induction of *lac* promoter	1 M	0.01-5

PROTOCOLS

Streak Plate for Elucidation of Colony Morphologies
Streak a plate of LB agar with a pure or mixed bacterial culture. Incubate inverted plate at organism's ideal temperature for 24 to 48 hours. Observe colony morphology. For more information on colony morphology, see Description of Colonial Morphology of Microorganisms.

Plating of Bacteriophage: See Plaque Assay entry.

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

COMMENTS AND TIPS

This section is to evolve as feedback on the protocol is discussed at ASMCUE. Please contact the project manager for further information.

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