Achieving Shaker Flask-Scale Cell Densities in Miniprep-Scale Cultures with Baffled Culture Tubes and Growth Medium H15

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Luria-Bertani (LB) or similar nutrient broths have been the most commonly used media for the routine growth of small-scale (1–5 mL) bacterial cultures for the preparation of plasmid DNA (1). In an effort to maximize cell densities and, thus, the yield of plasmid DNA, some laboratories have used “rich” media including Terrific Broth and SOC broth (4,5). More recently, medium H15 was reported to be among the most efficient of these rich media because the inclusion of biological buffers (MOPS and Tris) makes it possible for cells to undergo biphasic growth (2). The first phase of growth is driven primarily by the use of glucose, while the second phase utilizes casamino acids and yeast extract as the primary carbon and energy sources.

A significant obstacle to the adoption of H15 medium has been the difficulty of using it for small-scale overnight cultures common in molecular biology laboratories. The superior performance of H15 medium requires bacterial cultures (20 mL) to be grown in larger (300 mL) baffled flasks for maximal aeration (2). In the absence of adequate aeration, fermentation produces a rapid drop in pH to below 6.0, which causes the cells to enter stationary phase rather than undergo biphasic growth (3). However, when scaled down to 2-mL *E. coli* cultures in standard 15-mL (100 × 16 mm) culture tubes, H15 reproducibly failed to support the high cell densities achieved with baffled flasks. It was hypothesized that this resulted from a lack of adequate aeration combined with the vicissitudes associated with the preparation of the H15 broth. To circumvent these problems, we have developed a “baffled test tube” that provides for greater aeration, and we have optimized the conditions for the preparation and use of H15 with 2-mL bacterial cul-

tures. These modifications make it possible to obtain the same high yields of cells and plasmid DNA that are achieved in baffled flasks.

Bacterial cultures. *E. coli* strain DH5 α containing plasmid pGEM[®]-T (Promega, Madison, WI, USA) was used in the current study. Cultures were inoculated with 50 μ L of a liquid LB culture of *E. coli* at mid log phase (A_{600} = 0.6) and were incubated at 37°C with constant shaking (350 rpm) for 16 h. Spectrophotometric readings (A_{600}) were taken at 10, 12, 14, and 16 h. All cultures had reached stationary phase by 16 h post-inoculation, and no significant increases in cell densities were seen at 20 or 36 h post-inoculation. All experiments were performed in triplicate. Because of the extremely high cell densities supported by H15, a standard curve relating cell density to optical absorbance at 600 nm was generated by serial dilution of a 16-h liquid culture (Figure 1). The relationship between A_{600} values and cell density is described by the log equation [$y = 1.77 + 1.08 \log (x)$]. All comparisons of cell density were calculated by interpolation of this line. The coefficient of determination for this line ($r^2 = 0.992$) is virtually indistinguishable from the idealized value of 1.0 for this statistic. This made it possible to accurately compare cell densities across a broad range of optical absorbance values.

H15 medium preparation. Table 1 shows the composition of H15 medium, which was prepared in 100-mL aliquots. The RNA (ICN Biomedicals, Costa Mesa, CA, USA), yeast extract, casamino acids, and RNase A were added to 80 mL sterile water and stirred until dissolved (15–30 min). MOPS and Tris were then added simultaneously to avoid extremes in pH. The conditions under which H15 is prepared and stored impact the performance of the medium. First, the addition of glucose before autoclaving, or its addition to hot H15 after autoclaving, results in the formation of unstable products accompanied by a precipitate, which completely inhibits *E. coli* growth (2). However, even when glucose is added to cool H15 and stored at 4°C, a precipitate forms within a week with concomitant inhibition of cell growth. To ameliorate this problem, glucose-free

Benchmarks

H15 medium can be prepared and stored (-20°C) to which sterile glucose is added immediately before use. Even when prepared without glucose, however, the performance of autoclaved H15 medium was often inconsistent. Optimal and reproducible performance was only achieved by sterilization of H15 through a 0.45- μ m pore size filter (Millipore, Bedford, MA, USA).

Baffled culture tubes. In the original report on H15, bacteria were cultivated in 20 mL media in baffled 300-mL flasks for maximal aeration (2). A search of glassware suppliers, however, failed to turn up baffled culture tubes that would allow for maximal aeration of 2-mL liquid cultures more commonly used in molecular biology laboratories. We therefore modified standard (100 $\times$ 16 mm) glass culture tubes (Fisher Scientific, Pittsburgh, PA, USA) to create a baffled tube. This was done by heating the base of the tube with a Bunsen burner until the glass had softened. The sharp corner of a razor blade was then rapidly pressed into the wall of the tube producing a triangular indentation approximately 15 mm above the base of the tube (Figure 2).

To assess the efficacy of the baffled tubes, bacteria cultivated in LB and H15 broth in standard (smooth wall) tubes were compared to identical cultures

Table 1. Composition of H15 Growth Medium

Component	Concentration
Torula Yeast RNA	1% w/v
Yeast Extract	5% w/v
Casamino Acids	1% w/v
Glucose	2% w/v
MOPS	250 mM
Tris	289 mM
RNase A	10 μ g/mL
(pH medium to 7.6 with HCl)	

grown in baffled tubes. Figure 3 presents the results of these comparisons. The performance of the optimized H15 medium was found to be superior to that of LB medium regardless of the type of culture tube used. In standard culture tubes, cell densities supported by H15 medium were up to 330% of the densities recorded for cultures grown in LB medium at matching time points. Similar results were seen with baffled tubes in which cell densities with H15 were up 380% of those recorded for corresponding cultures in LB broth. When the results obtained with H15 in standard tubes were compared to those from baffled tubes, a two-way analysis

of variance (ANOVA) demonstrated that the use of baffled tubes produced significantly ($F_5: 488.0$; $df: 1, 16$; $P < .0.001$) greater cell densities. Conversely, the cell density of cultures grown in LB was virtually identical regardless of whether standard or baffled tubes were used. This was not unexpected because LB medium does not include glucose as a carbon source, thereby avoiding the potential for a rapid fermentation-driven drop in pH. Most importantly, the cell density of 2-mL *E. coli* cultures grown for 16 h in baffled culture tubes using the optimized H15 broth ($A_{600} = 1.815$) was indistinguishable from that of a 20-mL H15 *E. coli* culture grown in a baffled 300-mL flask as a control ($A_{600} = 1.810$).

Additional considerations. Beyond modifications to the preparation of the H15 medium and the use of baffled culture tubes, we noted one additional consideration for the use of H15 in molecular biology laboratories where the use of X-gal in blue/white selection screens is common. Inoculation of H15 medium (which does not contain X-gal) with colonies grown on agar containing ampicillin (50 μ g/mL) and X-gal (33 μ g/mL) resulted in a significantly longer lag phase and lower cell density at 10, 12, and 14 h post-inoculation than was observed for colonies taken from agar containing ampicillin only (Figure 4). A two-way ANOVA demon-

Figure 1. Standard curve of optical density at 600 nm plotted against serial dilutions of cells from a 16-h culture of *E. coli* grown in H15 broth in a 15-mL baffled tube shaken at 350 rpm.

Figure 2. Photograph of a 15-mL baffled tube (left) and 15-mL smooth wall tube (right).

strated that this difference in the two growth curves was statistically significant (F_s : 522.6; df:1, 16; $P < 0.001$). The reason for this phenomenon is unknown, but by 16 h, both cultures had reached nearly equivalent cell densities.

In conclusion, modifications to the preparation of H15 broth, including the preparation of glucose-free medium and filter sterilization, significantly improve

the storage and reliability of H15 growth medium. Furthermore, the use of baffled glass culture tubes enhances aeration and, thus, the performance of the H15 medium in small-scale bacterial cultures. As measured by optical absorbance, use of H15 broth in 2-mL overnight cultures now supports growth to the same high cell densities (i.e., nearly four times greater than that with

LB media) that previously were only attainable using much larger and more expensive baffled flasks. These modifications offer researchers the opportunity to significantly boost the efficiency and yield of the small-scale bacterial cultures that are used for a wide variety of applications including the preparation of plasmid DNA and the heterologous expression of proteins.

Figure 3. Graph of cell densities, based on optical absorbance (600 nm), of 2-mL cultures of *E. coli* measured at 10, 12, 14, and 16 h after inoculation of either LB or H15 media in 15-mL standard culture tubes or 15-mL baffled tubes. (●) LB in standard tubes; (○) LB in baffled tubes; (■) H15 in standard tubes; (□) H15 in baffled tubes.

Figure 4. Graph of cell densities based on optical absorbance (600 nm) measured at 10, 12, 14, and 16 h after inoculation with *E. coli* taken from agar containing: (●) ampicillin only or (○) ampicillin and X-gal into H15 broth in baffled 15-mL tubes.

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