Media Matters: Endotoxin-Free ClearColi® BL21 (DE3) for Protein Expression

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E. coli bacterial strains are used widely as expression hosts for protein production. *E. coli*'s accelerated growth rate, low media cost, and ease of genetic manipulation allow affordable protein production at both research and commercial scales. However, lipopolysaccharide (LPS), the major outer membrane component of Gram negative bacteria, triggers a strong endotoxic response in mammalian cells and must be removed or reduced prior to use as in recombinant FDA-approved biologics, cell culture additives, or cell-based assay ligands. Purifying the protein product from contaminating LPS is costly, time-consuming, and can reduce product yield.

ClearColi BL21 (DE3), a genetically modified strain of BL21 (DE3), eliminates LPS contamination at the source. This highly engineered cell line produces lipid IV_A, a non-glycosylated LPS precursor, in place of LPS. Seven null mutations in the LPS biosynthesis pathway (ΔgutQ, ΔkdsD, ΔlpxL, ΔlpxM, ΔpagP, ΔlpxP and Δept) remove two of the six acyl chains and the oligosaccharide chain (Figure 1). An eighth compensating mutation (msbA148) supports cell viability. The lipid IV_A molecule does not trigger the endotoxic response. The modified cellular membrane, which is more hydrophobic, can lead to slower growth rates for the cells in hypotonic media. The modified outer membrane is more permeable, rendering the cells osmosensitive and further reducing growth rates in conditions of low ionic strength. To further understand ClearColi's media requirements, we investigated the effect of media formulations and common additives on the cell line's growth rate and doubling time.

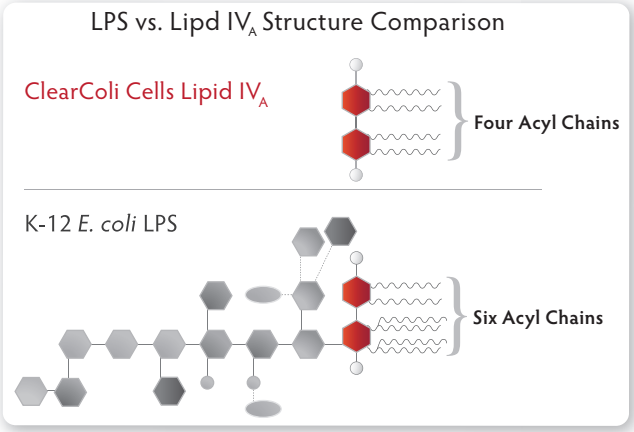

Figure 1. The LPS of a normal *E. coli* cell compared to the genetically modified Lipid IV_A from ClearColi cells. In ClearColi, the oligosaccharide chain has been deleted, and two of the six acyl chains have been removed to disable the endotoxin signal.

Methods

Electrocompetent ClearColi BL21(DE3) (Cat. # 60810-1) were transformed with a kanamycin-resistant plasmid using Lucigen's recommended protocol, and cells were plated on LB-Miller agar plates supplemented with 30 µg/mL kanamycin. Single colonies were picked for expansion in liquid media. Starter cultures (25 mL) grew overnight in LB-Miller supplemented with 30 µg/mL kanamycin with shaking at 250 rpm. The cells were then diluted to OD_{600} of 0.1 in 25 mL test media, and grown for 6 to 8 hours at 250 rpm unless otherwise specified. The optical density was monitored hourly or every two hours, and growth rate and doubling time were calculated using the following equations:

$$\text{Growth rate } h^{-1}\ (\mu) = 2.303(\log OD_2 - \log OD_1)/(t_2 - t_1)$$

$$\text{Generation rate or doubling time } (t_d) = \ln 2/\mu$$

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Results

We tested different strengths of LB-Miller medium, to determine the effect of richer medium on growth rate (**Figure 2**). We saw a concentration dependent increase in growth rate up to 1.5X LB.

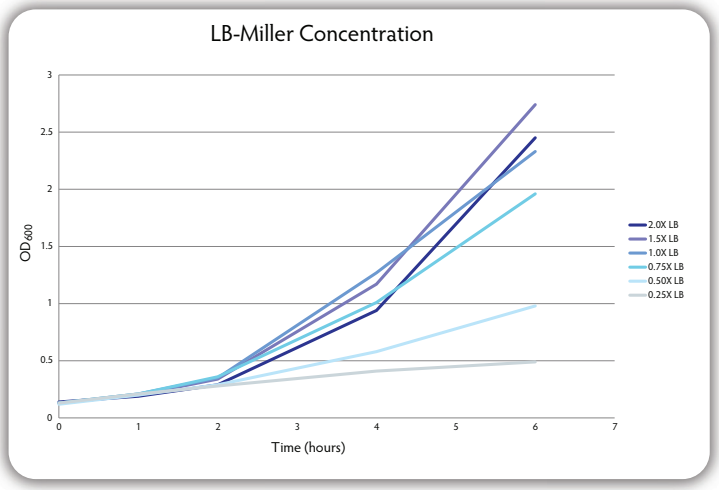

Figure 2. Increased LB concentration leads to increased growth rates up to 1.5X LB. ClearColi BL21(DE3) was grown in the specified medium for 6 hours. OD_{600} readings were taken every two hours.

Sources of calcium and magnesium are common additives to minimal media formulations. We tested these divalent cations at 2 mM and 10 mM concentration, alone and in combination. Mg^{2+} had no significant effect on growth rate at concentrations up to 10 mM (**Figure 3**). Ca^{2+} had a negative impact on ClearColi, even at the lowest tested concentration. This effect was partially rescued by addition of Mg^{2+}.

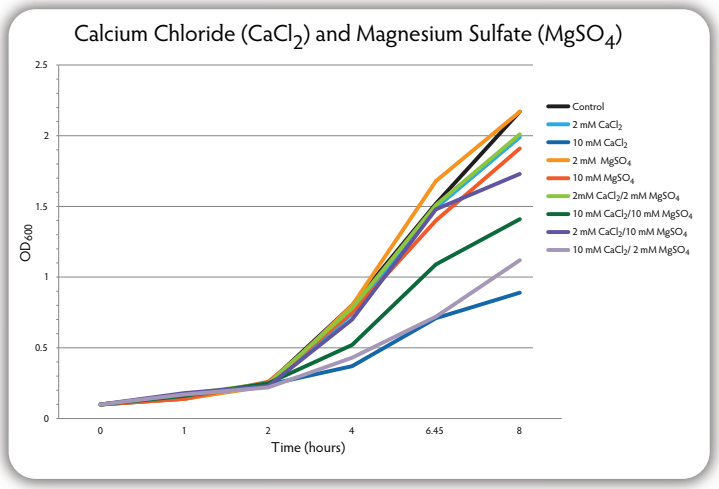

Figure 3. Mg^{2+} does not affect ClearColi at low concentrations, Ca^{2+} has deleterious effect on growth rate. ClearColi BL21 (DE3) was grown in LB-Miller medium supplemented with specified amounts of $CaCl_2$ and $MgSO_4$. OD_{600} readings were taken every two hours.

LB contains a low concentration of fermentable sugars. We grew ClearColi in LB-Miller media supplemented with glycerol and glucose as carbon sources (**Figure 4**). The carbon source did not appear to have a large effect on growth rate.

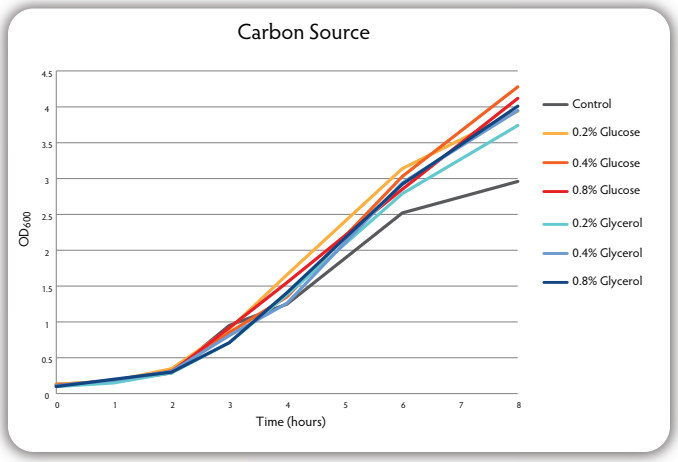

Figure 4. Glucose and glycerol increase cell densities. ClearColi BL21 (DE3) was grown in LB-Miller medium at 100 rpm supplemented with specified amounts of glucose or glycerol (v/v). OD_{600} readings were taken every other hour.

Media salt concentration effects the growth rate of ClearColi (**Figure 5**). LB-Miller (10 g NaCl/L) and LB-Lennox (5 g NaCl/L) formulations contain different amounts of sodium chloride (NaCl). The low-salt concentration of LB-Lennox had a deleterious effect on ClearColi's growth rate when compared to LB-Miller medium. The combination of a concentrated LB-Miller formulation and additional carbon source yielded higher cell densities.

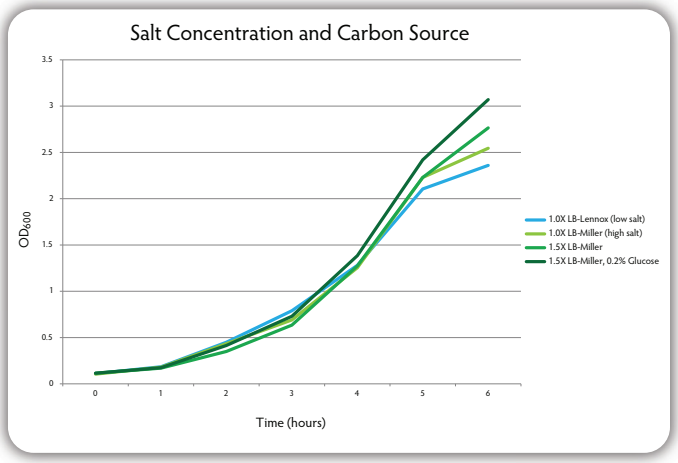

Figure 5. LB-Miller yields higher cell densities than LB-Lennox. ClearColi BL21 (DE3) was grown in 1X or 1.5X LB-Miller (10 g NaCl/L) or LB-Lennox (5 g NaCl/L) medium or in supplemented with specified amounts of glucose or glycerol (v/v). OD_{600} readings were taken every hour.

ClearColi BL21 (DE3) cells should be grown to an OD_{600} of 0.6-0.8 before induction of protein expression. ClearColi exhibits a longer initial lag phase than other BL21 (DE3) strains, so slightly longer growth

periods are required to reach desired cell densities before IPTG induction. After the first three hours of growth, ClearColi's growth rate and doubling times closely matched that of BL21 (DE3) (**Figure 6**). The growth rates within the 3-4 hours timepoints were accelerated in high-salt LB-Miller media.

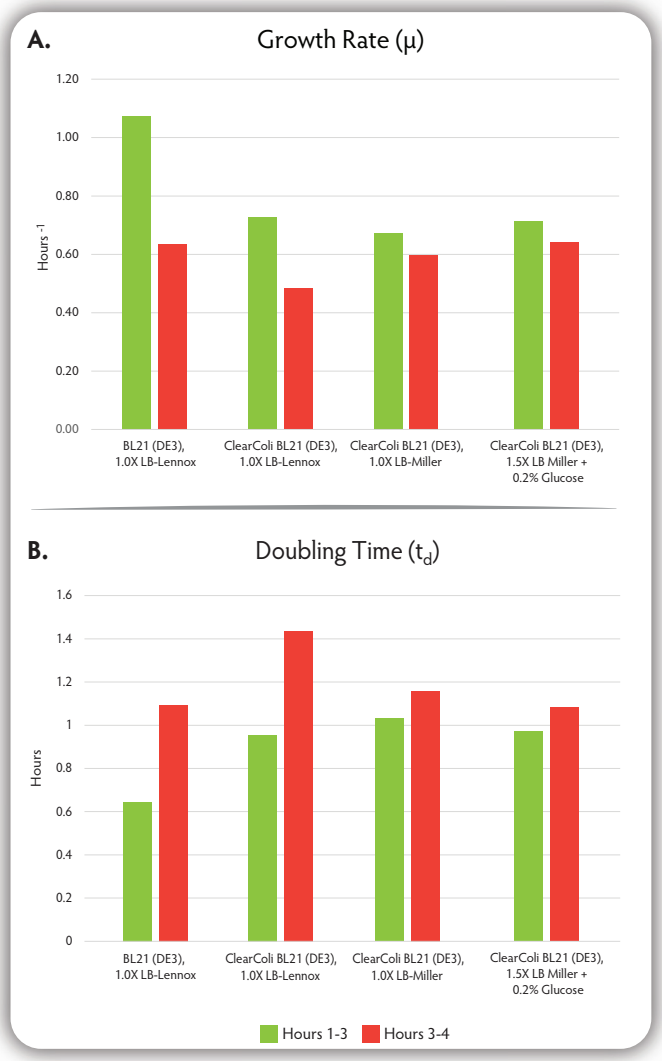

Figure 6. Growth rate (μ) and doubling time (td) for ClearColi and standard BL21 (DE3). ClearColi BL21 (DE3) or standard BL21 (DE3) were grown in specified medium at 250 rpm. Growth rates and doubling times were calculated based on OD_{600} readings taken every hour.

Conclusions

We have created ClearColi BL21 (DE3) for endotoxin-free protein expression. The highly engineered cells exhibit slightly slower growth rates compared to common LPS-positive BL21 (DE3) strains, but reach densities appropriate for protein induction within 3-4 hours after dilution to OD_{600} of 0.1. Common media supplements such as glucose and $MgCl_2$ may be beneficial in some cases, but Ca^{2+} should be avoided. Due to the osmosensitive nature of ClearColi it is important to have salt concentrations of at least 10g NaCl/L. High-salt LB-Miller (10 g NaCl/L) media should be used for both agar plates and liquid media to avoid osmotic stress response and maintain higher growth rates. The growth characteristics of ClearColi BL21 (DE3) Electrocompetent Cells are amenable to common protein production workflows, allowing researchers to save time and cost by eliminating LPS contamination at the source.