

Optimization of **BIOEXPRESS** SUPPLEMENTATION of M9 CULTURES

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Uniform labeling of proteins with ^{15}N and ^{13}C has typically been achieved through the use of bacteria grown in minimal media, such as M9, which contain single nitrogen and carbon sources. While this arrangement facilitates the straightforward isotopic replacements of these elements, the growth characteristics of *E. coli* in these media are somewhat compromised compared to growth in rich media. These effects typically include a drop in maximum cell density, requiring that larger cultures be grown to produce sufficient quantities of protein for NMR study.

To avoid the increased expense and time required to prepare, grow and process such larger cultures, an alternative approach is to supplement minimal media with mixtures of isotopically-labeled biomolecules, such as cell lysates. Here we evaluate the effects of adding increasing amounts of one such mixture, BioExpress Cell Growth media from Cambridge Isotope Laboratories, to *E. coli* cultures grown in M9 minimal media. We characterize the beneficial effects of this supplementation on cell growth rates, maximal densities and protein expression and observe significant benefits in all three of these categories.

Methods:

Escherichia coli strain BL21(DE3) (Stratagene) was transformed with the plasmid GB1-STOP, encoding the Streptococcal protein G β 1 domain (G β 1) under control of a T7 RNA polymerase promoter, and plated on an LB/amp plate. A colony from this plate was inoculated into 5 mL of LB media and allowed to grow for 4 hr at 37 C with vigorous shaking. At this point, the culture was divided into equal parts, centrifuged, and pellets resuspended in M9 media (Table 1) supplemented with various quantities of BioExpress Cell Growth media. For an initial test of growth characteristics without protein induction, these media contained BioExpress at concentrations of 0.5%, 1%, 10%, 20% and a standard M9-only sample. A subsequent test of growth characteristics with protein induction used cultures containing 0.5%, 1%, 2%, 5%, 10% and a standard M9-only sample. For this latter set, protein expression was induced by the addition of 0.5 mM IPTG at a point during the middle of log phase growth (typical A_{600} values \sim 0.4-1.2) as deter-

mined from the prior test without induction. At all points through these growths, cultures were grown at 37 C with vigorous shaking and densities were monitored by turbidity at 600 nm (A_{600}). Induced cultures were harvested 3 hr post-induction, at

Na_2HPO_4	6 g/L	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	246 mg/L (1 mM)
KH_2PO_4	3 g/L	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	14.7 mg/L (0.1 mM)
NaCl	0.5 g/L	thiamine	10 mg/L
NH_4Cl	1 g/L	biotin	10 mg/L
glucose	3 g/L	ampicillin	100 mg/L

Table 1

which point they were analyzed for protein expression (SDS-PAGE) and total cell mass (wet weight).

Results:

Supplementing standard M9 media with BioExpress Cell Growth media provided several significant benefits, including decreased doubling times and increased total cell mass. These favorable characteristics were observed in cultures supplemented with BioExpress at amounts significantly below the recommended working level of 1% v/v. In detail:

Supplementing M9 media with BioExpress significantly shortened doubling times by up to 40%. As detailed in Table 2, BioExpress-supplemented M9 cultures had doubling times between 31-49 minutes, as compared to 57 minutes for a non-supplemented culture. The degree of doubling time shortening correlated with the amount of added BioExpress, with the most

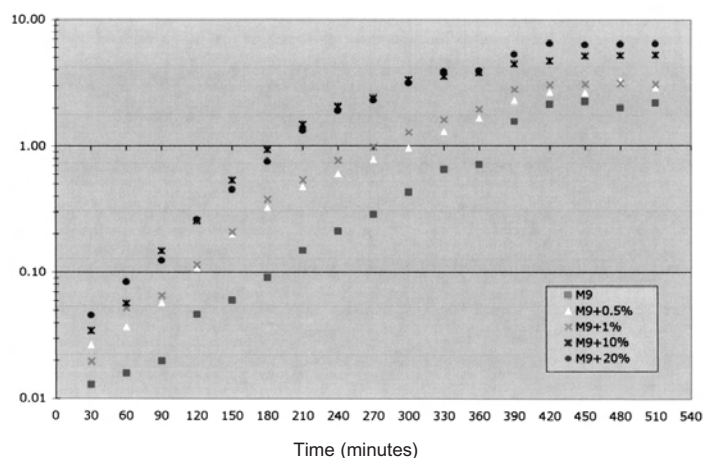
media	doubling time (min)	max A_{600} (non-induced)	A_{600} (3hr post induction)
Control (M9)	57.3 min	2.20	0.92
M9 + 0.5% v/v Bioexpress	48.7 min	2.86	1.75
M9 + 1%	38.2 min	3.09	1.63
M9 + 2%	34.7 min	n.d.	1.97
M9 + 5%	33.3 min	n.d.	1.90
M9 + 10%	31.6 min	5.26	2.81

Table 2

pronounced effects observed at concentrations of at least 1%.

Adding BioExpress significantly increased the maximum density obtained from these cultures, regardless of whether protein expression was induced or not. As shown in Table 2 and Figure 1, supplementation with BioExpress at levels as low as 0.5% v/v increased post-induction densities by factors of 2-3 fold compared to M9 controls.

BioExpress Growth Media evaluation:
Non-induced samples



BioExpress Growth Media evaluation:
Induced samples

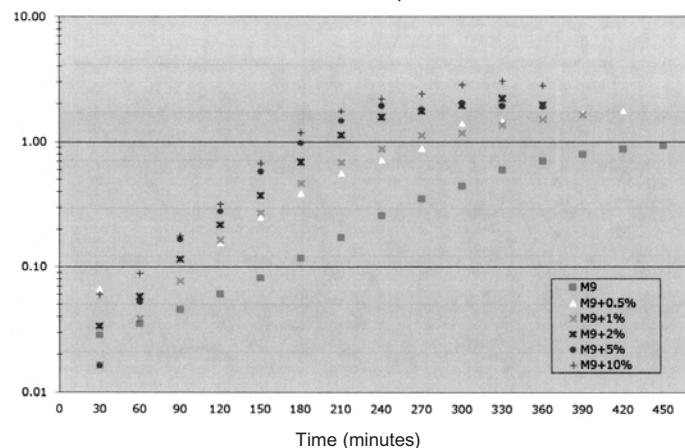


Figure 1

Growth curves of BL21(DE3) cells containing pGB1-STOP in M9 media supplemented by various concentrations of BioExpress Cell Growth Media. Protein expression in the induced cultures was triggered by the addition of 0.5mM IPTG at mid-log phase points that ranged from A_{600} -0.4 (M9) to A_{600} -1.2 (M9+10% BioExpress).

BioExpress-supplemented cultures also expressed higher amounts of the induced G β 1 protein on a per-cell basis, as shown in the SDS-PAGE analyses presented in Figure 2. Importantly, this effect is specific to the induced protein and does not represent an increase in the levels of all proteins, as shown by the equivalent staining of non-induced proteins across all of the samples in

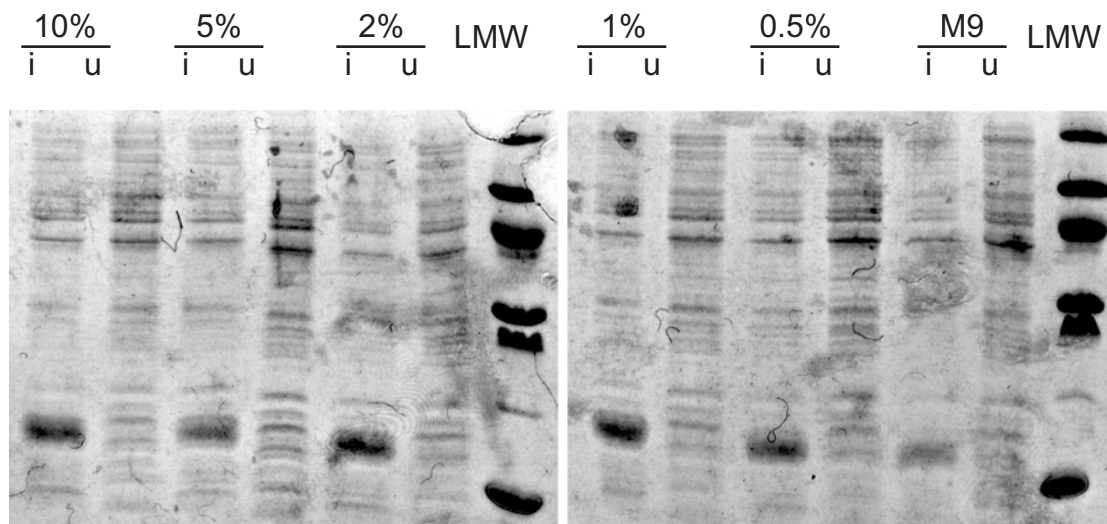


Figure 2

SDS-PAGE analysis of whole cells from non-induced and induced cultures of cells grown in M9 media containing varying concentrations of BioExpress Cell Growth media. Both uninduced (u) and induced (i) samples of cultures grown with each concentration of BioExpress were evaluated as identified by the percentage supplementation of BioExpress. All samples were corrected for cell density prior to loading. The band corresponding to Gβ1 is identified with an arrow on the left side of the figure. LMW: low molecular weight markers.

Summary:

The supplementation of standard M9 media with complex mixtures of biomolecules and their precursors, such as BioExpress Cell Growth media, offers a route to straightforwardly improve the yield of uniformly labeled protein from bacterial cultures. As shown here, this supplementation can improve three key aspects of this process: growth rate, maximal cell density, and the degree of overexpression of a recombinant protein. Significant improvements in each of these can be obtained by supplementing with modest (1% v/v) amounts of BioExpress, with further improvement possible at higher levels.



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