MEDIA COMPOSITION INFLUENCES RECOMBINANT PROTEIN ACCUMULATION IN E. COLI

Dr Sheldon E. Broedel

Escherichia coli has been the work-horse of gene expression for many years and is the first-line system for producing recombinant proteins. The reason for this is that many different host-vector systems are readily available, the organism is simple to culture, it grows rapidly, and recovery of the recombinant protein is relatively straightforward, particularly with the use of affinity tags. Commercially, E. coli is classified as a generally recognized as safe organism[1] with a good regulatory compliance history and has proved to be an economically viable means for producing protein products. However, not all proteins are accumulated to maximal levels in E. coli and production typically requires optimization.

The goal for optimizing production of recombinant proteins is to produce the highest amount of functional product per unit volume per unit time. For E. coli, as any other fermentation system, the level of intracellular accumulation of a recombinant protein is dependent on the final cell density and the specific activity of the protein, or, in other words, the level of accumulation relative to total protein. Four strategies are typically taken for optimizing the production of a recombinant protein. These are: The choice of culture medium, mode of cultivation, strain improvement, and expression system control (see Lee[2], Kleman and Strohl[3], and Gustafsson et al[4] for reviews on optimizing strategies).

Much of the effort aimed at increasing recombinant protein production in fermentations has been directed at maximizing the biomass production and often overlooked are the effects of media composition on the expression of specific recombinant proteins. However, it is well known that the production of secondary metabolites in microbial strains can depend on the composition of the medium in which the organism is grown. Despite this, little attention has been paid to the effects of medium formulation on the accumulation of recombinant proteins.

Statistical methods for developing the best medium formulation for maximizing the production of metabolites is a well-established practice[5]. In the course of optimizing the production of proteins in E. coli, we developed a set of media formulations that consistently increase the accumulation of recombinant proteins above the more traditional LB Broth recipe. Our observations have been that not all proteins are maximally expressed in any one medium. Rather, each protein accumulates to different levels in different media and it has not been possible to predict which medium would be most suitable for any given protein. Therefore, we devised a screen in which the production of a recombinant protein is examined when the host strain is grown in each of six media formulations. This simple and rapid screen allows for the selection of the most suitable medium for any given protein without laborious screening for the critical factors and subsequent optimization research.

The screen uses six different media to identify the formulation yielding the highest level of target protein production. The media include: Glucose M9Y, LB (Miller) Broth, Hyper Broth™, Power Broth™, Superior Broth™ and Turbo Broth™. The first two media are traditional formulations for cultivating E. coli. The latter four formulations were developed with the objective of increasing the volumetric production of recombinant proteins[6]. The host strain harboring the expression vectors is grown in each of the media, expression induced and the accumulation of the target protein measured as a function of total biomass and culture volume.

Once the best formulation is identified, the selected medium can be used at whatever fermentation size is needed to achieve the desired production levels. Examples of several proteins screened using this approach are shown in Figure 1.

The medium composition-dependent accumulation of recombinant proteins has several implications. The observation that each recombinant protein is expressed to different levels in different media suggests that there is a relationship between the respective protein and the composition of the medium in which it is produced. In the extreme case, the selection of one medium over another could be the difference between production and no production. The type of protein or its original source does not appear to predict which composition is most suitable. The medium producing the highest level of biomass also does not predict product accumulation. In our laboratory, Hyper Broth™ gives the highest biomass yields, yet this medium does not lead a priori to the highest level of recombinant protein accumulation. Consequently, a screen of several media formulations to determine which yields the best level of production would be advisable.

Despite the history of employing LB medium for the cultivation of E. coli, this is not necessarily the medium of choice for the production of recombinant proteins. LB does not contain a buffering system. Therefore, its use in a fermentation system, particularly with feed-batch or continuous culture protocols, is limited. Typically, buffered media formulations are employed in fermentations and most traditional fermentation media are of defined compositions[7,8]. Thus, when LB is used in a bench-scale setting to produce recombinant proteins, a different medium must be employed when the production is shifted to a fermentor. This requires additional research work to determine the best medium composition. While it can be argued that such work is necessary when regulatory compliance for commercial production is an issue, for most research and development, pre-clinical or pre-commercial work, this is an unnecessary expense. Selecting a more suitable medium which is more readily scalable, as is the case with Glucose M9Y, Hyper Broth™, Power Broth™ Superior

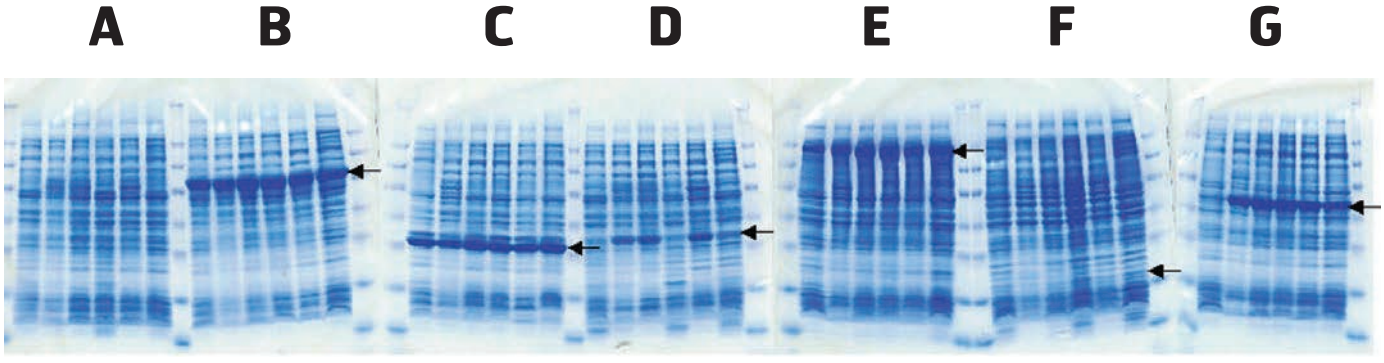

Figure 1. SDS-PAGE analysis of total protein from each of six different host-vector strains. Each strain was grown in 25 mL of the respective medium to an OD_{600} of 0.6, expression induced with 1 mM IPTG and a 1 mL sample removed at 3 h post-induction. The sample was adjusted to give 10D/mL and a portion analyzed by SDS-PAGE. Panel A – Strain JM109 without a recombinant protein; Panel B to G – Strains harboring plasmids that yield MalE, GST, GFP, I27δ, TesA, and LypA proteins respectively. Arrows denote the location of the respective recombinant protein. Marker proteins are shown to the left and right of each set of cellular proteins. From left to right in each panel are samples from cells grown in Glucose M9Y, LB (Miller), Hyper Broth™, Power Broth™, Superior Broth™ and Turbo Broth™.

Broth™ and Turbo Broth™ (all of which have a buffering system), reduces the time and effort needed to scale the protein production.

The observation that the medium composition can affect the accumulation of soluble protein is significant. The accumulation of recombinant proteins as insoluble products is a major shortcoming to using E. coli-based expression systems. In the example of LypA production, the increased amount of soluble protein, as judged by functional activity, did not follow the rule of more total product gives more soluble product.[8] In fact, the amount of soluble protein could not be predicted by the relative level of LypA accumulation. Similar results have been reported for the Candida albicans glucosyltransferase where Power Broth™ was found to yield soluble product whereas the other media did not. Other approaches reported to increase the relative accumulation of soluble protein include lowering the temperature during induction[10,11,12], reducing the concentration of inducer[13,14], using mutant strains which affect protein folding[15], fusing the target protein to a bacterial protein[16,17,18], and employing a variety of molecular chaperones[19]. Each of these factors is believed to affect protein folding in one way or another[20]. It may be that medium composition may affect protein folding too. Most likely the effect is through modulation of expression of the chaperones or accessory proteins involved in protein folding. Since a given chaperone only works on a subset of proteins it is reasonable to suggest that for any given recombinant protein the set of chaperones which influences its folding may or may not be at sufficient levels in any given medium. Until all of the chaperone-protein interactions have been defined, it remains beyond the scope of current knowledge to predict which set of chaperones are needed to correctly fold a protein and thus impossible to determine in advance which medium formulation is most suitable for the target protein. Therefore, the best medium for producing a given protein must be determined empirically.

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