Technical Brief:

Augmedium Case Studies

Sheldon E. Broedel, Jr., Ph. D.
Athena Environmental Sciences, Inc, Baltimore, MD

Athena Enzyme Systems™
1450 South Rolling Road
Baltimore, MD 21227
www.athenaes.com

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Augmedium™ is a medium additive used to precondition cultures for improving the expression of recombinant proteins in *E. coli* which tend to form insoluble products, inclusion bodies and aggregates. Frequently, heterologous proteins, when highly expressed in *E. coli*, accumulate as insoluble products. The protein produced under these circumstances is most often inactive, and, furthermore, it can be difficult or impossible to recover functional protein from the insoluble material. While techniques are available for purifying and refolding proteins that are produced as inclusion bodies this is not always desirable.

The role of molecular chaperones in protein folding has been extensively studied.[1,2,3] In *E. coli* the two primary chaperone networks are DnaK-DnaJ-GrpE and GroEL-GroES. In addition to these two networks there are several minor chaperones the expression of which are induced when the cells are under heat, chemical and oxidative stress. The chaperone proteins have been proposed to interact with nascent polypeptides and to facilitate the correct folding. Thus, it is not unexpected that when DnaK-DnaJ-GrpE or GroEL-GroES complexes are overexpressed the solubility of a number of aggregation-prone proteins is improved.[4,5,6,7,8,9,10,11,12,13,14] However, not all insoluble proteins exhibit improved solubility with overexpression of DnaK-DnaJ-GrpE or GroEL-GroES. Moreover, it has been shown that the solubility of some proteins is increased when the cells are subjected to chemical, thermal and oxidative stresses before expression of the insoluble protein.[15,16,17,18] Therefore, it seems likely that other chaperones may be necessary for some proteins. However, the mechanism by which a given protein is recognized by any given chaperone protein is not known. Augmedium™ was thus designed to induce the expression of several different chaperone proteins thereby allowing for an improvement in the solubility of aggregate-prone proteins without the need for identifying a specific chaperone effector. Below are two case studies where Augmedium™ was used.

The first case was an esterase from *Vibrio cholera*. This protein was expressed using pQE31 (Qiagen) with an N-terminal His tag in the strain M15. The protein accumulated to a large extent as an inclusion body with little of the protein accumulated in a soluble form. To increase the recovery of soluble enzyme, we first examined the effect of culture medium. A medium screen was performed according to the protocol of the Medium Optimization Kit™ (AthenaES™). Soluble protein was determined by measuring the level of enzymatic activity present in cells extracted with Y-Per Buffer (Pierce Chemical). It was found that the amount of enzyme activity recovered was medium-dependent and that Hyper Broth™ yielded the highest level of enzyme activity (Fig. 1). This was in contrast to LB (Miller) Broth where no enzymatic activity was detected.

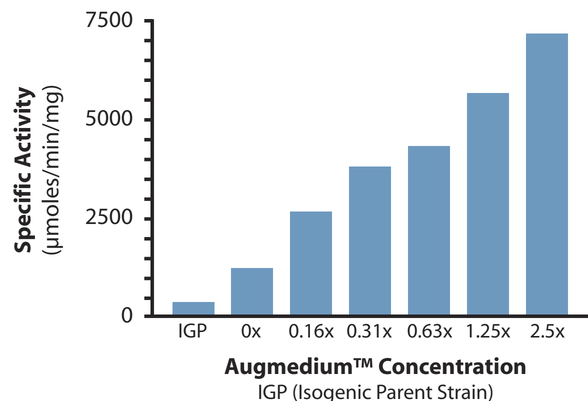

Figure 1. Augmedium™ - dependent increase in LypA activity. Medium-dependent accumulation of LypA after induction of expression. Augmedium™ was added at five different graduated concentrations. Cells were harvested after 3 hours of incubation. LypA activity was measured and the specific activity determined. A dose-dependent increase in enzyme activity with increasing Augmedium™ concentration was observed.

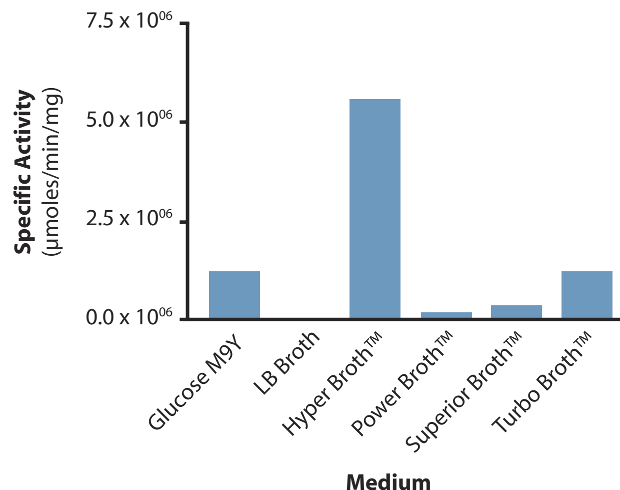

Figure 2. The above graph shows medium-dependent accumulation of LypA after induction of expression using five of AthenaES™'s proprietary expression media and industry standard LB Broth.

To determine whether Augmedium could improve the recovery of a protein in a medium giving poor expression, the expression of LypA was induced in cells grown in Power Broth™. This medium gave a low but measurable level of activity (Fig. 1). The effect of Augmedium™ on LypA activity was examined by culturing the cells in 25 ml of medium to a density of 1.0 OD_{600} and adding Augmedium™ to the culture at five different concentrations 20 min. prior to adding IPTG to 1 mM. After 3 hours incubation, the cells were harvested and the soluble enzyme released using 1 ml Y-Per Buffer™ (Pierce Chemical). LypA activity was measured and the specific activity determined. A dose-dependent increase in enzyme activity with increasing Augmedium™ concentration was observed (Fig. 2). The Augmedium™ at a concentration of 2.5x increased the yield of soluble esterase 5-fold over the non-treated culture.

In another example, AES8 (the functional properties of the protein can not be disclosed at this time due to its proprietary status), a somewhat more complex expression pattern was observed. As above, a screen of six medium formulations (Medium Optimization Kit™, AthenaES™) was used to determine the one yielding the highest level of soluble protein accumulation. Maximum levels of active protein in the soluble fraction were found when the cells were cultured in Glucose M9Y though the fraction of soluble AES8 protein produced remained less than 10% of the total accumulated. To increase the amount of soluble protein, the Augmedium™ concentration was titered in a matrix experiment (fractional factorial design) along with different IPTG concentrations and induction times. For this protein, both an enzyme assay and immunoassay were used to determine the level of soluble protein.

With regard to enzyme activity, a time- and Augmedium™ dose-dependent ("pre-condition") increase in protein accumulation was found (Fig. 3). Maximum activity was achieved after 6 h induction with 0.53 mM IPTG and 1x Augmedium™. With respect to AES8 mass accumulation (as measured by immunoblot), there appeared to be an interaction between the IPTG and Augmedium™ with maximum accumulation at the extremes of the dosing range and minimum in the mid-range doses (Fig 4.). These findings suggests that some portion of the protein that accumulates is not active. Therefore, when interpreting data on the production of a given recombinant protein, caution is advised against basing conclusions solely on mass accumulation data.

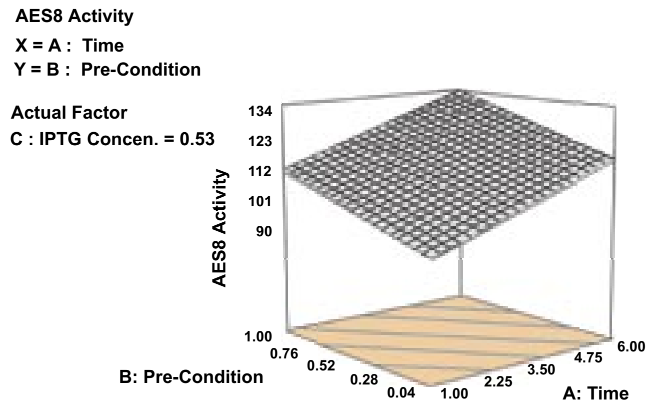

Figure 3. The above graph shows an increase in AES8 activity as a function of Augmedium™ concentration and induction time

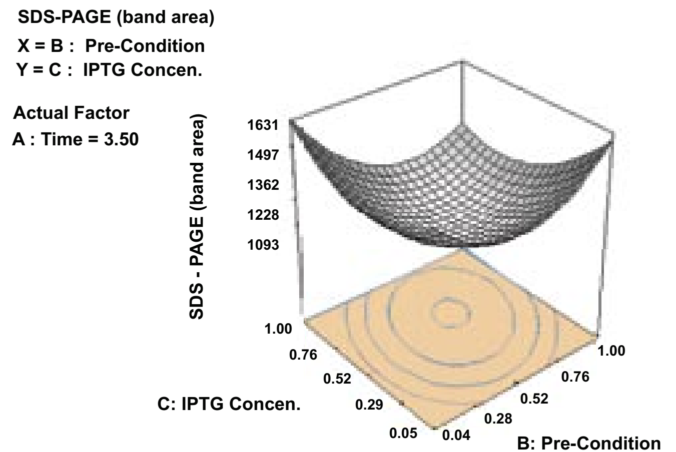

Figure 4. The above graph shows the accumulation of AES8 as a function of Augmedium™ and IPTG concentrations.

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