Technical Bulletin – Athena Enzyme Systems™ Group
Volume 2, January, 2001

The Selection of Optimum Media Formulations for Improved Expression of Recombinant Proteins In *E. coli*

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***Escherichia coli* is routinely used for the production of recombinant proteins both for research purposes and for commercial applications. Despite the extensive amount of knowledge gathered about the synthesis and accumulation of heterologous proteins and the well established principals affecting the production of secondary metabolites, little attention has been paid to the effects of medium composition on the production of recombinant proteins. In the course of optimizing the production of various recombinant proteins, we found that the level of accumulation of a given protein was composition-dependent. Since the best formulation for a protein could not be determined solely on the basis of the class of protein, a simple screening technique was developed to rapidly identify the best available medium for any give recombinant protein. The screen was tested on six different recombinant proteins and shown to be an effective means of empirically determining the best medium. It was further observed that the medium composition affected the relative level of soluble protein that accumulated. These results suggested that a medium screen should be employed as a routine part of developing bacterial strains which produce recombinant proteins. The application of such a screen could aid in improving the recovery of recombinant proteins.**

Introduction

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] 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*, or 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: choice of culture medium, mode of cultivation, strain improvement, and expression system control (see Lee[2] and Kleman and Strohl[3] for reviews on optimizing strategies). Much of the effort aimed at increasing recombinant protein production in bacterial strains has been directed at maximizing the biomass production and little is known about the effects of media composition on the expression of 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[4]. The basic approach used to develop an optimal medium formulation relies on empirical and trial-and-error processes. The use of statistical techniques for experimental design have provided a more elegant means of designing the best medium. Nevertheless, a rigorous and extensive research program is needed to devise the most suitable medium for the production of a given recombinant protein. The first step in developing the optimum formulation is to screen for the critical components. These are the factors which effect the production of the desired product. There are two experimental designs that can be applied.

The first is a Plackett-Burman design[5]. This is a 2-level matrix in which each factor is tested an equal number of times at its high and low values. Further, the design is balanced in that each factor is tested against the high and low of the other factors an equal number of times. This design is good when the number of factors is less than five and is well suited for detecting significant factors. It does not permit a test of interactions. The second type of experimental design used for screening factors is the so

Table 1. Bacterial strains.

Strain Name	Protein	Bacterial Strain	Vector	Promoter
AES-None	None	JM109	pUC19	*lacp*
AES-MalE	Maltose binding protein	JM109	pMalc2	*tac*
AES-GST	Glutathione S-transferase	JM109	pGEX2T	*tac*
AES-GFP	Green fluorescent protein	JM109	pGFP	*lacp*
AES-I278	Synthetic polyprotein of titin I27 domain	M15	pI278	*T5p*
AES-TesA	Thioesterase I (EC 3.1.1.5) of *E. coli*	M15	pTesA	*T5p*
AES-LypA	Lysophospholipase B (EC 3.1.1.5) of *Vibrio cholerae*	M15	pLypA	*T5p*

called fractional factorial design[6]. This is again a 2-level matrix design which can measure interactions. The factors are set at two levels, a high and a low, and consists of 2^{k-p} trials where k is the number of factors and $(1/2)^p$ is an integer of the fraction of full factorials. The main utility of this technique is that the main effects along with interactions can be examined.

Once the critical factors are determined, optimization can begin. First, curvature of the main effects is assessed by running replicate mid-points of the factor settings. If the mean of the mid-point outcome exceeds the mean of the factorial points the optimum is within the design space. Alternatively, if the mid-point mean is lower than the mean of the factorial points, then the optimum is outside the design space. The non-critical factors are set at their mid-points or on the best values based on the data. Mathematical models are then employed to describe the optimal process. Statistical software packages are available which greatly simplify this process. Typically, 2 to 5 factors are examined at 3 or 5 factor settings. A response surface modeling design is then used to develop the model[7]. The model developed predicts the optimal values for the factors in question which are tested in subsequent rounds of experimentation.

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 media optimization 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. In this paper, we describe the use of a media screen as a means of identifying the most suitable medium for the production of recombinant proteins in *E. coli*.

Materials and Methods

Chemicals. Chemicals and buffers were purchased from BD Biologicals (Baltimore, MD), Sigma Chemical (St. Louis, MO) or USBiological (Marblehead, MA). The media employed were manufactured by Athena Enzyme Systems™ of AthenaES (Baltimore, MD). The media included LB Broth (Miller), Glucose M9Y, Turbo Broth™, Power Broth™, Superior Broth™ and Hyper Broth™.

Bacterial strains and vectors. Table 1 lists the bacterial strains and plasmid vectors used to produce the recombinant proteins. The strains were cultured on solid TYE medium: 10g/L Tryptone, 5 g/L yeast extract, 8 g/L NaCl, 15 g/L microbiology grade agar supplemented with ampicillin to 100 µg/ml. The bacterial hosts were JM109 (*rec*A, *end*A1, *gyr*A96, *thi, hsd*R17, *sup*E44, *rel*A1, D*(lac-pro*AB*)*, F' *tra*D36, *pro*AB, *lac*I^{q}ZDM15) and M15 (Qiagen, Valencia, CA). Plasmids pMalc2 (New England BioLabs, Inc., Beverly, MA), pGEX2T (Amersham-Pharmacia, Piscataway, NJ) and pGFP (Clontech Laboratories, Inc., Polo Alto, CA) express the maltose binding protein (MalE), glutathione S-transferase (GST) and *Aequorea victoria* green fluorescent protein (GFP), respectively. AES-$I27_8$ produces a synthetic polyprotein composed of repeating human titin protein domains[8]. Plasmid pTesA was constructed by ligating the PCR amplified coding sequence of the *tes*A gene of *E. coli*[9] into pTrc99A (Amersham-Pharmacia, Piscataway, NJ) at the *Nco*I/*Eco*RI restriction sites of the vector. Plasmid pLypA was made by ligating the PCR amplified coding sequence of *lyp*A from *Vibrio cholera*[10] into pQE31 (Qiagen, Valencia, CA) at the *Bam*HI/*Pst*I sites. This construction added a poly-His tag to the amino-terminus of the LypA protein to facilitate purification of the enzyme.

Media screen. Overnight cultures were inoculated with a single colony of the recombinant strain into 2 ml of LB (Miller) Broth in a shake flask and incubated at 37°C. The overnight culture (0.1 ml) was used to inoculate 2 ml of each of the six media of the Media Optimization Kit™ which included Glucose M9Y, LB Broth (Miller), Hyper Broth™, Power Broth™, Superior Broth™ and Turbo Broth™ in six well culture dishes. The cultures were incubated at 37°C with shaking at 250 rpm until the OD_{600} reached 0.6 (about 2 h). Expression of the recombinant protein was induced by the addition of IPTG to 1 mM and the incubation continued for 3 h. A 1 ml sample was removed, the cells harvested by microfuging for 5 min., and the cells suspended in water to give 1.0 OD_{600} per ml. The cell suspension was stored at -20°C.

SDS-PAGE. SDS-PAGE analysis was performed using

8-16% Tris-glycine acrylamide gradient gels (Novex, San Diego, CA). The culture samples were prepared by mixing 25 µl of 2x loading dye with 25 µl of each cell suspension. The solutions were mixed well and heated at 100°C for 5 min. To each lane of the gel, 10 µl of dye-sample mixture was loaded and the electrophoresis performed in SDS-Tris-Glycine buffer at 130 V constant until the dye front reached the bottom of the gel. The protein bands were stained with GelCode Blue™ (Pierce Chemical).

Esterase assays. Enzymatic activity was measured in cell-free extracts or toluenized cells. The extracts were prepared using a lysozyme/freeze-thaw method. A 250 µl aliquot of each cell suspension was incubated with 250 µg lysozyme for 1 hour on ice. After three rounds of freezing in a dry ice/ethanol bath and thawing at 37°C, the extracts were incubated with 10 units DNaseI for 30 min on ice. The extracts were clarified by centrifugation at 12,000 xg for 10 min. and stored at 4°C. The protein concentration was determined using the Bradford dye-binding assay[11]. Toluenized cells were prepared as described[12].

Thioesterase activity was determined as described by Cho and Cronan[13] using a microplate format. To duplicate wells of a 96-well microtiter dish (PolySorp™, Nunc), 10 µl of cell-free extract or tolueninzed cells was mixed in 90 µl 50 mM potassium phosphate buffer pH 7.5. The reaction was started by adding 100 µl 2x reaction cocktail which was composed of 125 mM potassium phosphate buffer pH 7.5, 250 mM deconyl-CoA, 250 mM 5, 5'-dithio-*bis*(2-nitrobenzoic acid). The increase in absorbance at 405 nm over a 10 min. period was monitored using a UVMax microplate reader (Molecular Devices). Reaction rates were calculated from the initial rate curves.

Carboxylesterase activity was determined using *p*-nitrophenyl butyrate according to Stoops et al.[14] using a microplate format. To duplicate wells containing 90 µl of 50 mM potassium phosphate buffer pH 7.5, 10 µl of crude extract was added, mixed well, and 100 µl 100 mM *o*-nitrophenyl butyrate added. The increase in absorbance at 405 nm was monitored spectrophotometrically. The initial reaction rate was calculated from the first 2-4 min of the reaction.

Results

Table 2 lists the typical wet weight yields of JM109 grown in each of the media using shake flask cultures. Each of the four media specially formulated to increase the production of recombinant proteins, Hyper Broth™, Power Broth™, Superior Broth™ and Turbo Broth™, gave higher biomass yields than the reference medium, LB Broth. If the levels of accumulation of recombinant proteins are the same and independent of media composition, then the increase in biomass yields alone will serve to increase the production of a given recombinant protein. Thus, if the medium composition were to increase the relative level of accumulation of a given protein, then a higher yield per liter could be achieved if the most suitable formulation could be identified.

To examine the effect of media composition on the production of each of six recombinant proteins, the relative level of expression was analyzed using the six different media formulations. The respective cultures were grown and expression induced with IPTG as described in the Materials and Methods section. The synthesis of the recombinant proteins was induced at equal cell densities with the cells in exponential growth phase. This eliminated effects caused by differences in expression due to cell growth stage. Nonetheless, the final cell densities were different due to the different growth-rates achieved in each of the media. Recombinant protein accumulation relative to total protein was assessed by SDS-PAGE. Each lane of the gel was loaded with an equal amount of protein. Thus, observed differences in the stain intensity of the recombinant protein reflects a higher or lower level of accumulation relative to total protein. In this way qualitative differences in the specific activity of each protein could be measured.

Figure 1 shows the SDS-PAGE analyses of six recombinant proteins. Each panel shows the result of expressing a different recombinant protein in each of the six media of the screening kit. Panel A is the reference strain which does not harbor a plasmid expressing a recombinant protein. In this strain, differential expression was observed for several proteins, clearly indicating that differences in media formulation affects the expression of host genes. As expected, due to the nature of the protein and differences between the various expression vectors employed, the level of production of each protein was different. In each case variations in the relative level of accumulation were observed between the different media for a given protein. For example, MalE was maximally accumulated when grown in Power Broth™, GST in Hyper Broth™, GFP in Turbo Broth™, $I27_8$ in Superior Broth™, and LypA in Power Broth™. Accumulation of GFP was not observed in glucose M9Y, Hyper Broth™ or Superior Broth™ grown cells. Similarly, LypA was not observed in glucose M9Y grown cells. In contrast, MalE and GST were expressed well in glucose M9Y. In all cases, LB Broth was not the medium yielding the highest level of recombinant protein accumulation. These data show that medium composition has a significant effect on the accumulation of recombinant proteins and, importantly, that the accumulation of various recombinant proteins may be affected differently.

Table 2. Biomass yields (wet weight) in each of the six medium.

Medium	Biomass Yield (g/L)
Glucose M9Y	16
LB Broth	10
Hyper Broth™	36
Power Broth™	24
Superior Broth™	18
Turbo Broth™	30

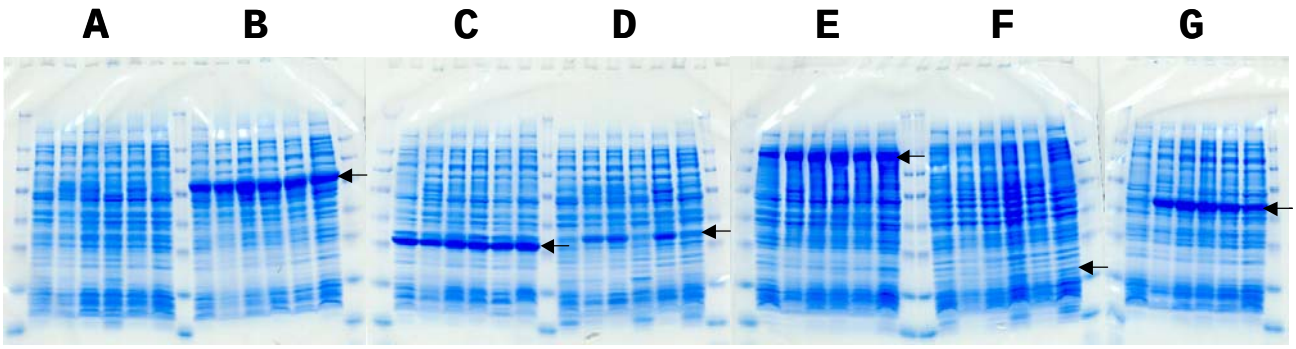

Figure 1. SDS-PAGE analysis of total protein from each strain in Table 1. Samples were prepared as described in the Materials and Methods section. Panel A - reference strain without a recombinant protein; Panel B to G - MalE, GST, GFP, I278, TesA, LypA, respectively. Arrows denote the location of the respective 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™.

TesA polypeptide could not be identified by SDS-PAGE. Because TesA is expressed with its signal sequence, it is likely to be exported to the periplasmic space. This would limit the amount of protein that can accumulate and levels of the protein would not be expected to be dramatically higher than in the wild-type strain. As shown in Figure 2, however, expression of the protein above wild-type levels was observed when a functional assay was used to track protein accumulation. Thioesterase activity was detected in all extracts from cells grown in all of the six media. Accumulation was highest, 2.5-fold above that in LB Broth, in glucose M9Y, Power Broth™ and Turbo Broth™. A similar pattern of expression was observed in toluenized cells (Fig. 2).

LypA was observed to be accumulated as an insoluble product. Microscopic inspection of post-induction cells revealed prominent inclusion bodies. Further, the protein was observed in the insoluble fraction of lysed cells. LypA enzymatic activity was measured in cell-free extracts as a means of determining whether a portion of the enzyme was in the soluble fraction and retained function (LypA has not been demonstrated to have thioesterase activity, therefore, only the carboxylesterase activity was assayed). Significant levels of enzyme activity (55,800 Units/mg compared to non-detectable in control cells) were detected in the extract prepared from cells cultured in Hyper Broth™ (Fig. 3). Some activity, about 6-fold above endogenous levels, was detected in extracts from cells grown in glucose M9Y and Turbo Broth™. However, the specific activity was 1/6 of that found in Hyper Broth™ grown cells. Interestingly, Hyper Broth™ did not yield the highest level of protein accumulation. In fact, it ranked second to last (Fig. 1 Panel G). Glucose M9Y and Turbo Broth™ ranked last and third to last, respectively, for relative LypA accumulation. Importantly, the amount of protein produced in Glucose M9Y grown cells was not readily visible in SDS-PAGE analysis whereas it was for the other two media. These results contradict the general rule that the amount of insoluble product formed by hyper-expressed proteins can, in some cases, be decreased by lowering the level of expression. Rather, it would seem that the composition of the medium not only affects the amount of recombinant protein accumulated, but it also affects the relative fraction that remains soluble.

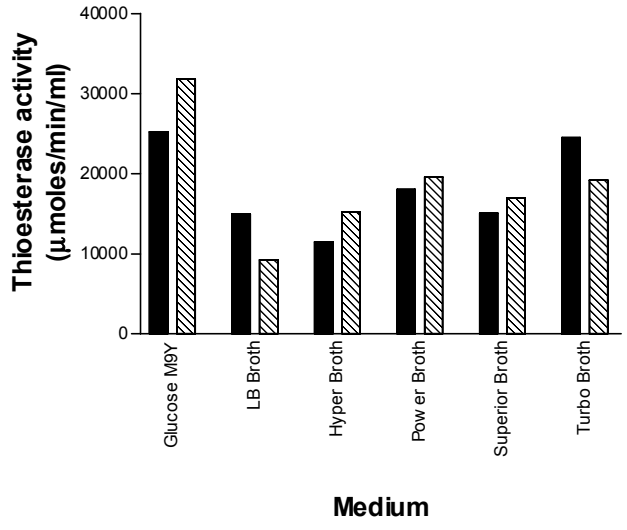

Figure 2. TesA thioesterase activity in crude extracts prepared from cells grown in the six different media. Thioesterase activity was measured as described in the Materials and Methods section. Left bars are the activity found in toluenized cells and the right bars activity found in the soluble fraction of lysed cells.

Discussion

The medium composition-dependent accumulation of recombinant proteins has several implications. As the data clearly demonstrated, each recombinant protein was expressed to different levels in different media suggesting that there is a relationship between each 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

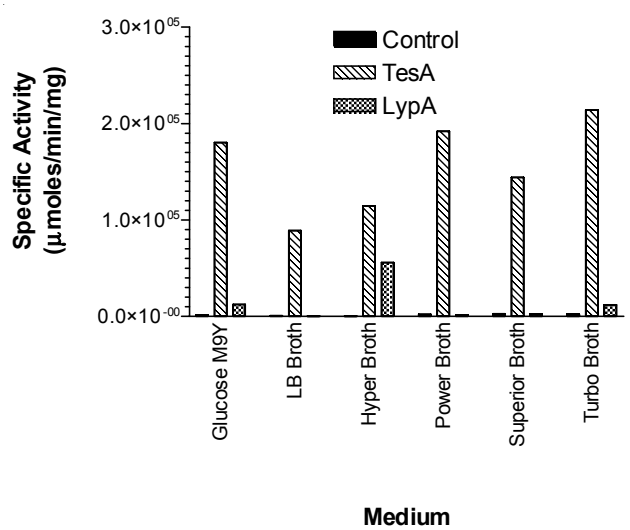

Figure 3. Specific activity of LypA and TesA in cell-free extracts after 3 hours induction with IPTG. Carboxyesterase activity was measured using ρ–nitrophenyl butyerate substrate as described in the Materials and Method section.

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 as shown above, 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[15,16]. 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 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. In fact, the amount of soluble protein could not be predicted by the relative level of LypA accumulation. While this report only presents one example, recent work by others found a similar result for the *Candida albicans* glucosyltransferase where Power Broth™ was found to yield soluble product whereas the other media did not[17]. Other approaches reported to increase the relative accumulation of soluble protein included lowering the temperature during induction[18,19,20], reducing the concentration of inducer[21,22], using mutant strains which affect protein folding[23], fusing the target protein to a bacterial protein[24,25,26], and employing a variety of molecular chaperones[27]. Each of these factors is believed to affect protein folding in one way or another. The subject of recombinant protein folding is reviewed by Baneyx[28]. It now seems that medium compositon 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 must be determined empirically. The described media screen should aid in simplifying this process.

References

1. Guidelines for Research Involving Recombinant DNA Molecules, January 2001, Office of Biotechnology Activities, National Institutes of Health, http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html.

2. Lee, S. Y. 1996. High Cell-density culture of *Escherichia coli*. Tibtech 14:98-105.

3. Kleman, G. L. and W. R. Strohl. 1994. Development in high cell density and high productivity microbial fermentation. Current Opinion in Biotech. 5:180-186.

4. Strobel, R. J. and G. R. Sullivan. 1999. Experimental design for improvement of fermentations. *In* Manual of industrial Microbiology and Biotechnology, A. L. Demain and J. E. Davies, eds., ASM Press, Washington, DC, pp. 80-93.

5. Plackett, R. L., and J. P. Burman. 1946. The design of optimum multifactorial experiments. Biometrika 33:305-325.

6. Haaland, P. d. 1989. Experimental Design in Biotechnology. Marcel Dekker, Inc., New York, NY.

7. Box, G. E. and N. R. Draper. 1987. Empirical Model-Building and Response Surfaces. John Wiley & Sons, Inc., New York, NY.

8. Carrion-Vazquez M., A.F. Oberhauser, S.B. Fowler, P.E. Marszalek, S.E. Broedel, Jr**.** J. Clarke, and J.M. Fernandez. 1999. Mechanical and chemical unfolding of a single protein: a comparison. Proc Natl Acad Sci U S A. 96(7):3694-3699.

9. Cho, H. and J. E. Cronan, Jr. 1993. *Escherichia coli* thioesterase I, molecular cloning and sequencing of the structural gene and identification as a periplasmic enzyme. J. Biol. Chem. 268(13):9238-9245.

10. Whayeb, S. A., K. Yamamoto, H. Tojo, T. Honda. 1996. Genetic analysis of the chromosomal region encoding lysophospholipase L2 of *Vibrio cholerae* O1. Biochim. Biophys. Acta 1300:1-4.

11. Bradford. M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

12. Schleif, R. F. and P. C. Wensink. 1981. Practical Methods in Molecular Biology. Springer-Verlag, New York, NY.

13. Cho, H. and J. E. Cronan, Jr. 1993. *Escherichia coli* thioesterase I, molecular cloning and sequencing of the structural gene and identification as a periplasmic enzyme. J. Biol. Chem. 268(13):9238-9245.

14. Stoops, J.K., D.J. Horgan, M.T. Runnegar, J. De Jersey, E.C Webb, and B. Zerner. 1969. Carboxylesterases (EC 3.1.1). Kinetic studies on carboxylesterases. Biochemistry 8(5):2026-2033.

15. Trilli, A. 1986. Scale-up fermentations. *In* Manual of Industrial Microbiology and Biotechnology, A. L. Demain and N. A. Solomon, eds., ASM Press, Washington, DC, pp. 277-307.

16. Hosobuchi, M. and H. Yoshikawa. 1999. Scale up of microbial processes. *In* Manual of Industrial Microbiology and Biotechnology, A. L. Demain and J. E. Daves, eds., ASM Press, Washington, DC, pp.236-239.

17. McElheny-Feser, G. and R. L. Cihlar, Georgetown University, personal communication.

18. Piatak, M., J. A. Lane, W. Laird, M. J. gjorn, A. Wang, and M. Williams. 1988. Expression of soluble and fully functional ricin A chain in *Escherichia coli*. J. Biol. Chem. 263:4837-4843.

19. Schein, C. H., and M. H. M. Noteborn. 1988. Formation of soluble recombinant proteins in *Escherichia coli* is favored by lower growth temperatures. BioTechnology 6:291-294.

20. Cabilly, S. 1989. Growth at sub-optimal temperatures allows the production of functiona, antigen-binding Fab fragment in *Escherichia coli*. Gene 85:553-557.

21. Takagi, H. Y. Morinaga, M. Tsuchiya, H. Ikemura, and M. Inouye. 1988. Control of folding of protein secreted by high expression secretion vector, pINIIIompA: 16-fold increase in production of active subtilisin E in *Escherichia coli*. BioTechnology 6:948-950.

22. Vasina, J. A. and F. Baneyx. 1997. Expression of aggregation-prone recombinant proteins at low temperature: a comparative study of the *Escherichia coli cspA* and *tac* promoter systems. Protein Expr. Purif. 9:211-218.

23. Miroux, B. and J. E. Walker. 1996. Over-production of protein in *Escherichia coli*: mutant hosts that allow synthesis of some membrane proteins and globular proteins at high levels. J. Mol. Biol. 260:289-298.;

24. Kapust, R. B. and D. S. Waugh. 1999. *Escherichia coli* maltose-binding protein is uncommonly effective at promoting the solubility of polypeptides to which it is fused. Protein Sci. 8:1668-1674.

25. Forrer, P. and R. Jaussi. 1998. High-level expression of soluble heterologous protein in the cytoplasm of *Escherichia coli* by fusion to the bacteriophage lambda head protein D. Gene 224;45-52.

26. Sachdev, D. and J. M. Chirgwin. 1998. Order of fusions between bacterial and mammalian protein can determine solubility in *Escherichia coli*. Biochem. Biophys. Res. Commun. 244:933-937.

27. Baneyx, F. 1999. Recombinant protein expression in *Escherichia coli*. Current Opinion in Biotechnology. 10:411-421.

28. Baneyx. F. 1999. In vivo folding of recombinant proteins in *Escherichia coli*. *In* Manual of Industrial Microbiology and Biotechnology, A. L. Demain and J. E. Daves, eds., ASM Press, Washington, DC, pp. 551-565.

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