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Poster Presentation

Cost-effective production of labeled recombinant proteins in *E. coli* using minimal medium

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Background

Application of NMR spectroscopy in drug discovery often requires large quantities (hundreds mg) of recombinant proteins labeled with stable isotopes (nitrogen-15, deuterium and carbon-13 in various combinations). Expression of recombinant proteins in *E. coli* is traditionally carried out using rich media such as LB due to simplicity, low cost and familiarity. Use of commercial labeled rich medium with the same characteristics as a conventional LB is a very attractive option for small-scale production of labeled proteins. However, larger scale cultivation with such media could become cost-prohibitive. In this study we describe how minimal medium was used for large-scale cost-effective production of labeled recombinant proteins in *E. coli*.

Results

Uniform high-level labeling was achieved by using a labeled substance as a sole source of the corresponding element, i.e. ammonium salts as a nitrogen-15 source and glucose as a carbon-13 source. In the experiments requiring deuteration, deuterium oxide (99.9%) and orthophosphoric acid were used as deuterium sources. The degree of deuterium incorporation in recombinant protein achieved by this method ranges from 84 to 89 per cent (remaining hydrogen atoms are derived from glucose). Use of these labeled substances allowed large savings of medium costs compared to commercially available rich medium. The medium cost savings were 6-fold for triple labeled (^{2}H, ^{15}N, ^{13}C) and 20-fold for ^{15}N-labeled medium. Achieved savings for production of 100 gram of triple-labeled biomass, for instance, can reach tens of thousands EUR.

While rich medium is an ill-defined complex mixture, a minimal medium is composed of defined components, which are consumed at constant specific rates by known metabolic pathways until one of the essential nutrients is exhausted. In labeling experiments the concentration of the most expensive medium component is set to be limiting, thus minimizing the cost. Nitrogen requirement can be easily calculated due to its relatively constant content in biomass (14%) [1]. This value can increase only slightly during protein overexpression due to higher nitrogen content in protein (18%) [2]. Carbon demand (as glucose) cannot be predicted as easily. Although carbon content in

Table 1: Biomass yield coefficient (OD per g/L glucose) observed during production of 3 recombinant proteins in batch culture with minimal medium.

	BL21(DE3) Protein A	BL21(DE3)* Protein B	BL21(DE3) Protein C	BL21(DE3) Protein C
before induction	1.5	1.2	1.3	1.3
after induction	1.5 (0.1mM IPTG)	1.1(0.5mM IPTG)	1.3 (0.05mM IPTG)	0.9 (0.5mM IPTG)

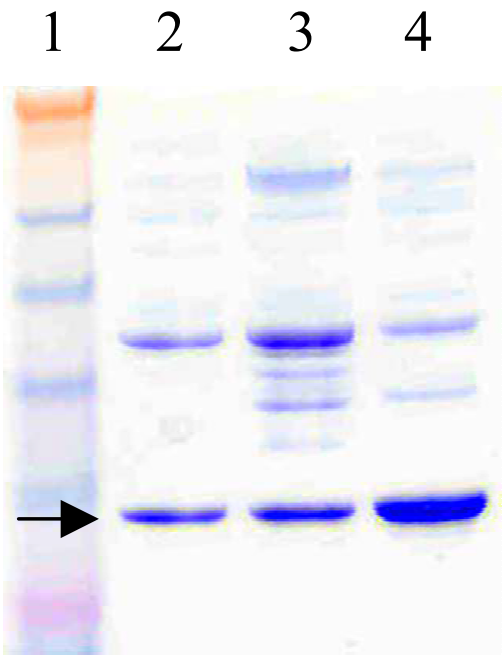

Figure 1
Expression of 27.5 kDa soluble recombinant protein (marked by arrow). Expression is similar for LB and minimal medium for shake flask cultures and the same induction time. Fermenter cultivation allows a higher expression level due to longer induction time. Soluble cell fractions were prepared by chemical/enzymatic lysis. Induction 40 μM IPTG at 20°. Lane 1. SeePlus 2+ MW ladder. Lane 2. Shake flask LB, time after induction 17 h. Lane 3. Shake flask, minimal medium, time after induction 17 h. Lane 4. Fermenter ^{2}H, ^{15}N 5-litre scale fermentation, minimal medium, time after induction 40 h.

biomass is fairly constant at 50%, glucose also serves as an energy source and is degraded into carbon dioxide and other by-products. These energy requirements for biomass biosynthesis, as well as for maintenance, vary depending on the plasmid, host strain and induction conditions (Table 1).

The culture growing on minimal medium with glucose in shake flask is much less prone to oxygen limitation as opposed to rich medium due to a greatly reduced specific growth rate (1.5 vs. 0.5 1/h) [3]. This makes a shake flask culture more suitable for small-scale experiments with a good potential for scale-up if needed.

Cells growing on minimal medium in batch culture metabolize glucose with a constant stoichiometry. Therefore, biomass growth is directly proportional to carbon dioxide evolution and oxygen and alkali consumption. These parameters can be used for indirect estimation of biomass in bioreactor and for triggering pre-programmed events such as induction and temperature changes. Unattended operation reduces a need for overtime and to increase reproducibility of the process.

Conclusion

Use of minimal medium significantly reduced the costs of labeled protein production. Defined physiological conditions, better process control and monitoring of nutrients enabled to achieve higher expression yields in fermenter compared to flasks with LB medium (Figure 1).

References

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