Effect of culture medium on recombinant Fab antibody fragment production

ENPRESSO

Application Note

Summary of published results [1], by Katharina Lehmann

Introduction

Fragments of immunoglobin molecules, such as Fab (Fragment antigen binding) are commonly used in therapy and diagnosis. Even though these fragments are small enough to be produced in E. coli, expression yields of functional bacterial antibody fragments are relatively low. Fab fragments are usually directed to the oxidizing periplasmic space for correct folding and leak into extracellular medium to be purified. To enhance correct folding and leakage into the culture medium, different modifications of cultivation conditions have been investigated [2].

In this application note the effects of host strain, culture medium and aeration conditions on the production and extracellular leakage of Fab fragments in shaken E. coli cultures are investigated, exemplary with Fabs binding NTproBNP, an important diagnostic marker of heart failure [3].

Cultivation and analysis

Three different media, EnBase medium, Super Broth medium and ZYM-5052 autoinduction medium were compared. Expression and accumulation were investigated into either medium or periplasm with two different E. coli strains for each media.
By using the EnBase system with enzyme-based glucose release from soluble polysaccharide, fed-batch-like conditions were provided. The EnBase medium consists of mineral salts, $MgSO_4$, thiamine, trace elements solution, soluble polysaccharide substrate, a low amount of complex nutrients and is supplemented with 1 g/l glucose. Cultures in shake flasks were supplied with 0.6 U l-1 of the glucose-releasing biocatalyst (solution A) before inoculation.
For deep well plate (24dwp) cultivation and flask-scale expression, fed-batch media were grown overnight, followed by induction with 0.2 mM IPTG inducer and simultaneous addition of EnBase Booster concentrate and the biocatalyst (3 U l-1).
In an additional experiment, slightly modified cultivations were performed with online recording of dissolved oxygen tension (DOT) in 5-minute intervals. Cell density was determined by measuring optical density at 600 nm (OD_{600}). The quantity of functional Fab in the cell lysates and broth supernatants were determined to define Fab yields.

Table 1 Cell density, pH and percentage of extracellular Fab in different media in 24dwp

		% of Fab in medium	OD_{600}			pH	
			19 h	24 h	42 h	19 h	42 h
RV308	EnBase	65.3 ± 2.6		21.7 ± 1.9	26.9 ± 2.7		7.07 ± 0.01
	ZYM-5052	6.8 ± 1.6	12.4 ± 3.7		14.1 ± 0.8		6.90 ± 0.02
	Super Broth	96.1 ± 2.4	13.8 ± 0.5			8.31 ± 0.18	
BL21(DE3)	EnBase	69.6 ± 2.6		19.8 ± 1.8	21.7 ± 6.5		6.70 ± 0.06
	ZYM-5052	39.8 ± 15.2	16.8 ± 5.1		17.5 ± 7.5		7.15 ± 0.02
						8.5 ± 0.04	

Table 1 Ukkonen et al., 2013

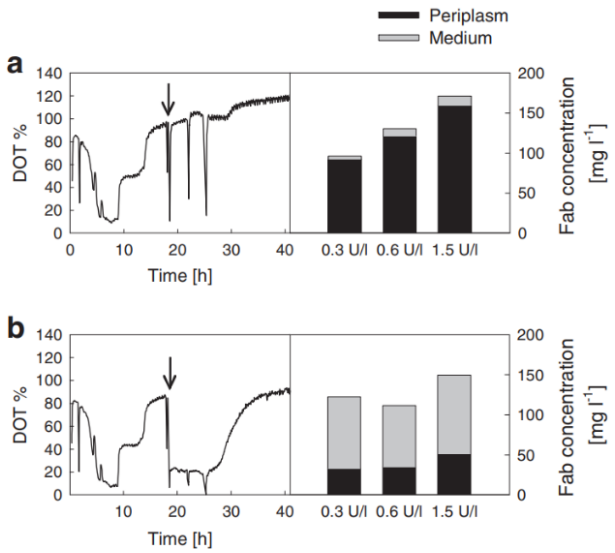

Figure 1 Ukkonen et al., 2013

Results

Expression in a medium with fed-batch-like glucose feeding provided highest total and extracellular yields caused by higher cell density (OD of F1 shown in Table 1) compared to ZYM. Compared to autoinduction and fed-based media, in SB media total Fab yields decreased and pH rose to unfavourable conditions (8.0 - 8.5). A reduced oxygen supply and dissolved oxygen tension may cause an increase in Fab leakage due to changes in growth rate, influencing the cell membrane [4] Dissolved oxygen tension (DOT) measurements indicated the increased oxygen consumption by addition of complex nutrients and biocatalyst at induction, leading to increased glucose release, associated with higher Fab activity in the extracellular medium (66-73% of total Fab activity). In the fed-batch medium the ratio of periplasmic and extracellular Fab can therefore be drastically changed by modifying the availability of carbon and nitrogen substrates after induction.
Cell densities demonstrated that leakage in the EnBase fed-batch medium begins several hours before significant lysis, enabling better harvest of extracellular Fab by optimizing the harvest time.
Unexpectedly, flask cultivation at 150 rpm shaking speed resulted in higher yield and accumulation of Fabs into culture medium as compared to cultivation at 250 rpm, with an increase of extracellular fraction from about 2-17% up to 75% of total in fed-batch mode.

Conclusions

Most favourable conditions for Fab production, such as high overall yield, efficient transport to extracellular medium and strain type robustness could be shown in the EnBase fed-batch medium. A maximised productivity of Fab proteins in fed-batch-like conditions and in autoinduction medium is achieved under sufficiently oxygen-limited conditions highlighting the importance of maintaining consistent aeration conditions during scale-up to avoid changes in yield or localization. The findings of cultivation improvements may have practical implications for screening applications and small-scale production.

References

[1] Content summarized from: Ukkonen K, Veijola J, Vasala A, Neubauer P: Effect of culture medium, host strain and oxygen transfer on recombinant Fab antibody fragment yield and leakage to medium in shaken *E. coli* cultures. Microbial Cell Factories 2013, 12:73. http://www.microbialcellfactories.com/content/12/1/73

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