Leveraging media and supplements for desired protein glycosylation

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INTRODUCTION

Obtaining desirable and consistent monoclonal antibody (mAb) protein quality attributes, such as glycosylation, is critical in today's bioproduction industry, especially for biosimilar molecules. The cell line, cell culture media, and process all contribute to the glycosylation profile. While modifications to the cell line and process can require extensive time and effort, optimization and supplementation of cell culture media offers a rapid option for achieving the desired glycosylation profile. Here, we highlight a case where we use high throughput screening (HTS) to evaluate mAb production and glycosylation for a Chinese hamster ovary (CHO) cell line in a chemically defined (CD) media panel. Selection of optimal media required considerations of both production and galactosylation. Further, mathematical modeling approaches were used for the identification of media components, which correlate to galactosylation and production, thereby giving a more in-depth understanding of the role that media components can play on these critical attributes. Additionally, we also highlight development of titratable protein quality media supplements for increasing mAb galactosylation, without impacting cell growth and mAb production. Such supplements provide a flexible solution to increasing protein galactosylation, where through the modulation of media, both desired galactosylation and production could be achieved.

MATERIALS AND METHODS

Cell culture was performed in 24-well shaking deep-well plates (Enzyscreen) and in 125 mL shake flasks for a CHO-K1 line and a CHO–DHFR line. Batch and fed batch studies were performed in the ambr™ 15 micro bioreactor system in 15 mL culture volume. Cell growth was determined using a Vi-Cell™ XR. mAb production was determined using an Octet™ QKe system. For N-glycan analysis, mAb purification was performed using Protein A Cartridges on the AssayMAP™ Bravo™ Platform. N-glycans were 2-AB labeled and analyzed using fluorescence detection on Waters UPLC™ system. Data analysis was done using Minitab™ software.

RESULTS

CD media screen and mAb glycosylation: understanding the impact of media components through mathematical modeling

CD media were screened for both production and N-glycan profiles of a mAb produced by a CHO-DHFR cell line. Figure 1A shows total % galactosylated species and production for different CD media conditions from the screening study.

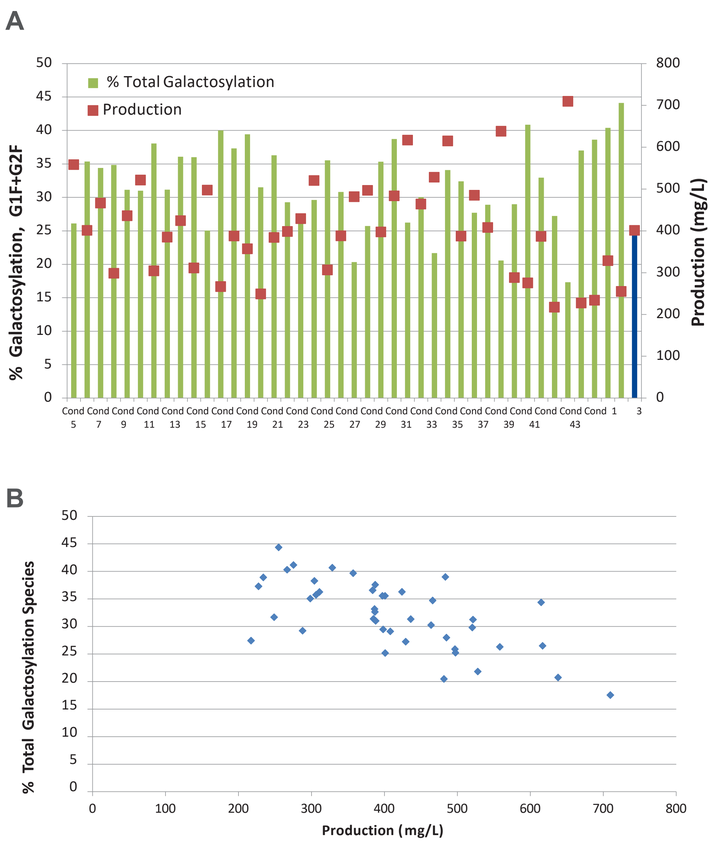

Figure 1: CD media screen for a CHO-DHFR line
(A) Percent galactosylation and mAb production for a 42 CD media library for a CHO-DHFR cell line. Different media conditions result in different production and % total galactosylation (sum of galactosylated species). The blue bar indicates the reference control media. (B) Negative correlation between total % galactosylation and production. The data highlights that it is critical to look at the impact of the formulations on cell growth, production, and protein quality early during media development itself for optimum medium selection.

To further evaluate the impact of media composition, a partial least squares (PLS) based model was fitted to screening data for identification of key components in the CD media formulations that have strong correlation to % galactosylation (Figure 2). PLS modeling also enabled the identification and classification of media components in the CD media library that have positive impacts on both % galactosylation and production and are potential component candidates for further optimization.

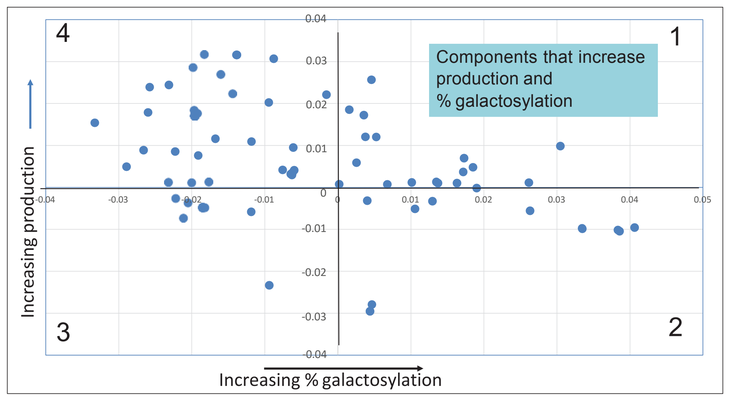

Figure 2: Impact of media components on galactosylation and production
PLS model score plots showing impact of various media components on % galactosylation and production. The 1st quadrant has media components which increase both production and % galactosylation. The 2nd quadrant has components which decrease production but increase % galactosylation. The 3rd quadrant has components which decrease both production and % galactosylation. The 4th quadrant has components which increase production and decrease % galactosylation.

Development of protein quality supplements for enhancing galactosylation

Different candidate protein quality supplements were evaluated over different CD media for a CHO-DHFR line and a CHO-K1 line in a shake flask format. Figure 3 shows the heat maps for supplement candidate evaluations. Selection of lead candidates was done using the overall score which included viable cell density (VCD), production, and galactosylation.

CHO-DHFR Cell Line				
Supplement	G1F+G2F	Average VCD	Production	Overall Score
13	46.49	6.52	424.38	3.09
2	46.57	6.62	405.38	3.01
9	45.84	6.78	405.60	2.99
12	45.61	6.76	401.68	2.92
16	45.67	6.24	431.88	2.91
7	46.02	6.41	399.05	2.80
10	45.04	6.42	408.88	2.75
1	46.09	6.27	380.63	2.62
5	45.96	6.12	390.90	2.61
4	46.17	6.04	382.18	2.54
6	46.05	5.94	389.25	2.53
3	45.6	5.75	399.35	2.47
11	31.1	7.48	392.45	1.47
14	31.84	6.44	427.45	1.44
8	29.19	6.67	390.53	1.15
17	26.4	7.04	407.83	1.03
15	26.74	6.76	398.28	1.00

CHO-K1 Cell Line				
Supplement	G1F+G2F	Average VCD	Production	Overall Score
2	49.01	7.01	76.63	1.81
9	47.38	7.18	77.45	1.75
13	49.07	7.68	66.83	1.73
1	46.24	6.71	76.48	1.54
5	48.97	6.74	67.35	1.53
7	47.39	6.76	70.08	1.49
4	47.65	7.05	66.18	1.48
14	46.96	7.43	64.20	1.47
3	46.39	7.28	67.08	1.47
11	46.46	6.91	69.85	1.46
8	45.48	7.13	69.78	1.44
10	41.5	7.76	75.45	1.41
6	47.29	6.68	65.88	1.38
16	40.6	7.26	68.70	1.15
12	41.76	6.88	68.18	1.15
15	39.09	7.03	67.10	1.01
17	37.47	7.49	67.88	1.00

Figure 3: Heat map for selection of protein quality supplement candidates
Considerations of VCD, production, and overall shift in galactosylation for the two cell lines evaluated (as judged by the overall score) led to the successful selection of supplements which can modulate protein galactosylation while maintaining cell growth and production.

The lead candidates with distinct profiles were developed into protein quality supplements which, when added to an existing base medium, can produce an increase in galactosylation without impacting mAb production and cell growth. These supplements can be titrated in order to achieve the desired galactosylation profile, as shown in Figure 4.

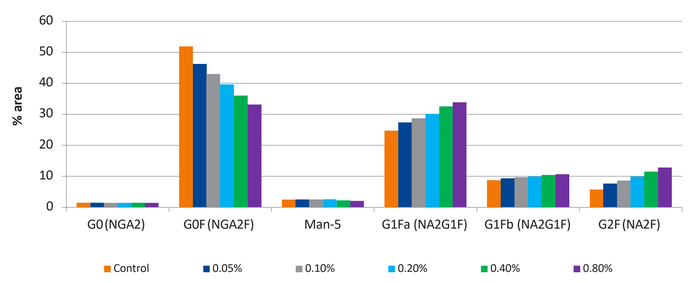

Figure 4: Modification of media through protein quality supplement titration produces changes in N-glycan species
A protein quality supplement that increases galactosylation was added at different concentrations on day 0 to a commercially available CD medium for a CHO-K1 cell line and evaluated in shake flasks. The titration resulted in higher galactosylation as seen by the increasing G1F and G2F. Growth and production were not impacted (data not shown).

Evaluation of these protein quality supplements in an existing CHO-K1 batch process, in shake flask and ambr 15 (Figure 5), resulted in a comparable increase in galactosylation without impacting cell growth and mAb production. This demonstrates that the effect of these supplements can be scaled to bioreactor conditions, with minimal need for changing the process.

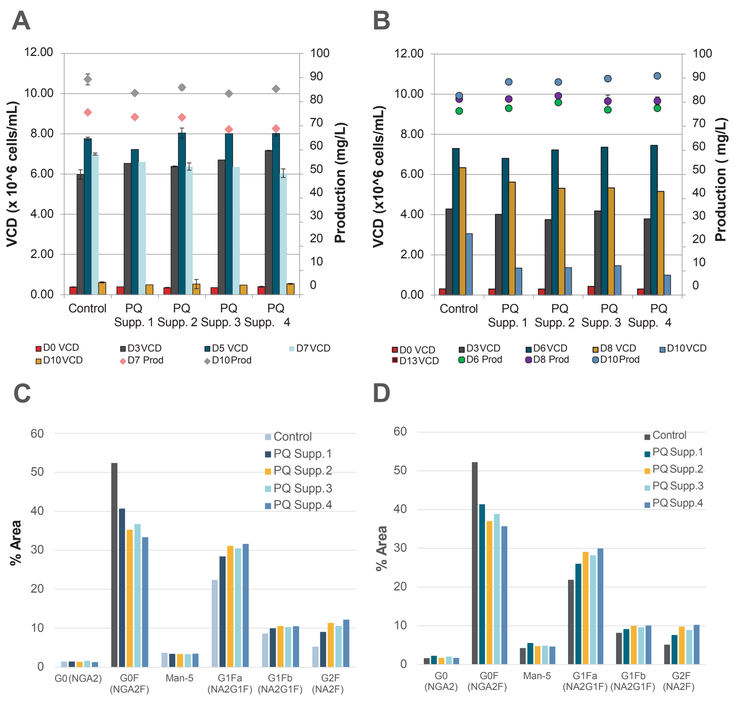

Figure 5: Shake flask and ambr 15 evaluation of protein quality supplements: VCD, production, and N-glycan profile
Four protein quality supplements, which increase galactosylation, were added to commercially available CD media for a CHO-K1 batch process evaluated in (A) shake flasks and in (B) ambr 15. No impact was seen on growth and production when compared to the control during the scale transfer. (C) The N-glycan profiles for shake flasks and (D) ambr 15. As seen from increasing G1F and G2F in both shake flask and ambr 15, these protein quality supplements increased galactosylation to a similar degree, demonstrating the scalability of these supplements.

CONCLUSIONS

It is critical to understand the impact of supplements and media components on both mAb production and key product quality attributes, such as glycosylation, during media development for selection of optimal media. Through the use of mathematical modeling, we are able to identify specific media components that modulate both production and N-glycan profile and can be leveraged for further optimization.

Additionally, protein quality supplements were developed that increase mAb protein galactosylation without impacting cell growth and production. The effects of these supplements were scalable to an ambr 15 micro bioreactor, which can mimic industrial bioreactors. These results demonstrate that supplementation of cell culture media enables modification of the glycosylation profile without the need to reengineer the cell line or change the production process.

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