Development of a chemically defined medium for *Escherichia coli*, Gibco™ Bacto™ CD Supreme Fermentation Production Medium (FPM) leveraging learnings from traditional peptone-based media

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Bioprocessing

INTRODUCTION

With the advent of new treatment modalities, such as conjugated monoclonal antibodies (mAbs) and DNA vaccines for cancer therapeutics, there has been a renewed interest in production platforms using *Escherichia Coli* (*E. coli*), which is an established workhorse for microbial-based recombinant bioproduction technology. Traditional media for *E. Coli* are often complex media consisting of yeast extract and peptones (protein hydrolysates derived from animal sources, such as casein, or plant proteins) and provide many benefits to growth and production. However, given the needs of the biopharmaceutical industry for driving consistency and the preference for animal origin-free components, a chemically defined (CD) medium alternative is desirable.

Here, we describe an approach for *E. Coli* CD media development that leverages understanding of the nutritive functionality of traditional peptone-based media for guidance in selection of targeted media components. The combination of this knowledge with statistical media design approaches resulted in the rapid development of CD medium; Gibco™ Bacto™ CD Supreme Fermentation Production Medium (FPM) that achieved higher density with minimal lag phase and supported enhanced protein and plasmid production.

MATERIALS AND METHODS

Bacterial culture was performed in 125 mL baffled shake flasks for *Escherichia coli* ATCC 33849™, ATCC 25922™ and One Shot™ BL21 (DE3). Bacterial growth was determined using Thermo Scientific™ SPECTRONIC™ 200, 20E spectrophotometer, and using Roche Cedex Bio Analyzer. GFP extraction was done using B-PER™ Complete Extraction Reagent with Halt™ Protease Inhibitor Cocktail. Fluorescence was measured on the VICTOR™ plate reader from PerkinElmer. Statistical designs were done in Minitab™ software. Plasmid quantification was done using GeneJET™ Plasmid Miniprep Kit and Genesys™ spectrophotometer. Bioreactor studies were done in Eppendorf BioFlo™ 320 in batch mode.

RESULTS

Traditional peptone media: a learning opportunity

Peptones, which are hydrolysates of plant or animal origin, are complex nutritional supplements (Figure 1) known to enhance various biopharmaceutical production processes. Unraveling the mystery of peptones through their analytical characterization and understanding of various components can guide in successful CD media design.

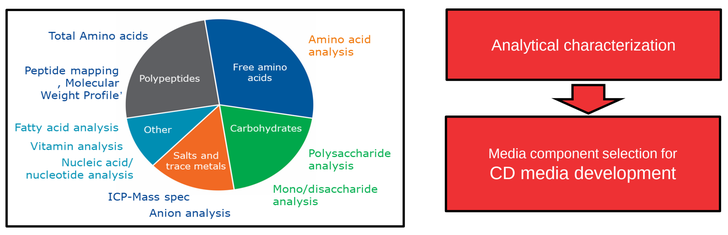

Figure 1: Typical composition of a peptone
Peptones provide numerous nutritional factors such as amino acids, peptides, sugars, growth factors, vitamins etc. that can enhance biopharmaceutical production. Characterization and quantification of these components and mimicking their functionality can provide media development opportunities.

Initial development and CD media screening

CD media candidates were screened for growth of a DH1 *E. Coli* strain and were found to be insufficient as compared to peptone media (Figure 2A). The lead candidate was further modified for buffering capacity and an additional group of components (Figure 2B) were tested which resulted in growth improvement. The lead candidates were chosen for further optimization and development (Figure 2C).

Figure 2: Initial CD media screen for *E. Coli* DH1
(A) *E. Coli* ATCC 33849 was screened for growth in initial CD media candidates with glucose in shake flasks. Media 5 was chosen for further enhancement. (B) The heat map table shows different groups of components evaluated at different concentrations in media 5. Green depicts a higher concentration and red a lower concentration. (C) The growth in improved media candidates with glycerol. The red arrows indicate the media candidates selected for further development. OD 600 = optical density at 600 nm, Hrs = hours.

Optimization of the CD media, using components found in peptones

The initial iteration of the CD media showed extended lag phase when compared to the peptone containing control medium. Various components typically found in peptone containing media (Figure 3A) were evaluated as CD additives for improvement in lag phase (Figure 3B). Group 4 components resulted in faster growth and the combination of group 4 and 5 resulted in both faster and enhanced growth, which was comparable to peptone containing media (Figure 3C). This developmental CD medium was further optimized and evaluated across different *E. Coli* strains (Figure 4) and the growth was found to be comparable to peptone containing media.

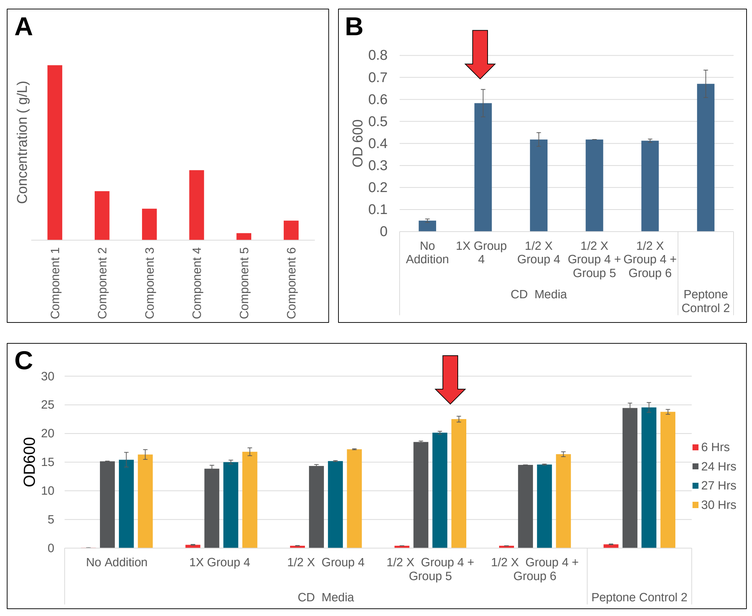

Figure 3: Evaluation of components found in peptones
Removal of lag phase (A) Various components which are contributed by a peptone in peptone containing media for *E. Coli*. (B) *E. Coli* ATCC 33849 was screened for growth with these components, as CD additives over the CD medium candidate. Growth shown at 6h post inoculation. Group 4 resulted in removal of lag phase and rapid growth. (C) Growth enhancement when group 4 and group 5 were added to CD media.

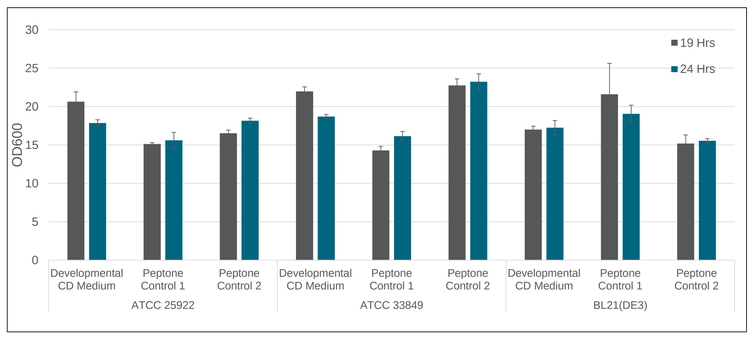

Figure 4: Evaluation of different strains in the developmental CD medium
The strains were evaluated in shake flask using glycerol as a carbon source. The growth achieved was similar to peptone based media controls across different strains.

Evaluation for protein production and plasmid production, small scale bioreactor scale-up

The developmental CD medium was further evaluated for production of recombinant GFP in One Shot BL21 (DE3) strain in a shake flask system. Enhanced protein production was achieved in CD medium when compared to peptone control (Figure 5). Additionally, plasmid producing DH-1 strain was evaluated in a shake flask and 3L bioreactor where good plasmid production was obtained demonstrating the scalability of the CD medium (Figure 6).

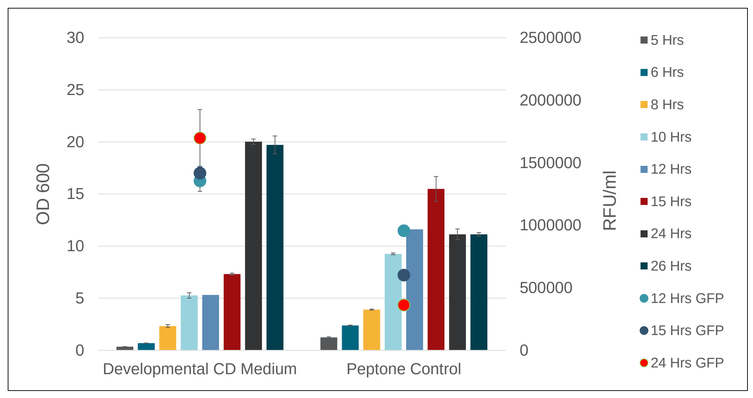

Figure 5: Evaluation of GFP production in developmental CD medium
BL21 (DE3) strain was evaluated in shake flask with glycerol as carbon source. The cultures were induced with 0.2mM IPTG and cell pellets were analyzed for GFP expression post induction. The growth for the strain was better when compared to peptone media and protein production was found to be 77% more in the CD medium.

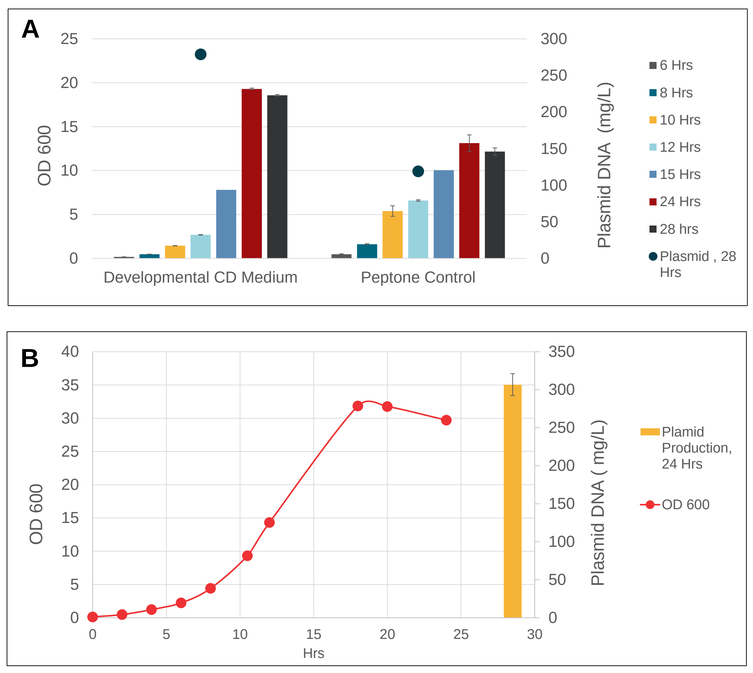

Figure 6: Evaluation of developmental CD medium for plasmid production.
DH1 strain bearing pUC19 plasmid was evaluated in shake flask (A). Good plasmid production was obtained when compared to peptone control, demonstrating suitability of the media for plasmid production. (B) The media was also evaluated in 3L bioreactor in batch mode with glucose as carbon source for plasmid production. Good growth and plasmid production was observed in the lab scale bioreactor, demonstrating the scalability of the media.

CONCLUSIONS

A CD *E. Coli* fermentation medium was developed using screening designs and leveraging our understanding of peptone containing media. The CD medium exhibits growth performance comparable to peptone containing media and demonstrates versatility (performance across various strains), enhanced protein production, plasmid production and scalability. Additionally, it has the advantages of consistency and low risk by virtue of its CD, animal origin-free nature.

These results demonstrate that such comprehensive media development approaches, where traditional media can be used as a learning tool for guiding new media development, can lead to robust media design for biopharmaceutical production needs. This approach enabled successful development of fully chemically defined media for E coli; Gibco™ Bacto™ CD Supreme Fermentation Production Medium (FPM)

TRADEMARKS/LICENSING