Bacto CD Supreme FPM consistently supports higher growth and plasmid production in *E. coli*

Introduction

Microbial cultures used in cloning, plasmid DNA preparation, and protein expression applications require specific and essential growth components. Microbial media have been available for many years, with the first Luria-Bertani (LB) broth formulation developed in the early 1950s [1]. With the development of new treatment modalities, such as conjugated monoclonal antibodies (mAbs) or mRNA and DNA vaccines, there is renewed interest in bioproduction platforms using *Escherichia coli* (*E. coli*), an established model for microbial recombinant bioproduction technology.

Traditional microbial media for *E. coli* often contain animal-origin components and are complex, consisting of yeast extracts and peptones to provide nutrients for growth and production. Overall, the biopharmaceutical industry has been driving toward achieving and supporting greater consistency in plasmid production for scale-up by developing animal origin–free (AOF) and chemically defined (CD) media. Additionally, it is desirable for manufacturers to have the flexibility to use autoclaving, steam-in-place (SIP), or membrane filtration procedures during media preparation.

The development of Gibco™ Bacto™ CD Supreme Fermentation Production Medium (FPM) supports process flexibility and consistency in production. Bacto CD Supreme FPM was evaluated for plasmid production with Thermo Scientific™ DH5α™ *E. coli,* and compared to Terrific Broth (TB), a traditional microbial medium. Additionally, consistency in growth and production was evaluated for scale-up from a shake flask to a benchtop stirred-tank bioreactor applying a batch or fed-batch process.

Materials and methods

Bacterial culture

Competent DH5α *E. coli* cells (Cat. No. EC0112), transformed with Thermo Scientific™ pUC19 plasmid (Cat. No. SD0061), were plated and incubated at 35ºC overnight (12–18 hours). Colonies were suspended in a saline solution to create a culture stock with an OD_{600} of 0.09 (Multiple colonies were suspended together in a saline solution in one tube to create a culture stock 10^8 CFU/mL).

Media

Bacto CD Supreme FPM (Cat. No. A4973701) and TB from an external vendor were assessed. Bacto CD Supreme FPM was prepared per label instructions and was sterilized by either using a 0.2 µm membrane filter or autoclaving at 121ºC for 15 minutes. TB was also prepared per label instructions and sterilized by autoclaving at 121ºC for 15 minutes. Shake flask conditions included Bacto CD Supreme FPM and TB supplemented with 10 mL/L glycerol, 5 g/L glucose, and 100 mg/L ampicillin (sodium salt). For the bioreactor batch and fed-batch studies, the medium was supplemented with glucose and ampicillin (sodium salt) to final concentrations of 20 g/L and 100 mg/L, respectively.

Shake flask (batch culture)

Using the prepared saline stock, cultures were seeded at 10^6 CFU/mL in 125 mL baffled shake flasks with a 30 mL working volume. The cultures were incubated at 35ºC on an orbital shaker set at 300 rpm. Bacterial growth was measured at 6, 24, and 28 hours. At 24 hours, a sample from each culture was processed for plasmid production.

Bioreactor (batch culture)

The BioFlo™ 320 Bioprocess Control Station, 3 L (Eppendorf), with a working volume of 2 L of medium was seeded to an OD_{600} of 0.25 from shake flasks. Cultures were maintained for 28 hours at 35ºC and 30% DO with air sparging at 0.3–4.0 sLPM and agitation at 200–1,200 rpm. pH was maintained at 7.0 ± 0.1 using 6 N ammonium hydroxide and 6 N phosphoric acid. An antifoam agent was added as needed. Cultures were harvested at 28 hours.

Bioreactor (simple fed-batch culture)

BioFlo 320 Bioprocess Control Stations with a working volume of 2 L of medium were seeded to an OD_{600} of 0.1 from shake flasks. Cultures were maintained at 35ºC and 30% DO with air sparging at 0.3–4.0 sLPM and agitation at 200–1,200 rpm. pH was maintained at 7.0 ± 0.1 using 6 N ammonium hydroxide and 6 N phosphoric acid. An antifoam agent was added as needed. Glucose levels were maintained between 5 and 10 g/L by adjusting the feeding rate to between 0.3 and 0.9 mL/min. The feed formulation was 400 g/L glucose, 4 g/L $MgSO_4$, and 220 mg/L thiamine HCl. Cultures were harvested at 34 hours.

Growth and plasmid production

Growth was measured using a Thermo Scientific™ SPECTRONIC™ 20E Spectrophotometer or a Cedex™ Bio Analyzer (Roche). Cultures were sampled for plasmid production at 24 hours for the shake flask (batch culture) and bioreactor (batch culture), and at 34 hours for the bioreactor (simple-fed batch culture). DNA was purified and quantitated using the Thermo Scientific™ GeneJET™ Plasmid Miniprep Kit (Cat. No. K0502) and Thermo Scientific™ GENESYS™ Spectrophotometer. Plasmid quality was assessed on an Invitrogen™ E-Gel™ Agarose Gel with SYBR™ Safe DNA Gel Stain, 2% (Cat. No. A45205) using the Invitrogen™ E-Gel™ Power Snap Electrophoresis System (Cat. No. G8300). The Invitrogen™ E-Gel™ 1 Kb Plus Express DNA Ladder (Cat. No. 10488091) and a pUC19 control linearized with Thermo Scientific™ FastDigest™ Hind III (Cat. No. FD0505) were also run on the gel for comparison.

Results

Depending on the lot of TB, Bacto CD Supreme FPM demonstrated comparable or significantly higher growth and plasmid production in the shake flasks (Figure 1). At 24 hours, Bacto CD Supreme FPM consistently achieved an OD_{600} above 20, while only one lot of the TB reached an OD_{600} above 20. The OD_{600} measurements for the remaining lots were below 15.

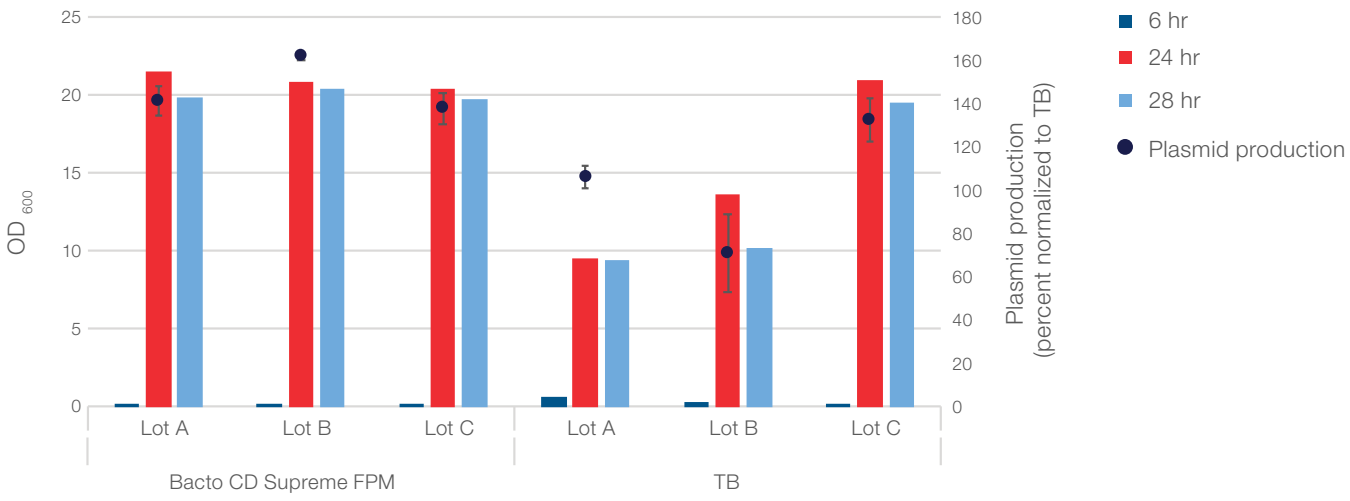

Figure 1. Consistency of growth and plasmid production in shake flasks across multiple lots of Bacto CD Supreme FPM. DH5α *E. coli* cells were cultured in shake flasks using three lots each of prepared Bacto CD Supreme FPM (filtered) and TB (autoclaved). Growth was measured at 6, 24, and 28 hours. Plasmid production was measured at 24 hours. On average, Bacto CD Supreme FPM outperformed TB by a 1.4-fold increase in growth and plasmid production.

Scale-up to a 3 L benchtop stirred-tank bioreactor with a 2 L working volume (batch process) demonstrated consistent growth performance with Bacto CD Supreme FPM, independent of the selected sterilization method (Figure 2A). Additionally, results showed the medium achieving higher growth (Figure 2A) and on average a 1.4-fold increase in plasmid production (Figure 2B) compared to the TB.

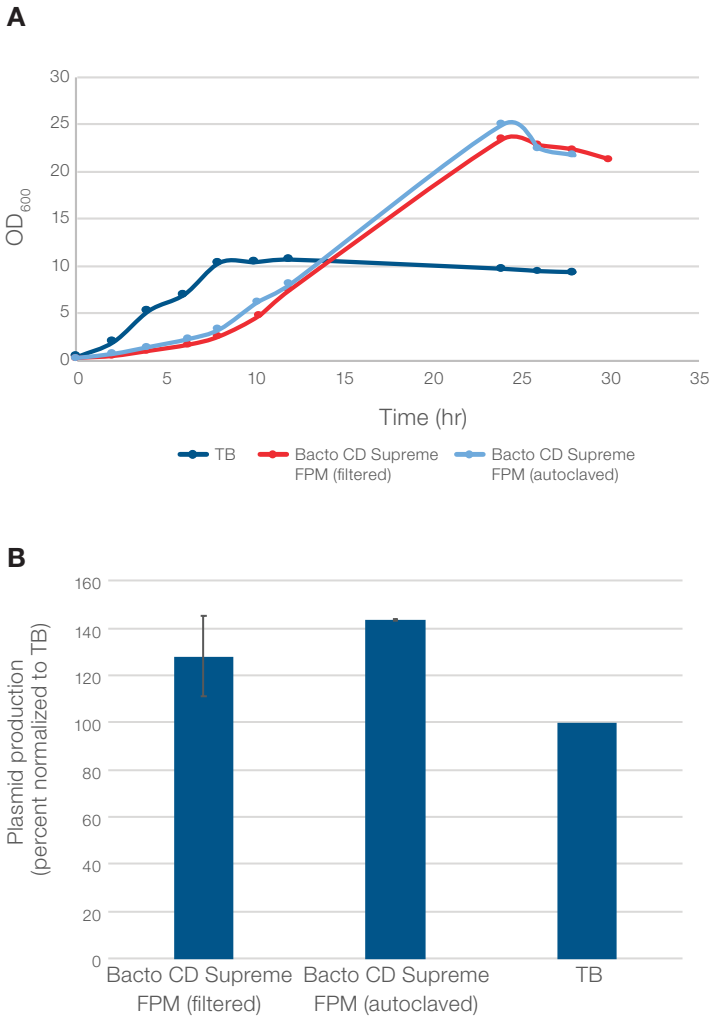

Figure 2. Improved growth and plasmid production with Bacto CD Supreme FPM compared to TB. (A) DH5α *E. coli* growth (OD_{600}) was measured in a 30-hour time course. Measurements at 24 hours revealed that Bacto CD Supreme FPM achieved a 2.40-2.60-fold increase in growth over TB when filtered or autoclaved, respectively. **(B)** DH5α *E. coli* plasmid production was calculated at 24 hours and was normalized to TB. Bacto CD Supreme FPM had a minimum of a 1.3-fold increase over TB.

Bacto CD Supreme FPM was designed to support high cell density. To evaluate this feature, a simple fed-batch process was performed in duplicate using a 3 L benchtop stirred-tank bioreactor (Figure 3). By incorporating the fed-batch process, the cultures achieved high cell densities up to an OD_{600} range of 90–95. For batch culture, an OD_{600} of ~23 was attained (Figure 3A). The fed-batch process also supported plasmid production, with a 3.5-fold increase over the batch process (Figure 3B).

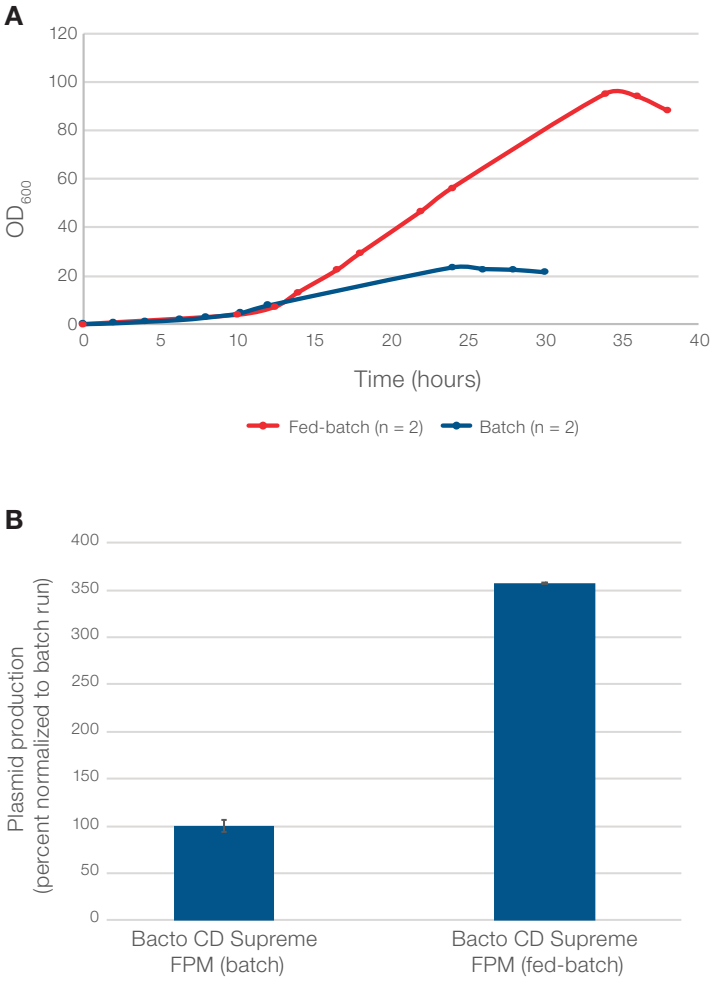

Figure 3. Bacto CD Supreme FPM supports scaling by increasing growth and plasmid production in fed-batch cultures. (A) DH5α *E. coli* growth curve (OD_{600}) using Bacto CD Supreme FPM for batch and fed-batch processes. **(B)** DH5α *E. coli* plasmid production was measured at 24 and 34 hours for the batch and fed-batch cultures, respectively, and reported as normalized to the batch culture.

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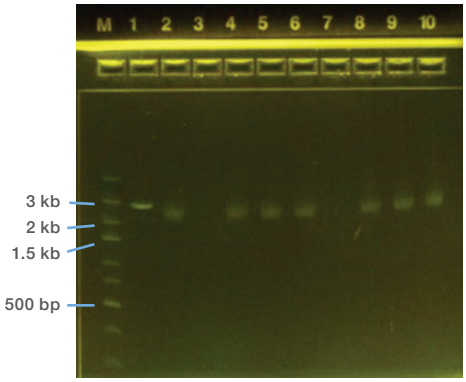
To verify plasmid quality from the various testing conditions used with Bacto CD Supreme FPM, plasmid preps were run on a 2% agarose gel along with linearized pUC19 for comparison (Figure 4).



M E-Gel 1 Kb Plus Express DNA Ladder
1 pUC19, FastDigest HindIII
2 pUC19 (undigested)
3 Empty
4 Shake flask, Bacto CD Supreme FPM—lot A (filtered)
5 Shake flask, Bacto CD Supreme FPM—lot B (filtered)
6 Shake flask, Bacto CD Supreme FPM—lot C (filtered)
7 Empty
8 Batch bioreactor, Bacto CD Supreme FPM (autoclaved)
9 Batch bioreactor, Bacto CD Supreme FPM (filtered)
10 Fed-batch bioreactor, Bacto CD Supreme FPM (filtered)

Figure 4. High-quality plasmid production in Bacto CD Supreme FPM. The quality of plasmid pUC19 (2,686 bp) produced using Bacto CD Supreme FPM in a shake flask or benchtop stirred-tank bioreactor (batch or fed-batch process) was compared to a commercially available control (pUC19). pUC19 was linearized with FastDigest Hind III (lane 1) for comparison to the supercoiled form expected from plasmid production. High-quality (supercoiled) plasmid was, produced from three lots of Bacto CD Supreme FPM in shake flasks, (lanes 4–6). Additionally, plasmid quality was assessed for cultures grown using different process methods (batch or fed-batch) media and sterilization methods (autoclaving or membrane filtration) (lanes 8–10).

Conclusions

Bacto CD Supreme FPM is chemically defined and provides consistent product yield and quality. The data presented demonstrate Bacto CD Supreme FPM to be a robust *E. coli* fermentation medium to support high growth and production. This medium also provides manufacturers and end users options for their sterilization process. Bacto CD Supreme FPM performed equivalently with membrane filtration and autoclave sterilization methods. While maintaining quality, filtration sterilization can help reduce turnaround time and overall energy costs, resulting in optimal equipment usage and increased facility productivity. Furthermore, in the fed-batch study, achieving high cell density while maintaining plasmid quality allowed for scale-up to support manufacturing plasmid quality and production demands.

Reference

1. Bertani G (1951) Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J Bacteriol* 62:293–300.

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