

Formulation of a semi-defined medium for high cell density cultivation of *Escherichia coli* in shake flasks

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Microbes for environmental research should be cultured in growth media with characteristics (e.g., pH, ionic strength, and organic and ionic composition) as close to their original habitat as possible. In addition, the medium should also enable high cell density to be obtained - needed for providing sufficient cells in subsequent experiments. This in-progress report describes the formulation of a medium with an environmentally-relevant composition (i.e., lack of complex organics), and that allows aerobic high cell density cultivation of *Escherichia coli* DH5 α (ATCC 53868) in shake flasks. The formulated medium comprises four components: a buffer system (K₂HPO₄: 12.54 g/L and KH₂PO₄: 2.31 g/L), vitamins (yeast extract: 12.0 g/L), salts (NaCl: 5.0 g/L and MgSO₄: 0.24 g/L), and carbon and nitrogen sources (D-Glucose: 6.0 g/L and NH₄Cl: 1.5 g/L). Notable characteristics of this medium were: high capacity phosphate buffer system (89 mM phosphate); 1:1 molar ratio between D-Glucose and NH₄Cl; and yeast extract providing trace elements and a secondary carbon and nitrogen source. Growth experiments revealed that an OD_{600nm} of 9 was obtained after 24 hours of cultivation at 37 °C. Glucose and NH₄Cl served as primary carbon and nitrogen sources for this phase of growth. After 48 hours, the OD_{600nm} reached 11, where carbohydrates, lipids and proteins in yeast extract provided the nutrients for biomass formation. Broth's pH varied between 5.5 and 7.8 during cultivation, which was in the range conducive for *E. coli* growth. In comparison, the OD_{600nm} of *E. coli* reached 1.4, 3.2, and 9.2 in three commonly used complex media; Nutrient Broth, LB Lennox, and Tryptic Soy Broth, respectively, over 48 hours under identical culture conditions. In addition, the formulated medium was able to maintain a large viable cell population for a longer period of time (three days) compared to Tryptic Soy Broth. Thus, preliminary data suggested that the formulated medium holds potential for use as a high cell density aerobic growth medium for Gram-negative bacteria.

Keywords: High cell density; shake flask; culture medium; aerobic; Gram-negative; bacteria;

Introduction

Cell culture is fundamental to biology research. While many complex media are commercially available for cultivating a wide variety of bacteria, they do not mimic the often nutrient poor natural environment that the bacteria of interest inhabit. In addition, the medium should also support the high cell density cultivation of bacteria even in simple culture systems such as shake flasks, which have intrinsic cost and maintenance advantages compared to fermenters. This extended abstract describes performance characterization of a formulated high cell density medium with environmentally-relevant composition.

Material

Nutrient broth (Difco), LB Lennox (Difco) and Tryptic Soy Broth (Merck) were purchased commercially and used as is. The composition of the formulated medium is as follows, [g/L]: K₂HPO₄, 12.54; KH₂PO₄, 2.31; NaCl, 5.0; Yeast extract, 12.0; NH₄Cl, 1.5; D-Glucose, 6.0; MgSO₄, 0.24.

Experimental

Escherichia coli DH5 α (ATCC 53868) was grown aerobically in the various growth media at 37 °C and 230 rpm in a temperature controlled orbital shaker incubator. Optical density was measured at 600 nm with a Shimadzu BioSpec Mini spectrophotometer and a quartz cuvette (pathlength = 1 cm) with appropriate dilution where necessary. Viable cell count assay was

performed using the pour plate method with phosphate buffer (pH 7.2) serving as the diluent during serial dilution of the samples. LB Lennox (15 g/L agar powder) was used as the solid growth medium. The inoculated agar plates were incubated at 37 °C for 24±2 hours.

Results and Discussion

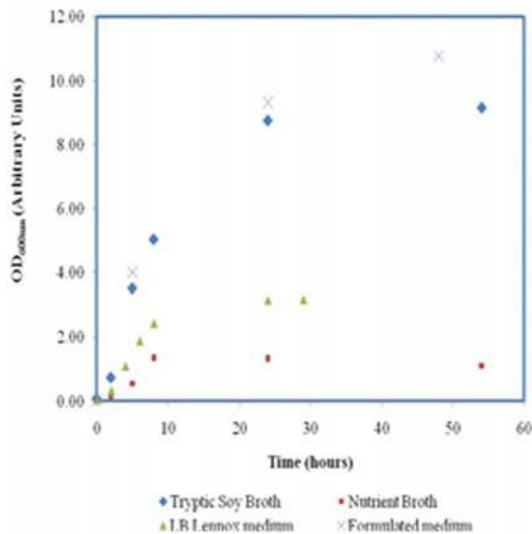


Fig 1: Growth curve of *E. coli* in different media. Larger version of figure is in supplementary information.

As can be seen in Figure 1, an OD_{600nm} of about 9 was obtained in the formulated high cell density medium after 24 hours of incubation, which increased to 11 at 48 hours. This was slightly higher than that obtained using Tryptic Soy Broth. LB Lennox and Nutrient broth were clearly unable to support high cell density cultivation of *E. coli* as the highest OD_{600nm} obtained; 3.2 and 1.4, respectively, were clearly far below that obtained using the formulated high cell density medium. In addition, the growth rate of *E. coli* in the formulated medium was higher compared to growth in commercial growth media.

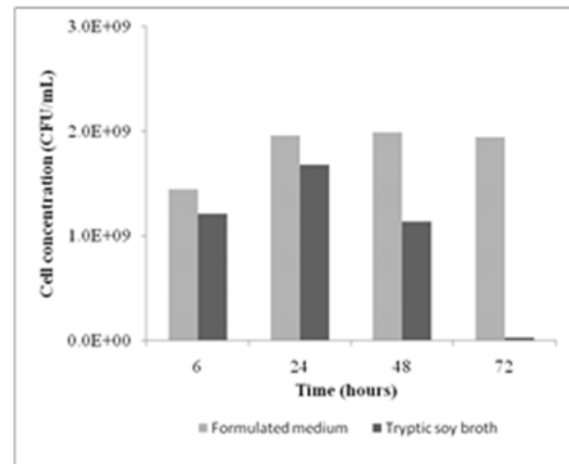


Fig 2: Viable cell population of *E. coli* at various time points when cultivated in high cell density medium and Tryptic Soy Broth. Larger version of figure is in supplementary information.

Figure 2 highlighted another advantage of the formulated high cell density medium, i.e., it was able to sustain a large viable cell population for a longer period of time compared to Tryptic Soy Broth. It could be seen that the large cell population in the high cell density medium remained stable and viable over a period of three days, whilst that in Tryptic Soy Broth decreased drastically after one day. Specifically, the viable cell population at the end of three days in Tryptic Soy Broth was about 1.6% of that on the first day. This could be due to the presence of high concentration of toxic metabolites generated during growth in Tryptic Soy Broth.

Conclusions

The formulated medium was able to support rapid aerobic high cell density cultivation of *E. coli*. In addition, the large viable cell population remained stable and viable for a period of three days with no sign of a drastic decrease such as that seen in Tryptic Soy Broth.

Acknowledgment

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