Development of a semi-defined medium for high cell density cultivation of *Escherichia coli* in shake flask culture system

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Microbes in environmental studies should be cultured in growth media with characteristics as close to their original habitat as possible, and which also allows a high cell density to be attained for providing enough cells in subsequent experiments. This in-progress report describes the formulation of a medium with an environmentally-relevant composition, and which also affords aerobic high cell density cultivation of *Escherichia coli* DH5α in shake flasks. The formulated medium comprises four components: a buffer system (K_2HPO_4: 12.54 g/L and KH_2PO_4: 2.31 g/L), vitamins (yeast extract: 12.0 g/L), salts (NaCl: 5.0 g/L and $MgSO_4$: 0.24 g/L), and carbon and nitrogen sources (D-Glucose: 6.0 g/L and NH_4Cl: 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 NH_4Cl; and yeast extract providing trace elements and a secondary carbon and nitrogen source. Growth experiments revealed that an OD_{600nm} of 9 was attained after 24 hours of cultivation at 37 °C. This phase of growth was largely fuelled by glucose and NH_4Cl. After 48 hours, the OD_{600nm} reached 11, which was fuelled by the mixture of carbohydrates, lipids and proteins in yeast extract. Broth's pH varied between 5.5 and 7.8 during cultivation, which was in the range conducive for growth of *E. coli*. In comparison, the OD_{600nm} of *E. coli* reached 1.4, 3.2, and 9.2 for 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) relative 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 biological 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. Additionally, 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 relative to fermenters. This extended abstract describes the experimental characterization of the performance 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]: K_2HPO_4, 12.54; KH_2PO_4, 2.31; NaCl, 5.0; Yeast extract, 12.0; NH_4Cl, 1.5; D-Glucose, 6.0; $MgSO_4$, 0.24.

Experimental

Escherichia coli DH5α (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

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performed using the pour plate method with phosphate buffer (pH 7.2) serving as the diluent during serial dilution of the samples. LB Lennox (5 g/L agar powder) was used as the solid growth medium. The inoculated agar plates were incubated at 37 ºC for 24±2 hours.

Results and Discussion

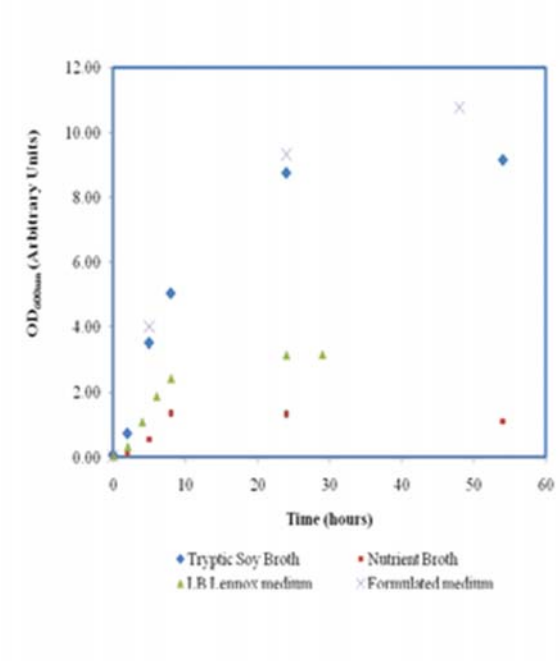

Fig 1: Growth curve of *E. coli* in different media Larger version of figure is in supplementary information.

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, 48 hours post-inoculation. 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 very rapid relative to growth in commercial growth media.

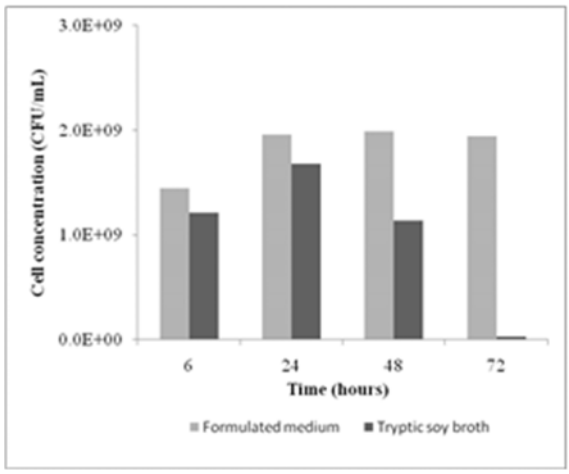

Fig 2: Viable cell population of *E. coli* at various time points when cultivated in high cell density medium and Tryptic Soy Broth. Larger version of figure is in supplementary information.

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 as 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, with the viable cell population at the end of three days 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 high cell density medium was able to support rapid aerobic high cell density cultivation of *E. coli*. Additionally, 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.

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