Viable cell count of *Escherichia coli* DH5α in LB Lennox medium supplemented with 2 g/L glucose

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Abstract

Supplementation of growth medium with glucose is a common approach for improving biomass formation given that most microorganisms are capable of utilizing glucose in preference to other carbon sources. LB medium is a common medium for the growth of *Escherichia coli*, and in this report LB Lennox medium was supplemented with 2 g/L glucose for the cultivation of *Escherichia coli* DH5α (ATCC 53868). Viable cell count assays were conducted to determine the performance of the medium in supporting an expanded cell population. Experiment results revealed that the glucose supplemented medium was able to support a larger viable cell population of 2.4 x 10^9 colony forming units (CFU)/mL compared to 1.6 x 10^9 CFU/mL in LB Lennox medium. Increase in viable cell population during initial growth phase in LB Lennox supplemented with 2 g/L glucose (LBG) was rapid with a 64 fold increase in viable cells in 6 hours. After attainment of maximal viable cell concentration, gradual decline in viable cell concentration was observed, but a significant viable cell population of 2.2 x 10^8 CFU/mL remained in the culture after 121 hours of incubation. Thus, LBG medium was able to support a larger viable cell population compared to LB Lennox medium. In addition, observation of slow, gradual decline in viable cell concentration during stationary phase revealed that there was no significant secretion of toxic metabolites by the cells during growth in LBG medium. Overall, LBG medium is suitable for use in cultivating *E. coli* DH5α to higher biomass yield in experiments where a large viable cell population is needed for extended periods.

Keywords: biomass formation, *Escherichia coli*, LB Lennox, viable cell count, glucose supplementation, toxic metabolites, stationary phase, diauxic growth,

Subject areas: microbiology, biochemistry, biotechnology, cell biology, bioengineering,

Background and summary

Glucose supplementation is commonly used to improve biomass formation during growth of bacteria in growth medium. In the case of *Escherichia coli*, LB medium is one of the most popular growth medium for the cultivation of the bacterium. Supplementation of LB medium with glucose has been reported to help improve biomass formation. In this report, LB Lennox medium was supplemented with 2 g/L glucose to help improve biomass formation in *Escherichia coli* DH5α (ATCC 53868). Viable cell count via the spread plate technique was used in assessing the biomass formation potential of LB Lennox medium supplemented with 2 g/L glucose. Experiment results revealed improved biomass formation of *E. coli* DH5α with

maximum viable cell count of 2.4 x 10^9 colony forming units (CFU)/mL compared to 1.6 x 10^9 CFU/mL for the same bacterium grown in LB Lennox medium (Figure 1).

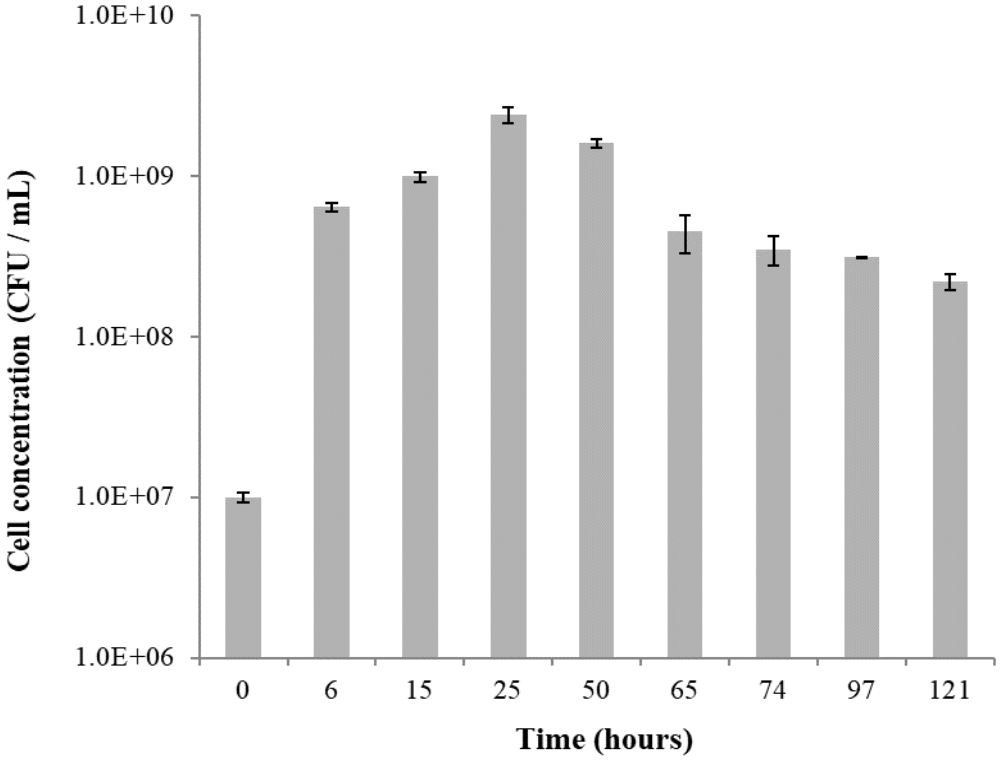

Figure 1: Viable cell count of *Escherichia coli* DH5α in LB Lennox medium supplemented with 2 g/L glucose.

Specifically, viable cell population increased from 1.0 x 10^7 CFU/mL at the start of the cultivation to 6.4 x 10^8 CFU/mL after 6 hours of cultivation. Viable cell count continued to increase till the attainment of maximal viable cell count at 25 hours of cultivation (2.4 x 10^9 CFU/mL). After the attainment of maximal viable cell concentration, viable cell count gradually decreased with the onset of stationary phase till 121 hours into the experiment where a viable cell concentration of 2.2 x 10^8 CFU/mL was reported. Overall, supplementation of LB Lennox medium with 2 g/L glucose helped improve maximal viable cell count during exponential growth phase, and the supplemented medium could support a fairly large viable cell population for long duration of a few days. Hence, growth of *E. coli* DH5α in the glucose supplemented medium likely did not generate significant toxic metabolites that could impact on the viability of cells in the population. In summary, supplementing LB Lennox medium with 2 g/L glucose is a feasible approach for increasing biomass formation in *E. coli* DH5α without the generation of toxic metabolites that could impact on the viability of the bacterial cell population.

Materials and methods

Materials

LB Lennox medium was purchased from Difco and used as is. D-Glucose was purchased from Sigma-Aldrich. Phosphate buffer was used as diluent in viable cell counting. Composition of LB Lennox medium was [g/L]: Tryptone, 10.0, Yeast extract, 5.0, NaCl, 5.0. Composition of LB Lennox medium supplemented with 2 g/L glucose was [g/L]: Tryptone, 10.0, Yeast extract, 5.0, NaCl, 5.0, D-Glucose, 2.0. Composition of phosphate buffer was [g/L]: KH_2PO_4, 26.22; Na_2CO_3, 7.78. Composition of LB Lennox agar was [g/L]: Tryptone, 10.0, Yeast extract, 5.0, NaCl, 5.0, Agar, 15.0.

Growth of E. coli DH5α in growth medium

Stock cultures of *E. coli* DH5α were prepared in 40% glycerol and kept at -70 ^{o}C till use. One glycerol stock culture of *E. coli* DH5α was used as inoculum for 100 mL of LB Lennox medium in 250 mL glass conical flask in a starter culture. Incubation conditions were 37 ^{o}C and 230 rpm rotational speed in a temperature controlled incubator. After 8 hours of incubation, 1 mL of starter culture was used as inoculum for 100 mL of LB Lennox + 2 g/L glucose medium in 250 mL glass conical flask. Three biological replicates were performed. Incubation conditions for the experiment cultures were 37 ^{o}C and 230 rpm rotational speed in a temperature controlled incubator.

Viable cell count via spread plate method

1 mL of experiment cultures were aliquoted at appropriate time points and diluted with sterile phosphate buffer in a 15 mL polypropylene centrifuge tube. Serial dilution with the same buffer was performed. At appropriate dilution, 1 mL of the diluted cell suspension was used as inoculum on a LB Lennox agar plate. Spread plate inoculation was used in distributing the inoculum evenly on the surface of the agar. Inoculated agar plates were incubated at 37 ^{o}C in a temperature controlled incubator for 24 hours. Viable cell count was calculated from colony count on plates with 30 to 300 colonies multiplied by the dilution factor. Existence of pure culture on the agar plate was checked prior to commencement of viable cell count.

Conflicts of interest

The author declares no conflicts of interest.

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