

# Enzyme-based Method for Rapid Detection of *E. coli* K12 during the Lag Phase or Early Exponential Phase

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Rapid and inexpensive methods for foodborne pathogen detection are needed in the food industry to help reduce the incidence of outbreaks of illness. Rapid methods have the potential of preventing the distribution of contaminated food and hence decreasing the number of product recalls. Polymerase chain reaction (PCR) methods or immunoassay often require 8 to 18 hours of enrichment to reach the limit of detection for pathogen numbers. We have determined the shortest time needed for *Escherichia coli* K12 to produce  $\beta$ -galactosidase activity using a colorimetric method during the lag phase or early exponential phase in minimal medium plus selected sugars. Glucose, lactose, fructose, xylose, or galactose were selected as the primary carbon sources for bacterial growth. Using this enrichment, the time was reduced to 6 hours, with a limit of detection in the order of 104 colony forming units per milliliter. This initial proof of concept study supports our hypothesis that selective enzyme activities can be used for early detection of bacteria.

Foodborne pathogens that contaminate fresh produce and processed foods represent serious threats to human health in the US and worldwide. Traditional culture and plate count methods are tedious and require at least 48 to 72 h. Rapid and inexpensive methods for detection of these microorganisms are needed in the food industry (CDC, 2011). Several methods have been developed for rapid detection of foodborne pathogens. Polymerase chain reaction (PCR) based or immunoassay methods can detect pathogens in about 24 h. However, they require expensive instrumentation and a skilled operator. PCR methods may give false positive results due to the influence of damaged or non-viable cells. Moreover, time for enrichment requires at least 18 h, which makes the total assay time of PCR more than 24 h (Malorny et al, 2009). Knowledge of bacterial metabolomics can be useful to design alternative rapid methods to detect the target pathogen presence in the culture media. A GC-MS based metabolomics method was reported for rapid detection of Escherichia coli O157:H7 and Salmonella spp. after growth in tryptic soy broth for 18 h. However, no specific biomarker was found during the lag phase or early exponential phase of each pathogen (Cevallos-Cevallos et al., 2011). Thus, metabolomics methods also require expensive instruments and are not practical in the food industry unless unique biomarkers are identified. Rapid enzyme methods for early detection of foodborne pathogens were reported in tryptic soy broth or modified Luria-Bertani medium. β-Galactosidase and C<sub>8</sub> esterase were selected as the specific enzymes for detection of E. coli O157:H7 and Salmonella spp. (Jokerst et al., 2012). However, these selective enzymes for the rapid detection of foodborne pathogens in minimal medium plus selected sugars during bacteria lag phase or early exponential phase have never been studied. In this study, we hypothesized that E. coli K12 produces selective enzyme activity during the lag phase or early exponential in minimal medium plus selected sugars. Non-pathogenic E. coli K12 was selected because the experiment was not conducted in a biosafety lab. As a first step to show proof of concept,  $\beta$ -galactosidase activity

was selected as it has been reported to be specific to *E. coli* species and it is not produced by *Salmonella* spp. or other lactose negative bacteria (Jacobson et al., 1994). The research objective was to develop a reliable enzyme method for rapid detection of *E. coli* K12 during the lag phase or early exponential phase. The use of minimal medium plus selected sugars is proposed as an additional layer of selectivity.

#### **Material and Methods**

STRAIN AND MEDIUM. The bacterial strain used was E. coli K12 MDD333. Tryptic soy broth, Bradford reagent, bovine serum albumin and selected sugars were purchased from Sigma (St. Louis, MO). Tryptic soy agar was purchased from Becton, Dickinson and Co. (Franklin Lakes, NJ). Minimal medium salts were purchased from Fisher Scientific (Pittsburgh, PA). The media included 6.8 g·L<sup>-1</sup> of Na<sub>2</sub>HPO<sub>4</sub>, 3.0 g·L<sup>-1</sup> of KH<sub>2</sub>PO<sub>4</sub>, 0.5 g·L<sup>-1</sup> of NaCl, and 1.0 g·L<sup>-1</sup> of NH<sub>4</sub>Cl, sterilized by autoclaving. Two mL of 0.24 g·L<sup>-1</sup> MgSO<sub>4</sub> and 100 mL of 0.01 g·L<sup>-1</sup> CaCl<sub>2</sub> were prepared by autoclaving and were added to the minimal medium. Twenty milliliters of 4  $g \cdot L^{-1}$  of glucose, lactose, fructose, xylose, or galactose were added as selected sugars. The  $\beta$ -galactosidase enzyme assay system with lysis buffer (E2000) was purchased from Promega (Madison, WI). Phosphate buffer solution was made of 8 g·L<sup>-1</sup> of NaCl, 0.2 g·L<sup>-1</sup> of KCl, 1.44 g·L<sup>-1</sup> Na<sub>2</sub>HPO<sub>4</sub> and 0.24 g·L<sup>-1</sup> of KH<sub>2</sub>PO<sub>4</sub> from Fisher Scientific. A fiberoptic spectrometer model HR4000CG-UV-NIR from Ocean Optic (Dunedin, FL) was connected with a fiberoptic cuvette holder inside the incubator for growth curve measurements.

**MICROBIAL GROWTH.** Cultures (24 h at 37 °C) in tryptic soy broth were serially diluted (10:1) and inoculated in 10 mL of minimal medium plus selected sugars. Cultures on agar were prepared by spreading the sample over tryptic soy agar ( $150 \times 15 \text{ mm}$ ) to produce a bacteria lawn and serially diluted in minimal media plus selected sugars. Growth was monitored by absorbance at 600 nm for 24 h at 37 °C using a fiberoptic spectrometer. Initial and final cell density was determined by plate count on TSA. Each

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growth in minimal medium plus selected sugars was measured in triplicate.

PROTEIN CONTENT AND ENZYME ACTIVITY ASSAY. The Bradford assay was used for determination of protein content produced by E. coli K12 during each phase of the growth curve and then extracted upon cell rupture. An aliquot of 1 mL of cell suspension was sonicated for 20 s at 40 kHz in a sonicator model FS20H (Fisher Scientific) and mixed with 1 mL of Bradford reagent for 5 min at room temperature. Absorbance was measured at 595 nm. A bovine serum albumin standard curve was performed for protein quantification. For cell harvest, the cells were washed by PBS buffer and resuspended in 500 µL of lysis buffer (E3971, Promega). β-Galactosidase assay was performed by adding 150 mL of assay buffer, which contains 1.33 mg·mL<sup>-1</sup> of ONPG (o-nitrophenyl- $\beta$ -D-galactopyranoside) to 150  $\mu$ L of lysate sample. The reaction was continuously monitored by spectrometer for 30 min at 37 °C. Initial and final cell density at each growth phase was determined by plate count on TSA. Each assay was performed in triplicate.

## **Result and Discussion:**

**MICROBIAL GROWTH.** The growth curve of *E. coli* K12 in minimal medium plus selected sugars is shown in Figure 1. *E. coli* K12 had a lag phase of almost 5 h in minimal medium plus any of the selected sugars. *E. coli* K12 grew faster on glucose than on any other single carbon source. Bacteria preferentially use the carbon sources that are most easily accessible and allow fastest



Fig. 1. Growth curves of *E. coli* K12 in minimal medium plus selected sugars. (A) Culture in suspension (B) Culture on agar; (■) minimal medium plus glucose,
(□) minimal medium plus lactose, (▲) minimal medium plus fructose, (×) minimal medium plus xylose, (\*) minimal medium plus galactose

growth. Compared to other carbon sources, glucose is considered the preferred carbon source in many of the model organisms that have been studied, including the *E. coli* species (Görke and Stülke, 2008). Diauxic growth of *E. coli* K12 was observed in minimal medium plus galactose around 5 to 10 h incubation. The diauxic shift observed in minimal medium plus galactose indicates that cells may produce the enzymes needed to metabolize the second carbon source, which needs further investigation.

PROTEIN CONTENT AND ENZYME ACTIVITY ASSAY. The protein content of E. coli K12 during the lag phase was not measured because the limit of detection of the Bradford assay is 1 mg·mL-1 of protein which is not sensitive enough for our purposes. β-Galactosidase activity was only detected in presence of minimal medium plus lactose or galactose because the synthesis of  $\beta$ -galactosidase does not occur due to the effect of catabolite repression caused by glucose or other carbon source (Deutscher, 2008). The inoculation level of E. coli K12 was approximately 0.8 Log CFU/mL in minimal media plus lactose or galactose. Figure 2 shows the reaction progress of β-galactosidase-catalyzed reaction at different incubation times in minimal media plus selected sugars. Enzyme activity was calculated from the slopes of the linear portion of these curves. Results indicate that E. coli K12 needed 6 h of enrichment to be detected in lactose (0.27 milliunit) (Fig. 2A) and 11 h of enrichment to be detected in galactose (0.14 milliunits) (Fig. 2B). The results shown in Figure 3 indicate that the limit of detection of E. coli K12 was approximately 4 Log CFU/mL in minimal medium plus lactose (Fig. 3A) and 7 Log CFU/mL in minimal media plus galactose (Fig. 3B). Similar enzyme assays for rapid detection of pathogen were reported in



Fig. 2. Progress reaction curves for enzyme-catalyzed reaction used to calculate  $\beta$ -galactosidase activity of *E. coli* K12 at different incubation times. (A) Minimal medium plus lactose after incubation time of ( $\blacksquare$ ) 5 h ( $\square$ ) 6 h ( $\blacktriangle$ ) 7 h ( $\times$ ) 8 h; (B) minimal medium plus galactose after incubation time of (\*)10 h, (-) 11 h, (O)12 h, (+) 13 h.



Fig. 3. Correlation of enzyme activity of *E. coli* K12 with cell density in minimal medium plus selected sugars (A) minimal medium plus lactose (B) minimal medium plus galactose.

tryptic soy broth with a limit of detection of 6 Log CFU/mL for *E. coli* O157:H7 after 4.5 h of incubation, 4 Log CFU/mL for *Salmonella* Typhimurium after 4 h of incubation and 8 Log CFU/mL for *Listeria monocytogenes* after 6 h of incubation (Jokerst et al., 2012). Other studies targeting enzyme activities for bioindication of a bacterium have been done during the stationary phase after enrichment in non-selective media (Jokerst et al., 2012). In this study we chose minimal medium plus selected sugars for differentiated bacteria growth, which would improve the overall selectivity of the assay. Results indicate that lactose served as a good selective carbon source to measure  $\beta$ -galactosidase activity

and to enhance the selectivity of the enzyme assay (Fiksdal and Tryland, 2008). Moreover, *Salmonella* or other pathogens may produce different durations of the lag phase in minimal medium plus selected carbon source, thus selective enzyme activities of different pathogens can be detected with selective incubation times. The results support the hypothesis that selective enzyme activity of *E. coli* K12 can be detected during the early exponential phase.

### Conclusion

We have shown proof of concept that the selective  $\beta$ -galactosidase activity produced by *E. coli* K12 can be detected during the lag phase or early exponential phase. This method has shorter enrichment time than culture or PCR methods. Future work should be focused on development of selective enzyme methods for rapid detection of *Salmonella* spp. or other pathogens in minimal medium plus selected sugars. Also, cross enzyme reactivity among pathogens needs to be studied.

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