Application Note

Microbiology

Monitoring Bacterial Growth under Different Environmental Conditions

Using the Agilent BioTek LogPhase 600 to provide high quality kinetic data

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Abstract

Microbial organisms, such as bacteria, serve critical roles in many different facets of modern day living. They are used for the production of medical products, environmental remediation, biomedical research, and food production, to name just a few different uses. The ability to manipulate and select different strains with specific characteristics has been paramount to their utility. Likewise, the development of new strains often requires monitoring the growth of strains under various conditions. Described here is the use of the Agilent BioTek LogPhase 600 microbiology reader to provide temperature control, suspension agitation, and monitor bacterial growth using light scatter in 96-well microplates.

Introduction

Bacteria play a critical role in industry in a number of ways that exploit their natural capabilities. They are used in manufacture of foods and production of antibiotics, probiotics, drugs, vaccines, starter cultures, insecticides, enzymes, fuels and solvents. In the foods industry, lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, and *Streptococcus* are used in the manufacture of dairy products such as cheeses and yogurt. Lactic acid bacteria and acetic acid bacteria are used in pickling processes for items such as olives, pickles and sauerkraut, while bacterial fermentations are used in the processing of soy sauce. In the pharmaceutical industry, bacteria are used to produce antibiotics, vaccines, and medically-useful enzymes. Soil and waste remediation can be augmented with the use of bacteria to metabolize specific pollutants.

To optimize their efficiency, bacterial strains that grow under specific environmental conditions have been selected. As a means to select bacteria with these attributes, bacterial strains are grown in suspension under various experimental conditions and strains with the highest growth rate identified. Growth of bacteria in suspension culture can be monitored using turbidity or light scatter measurements. As the number of cells increases, the solution becomes increasingly cloudy or turbid because light passing through it is scattered by the microorganisms present.[1] While not obeying Beer's law, as light scatter increases, the percentage of the total light beam reaching the detector diminishes and is recorded as absorbance.

Bacteria grown in suspension will increase in number over time. If the light scatter is monitored by absorbance, a sigmoidal shaped curve is observed (Figure 1). After the initial inoculation, bacteria numbers are quite small and the bacteria present are adapting to the new environment. This stage, referred to as the lag phase, demonstrates very little change in optical density (OD). In log phase, growth accelerates and bacterial number become significant, rapid changes in OD are observed. As nutrients become scarce and waste products accumulate, growth slows and the culture enter stationary phase.

The LogPhase 600 microbiology reader performs kinetic OD_{600} determinations, while providing temperature control and shaking for up to four separate 96-well microplates. The associate software application provides a simple user interface to input reading parameters. At the completion of the run, the application also provides assay metrics such as the length of the lag phase, maximal growth rate, and time

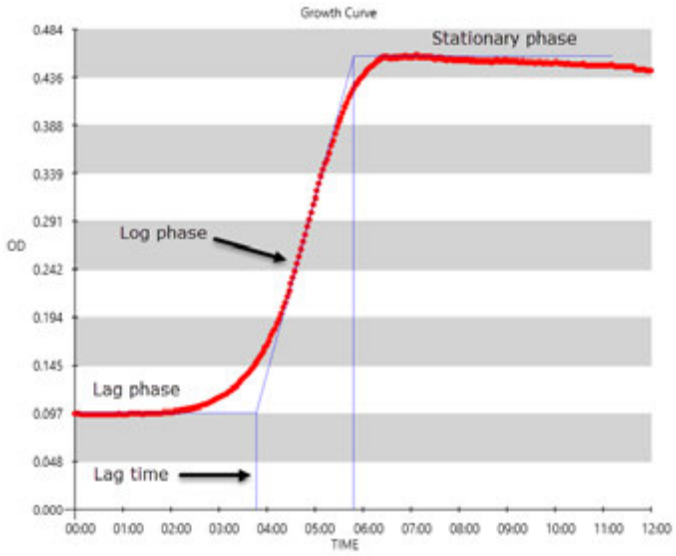

Figure 1. Typical bacterial kinetic growth curve.

to maximum optical density. The reader uses the capability of microplates to assess large number of experimental conditions within a small standardized footprint. The ability to accommodate four 96-well microplates simultaneously further increases the experimental possibilities.

Materials and methods

Media powders, dry chemicals, and ethanol were obtained from Sigma-Aldrich (St. Louis, MO). LB and 2XYT broth media were prepared as directed and sterilized by autoclaving. LB media consisted of 10 g tryptone, 5 g yeast extract, and 5 g NaCl per liter, while 2XYT consisted of 16 g of tryptone, 10 g of yeast extract and 5 g NaCl per liter of solution. Sterile flat bottomed clear microplates (part number 3598), were from Corning (Corning, NY). Optically clear TopSeal-A plate sealers, catalogue number 6005185, were obtained from PerkinElmer (Boston, MA). JM109 Strain of *E. coli* was obtained from Life Technologies (Carlsbad, CA).

All of the experiments followed the same general format. Overnight stock cultures (50 mL) were grown in 250 mL Erlenmeyer flasks at 37 °C with orbital shaking at 200 rpm. Prior to growth experiments, bacterial cultures were diluted to 1:500 with fresh media. The diluted cells were then plated as needed into Corning 3598 flat bottomed clear plates in a total volume of 150 µL. Measurements were made every 2.5 to 10 minutes with continuous orbital shaking (800 rpm) using an Agilent BioTek LogPhase 600 microbiology reader set to 37 °C for 12 hours. The reader was controlled and the data were collected using the Agilent BioTek Microbial Growth app.

All experiments required that a portion of the growth media in the well be replaced by the experimental compound(s), with the final volume of the microplate well always being 150 µL. Unless otherwise indicated, experimental additions were diluted in water. In each experiment all of the samples had the growth media diluted equally for comparison.

Results and discussion

Comparison of growth media formulations

Two different commonly used media formulations were initially tested for their growth characteristics. As demonstrated in Figure 2, both formulations provide significant and nearly equivalent growth curves when used undiluted. While 2XYT was able to grow *E. coli* to a slightly higher density and growth rate, the differences were not dramatic relative to the differences in formulation.

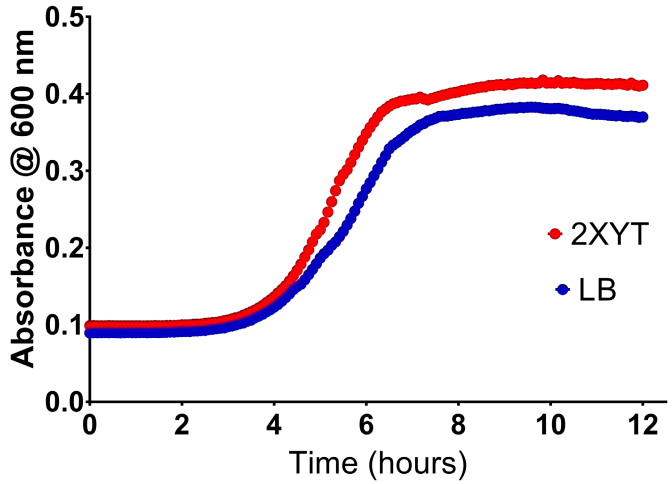

Figure 2. Comparison of *E. coli* growth in LB and 2XYT media formulations. Wells of a 96-well microplate were inoculated with *E. coli* and the absorbance monitored kinetically every 5 minutes for 12 hours. Data represent the mean of eight wells.

Because several experiments will require the media to be diluted to some extent for experimental purposes, both formulations were diluted with water to various percentages relative to the defined recipe. Under these conditions, 2XYT provided near optimal growth rates at much lower concentrations (Figure 3). This was not unexpected, as the formulation uses considerably more of the same constituents per volume as compared to LB media. All further experimentation used 2XYT media.

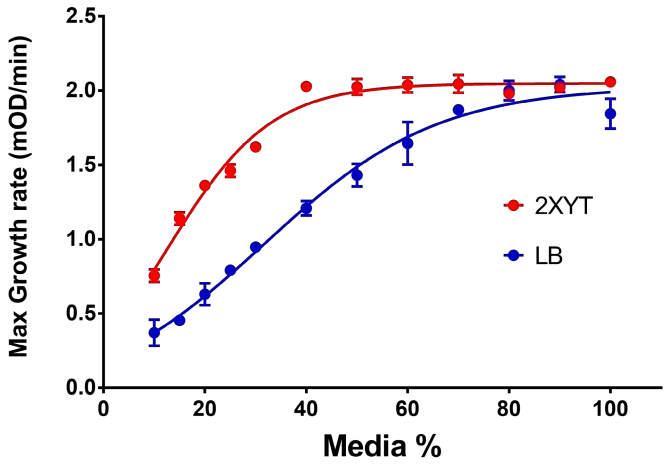

Figure 3. Growth rate of *E. coli* with various concentrations of bacterial media. LB and 2XYT media was diluted with water and inoculated with *E. coli* and growth monitored for kinetically. Maximum growth rate for each well was determined and plotted as a function of growth media concentration. Data represents the mean and standard deviation of eight determinations.

Effect of NaCl levels on growth rates

To examine the effect of NaCl on bacteria growth, a portion of the 2XYT media was replaced with dilutions of NaCl in water. As demonstrated in Figure 4, increasing the salinity of the environment reduces the growth rate of *E. coli*. At very high concentrations, there is virtually no bacterial growth.

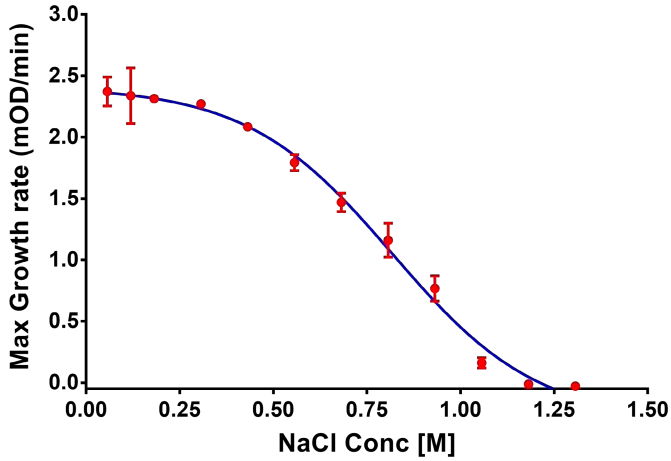

Figure 4. Effect of salt concentration on bacterial growth. *E. coli* growth in 2XYT media supplemented with various amounts of NaCl was monitored kinetically. The maximal growth rate for each well was determined and plotted as a function of NaCl concentration.

Effect of pH levels

Environmental pH can have a profound effect on bacterial growth. A wide range buffer system was used to maintain pH levels from 2.5 to 12.[3] A portion of the growth media was replaced with buffer and the wells inoculated with *E. coli*. As shown in Figure 5, there is virtually no growth at pH levels below 5.5. Growth rates rapidly increase as the pH is raised, with a maximal rate at pH 7.5. Increasing pH above 7.5 results in a reduction of the growth rate with at pH 12 approximately 60% of maximal (Figure 5).

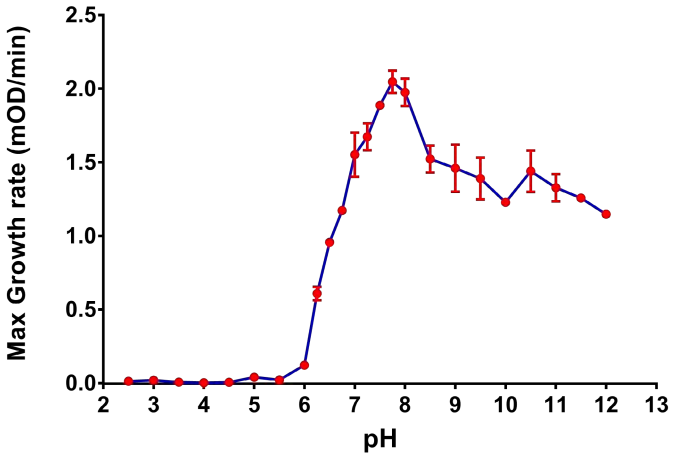

Figure 5. Effect of media pH on bacterial growth. 100 µL of dilute *E. coli* cultures in 2XYT media were added to wells of a microplate with 50 µL of universal buffer at various pH levels. Growth was monitored kinetically for 12 hours and the maximum growth rate plotted as a function of pH. Data represents the mean and standard deviation of eight determinations.

Effect of adjunct sugar sources on growth rates

Bacteria can often be differentiated by their ability to metabolize specific sugars. As shown in Figure 6, the JM109 strain of *E. coli* can use both glucose and galactose equally. Either of these two six-carbon monosaccharide sugars increase the maximal growth by 33% over the control wells.

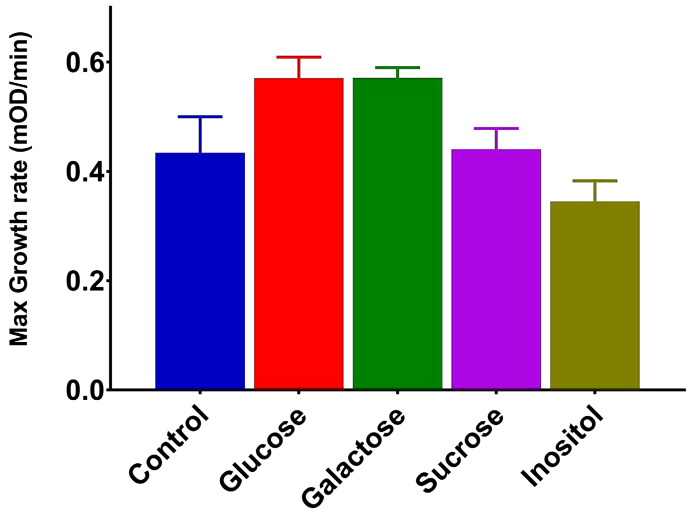

Figure 6. Comparison of bacterial growth in the presence of various sugar molecules. 2XYT media supplemented with 100 µM of the indicated sugar was inoculated with *E. coli* and the growth monitored kinetically. Bar graph represents the mean and standard deviation of eight determinations.

The disaccharide sucrose, which is composed of the glucose and fructose monosaccharides, does not confer the same increase in growth rate. This suggests that this strain lacks the ability to efficiently cleave the glycosidic linkage between the two monosaccharides or that the energy cost is equivalent to the gain conferred by the liberation of glucose. Inositol, a metabolic byproduct of glucose by the kidney, results in the decrease in growth rate when added to *E. coli* cultures.

A number of nonsugar carbohydrate molecules were also used to supplement *E. coli* cultures. Interestingly, the addition of 50 µM of the three-carbon molecule pyruvate resulted in a 38% increase in the maximal growth rate as compared to the control wells (Figure 7). Acetate, a two-carbon moiety, resulted in a 26% decrease and citrate completely abolished growth at similar concentrations. These changes in growth rates were not the result of changes in pH caused by the addition of these weak organic acids, as the cultures also contained 50 mM Hepes (pH 7.5) as a means to mitigate pH as a variable.

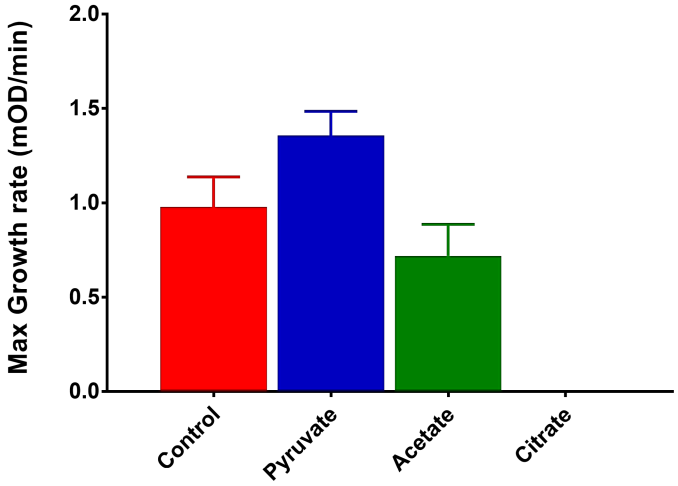

Figure 7. Comparison of bacterial growth rate in the presence of small carbon molecules. 2XYT media, supplemented with 50 µM of the indicated molecule and 50 mM Hepes (pH 7.5), was inoculated with *E. coli* and the growth monitored kinetically. Bar graph represents the mean and standard deviation of eight determinations.

Inhibition of growth rates by addition of solvents

The presence of solvents can effect bacterial growth. As demonstrated in Figure 8, increasing amounts of several solvents commonly used in biological systems can have a marked influence on growth rate. The presence of tetrahydrofuran in excess of 0.5% abolished bacterial growth, whereas the presence of 5% DMSO is tolerated. Interestingly, low concentrations of DMSO increase growth rate as compared to untreated controls.

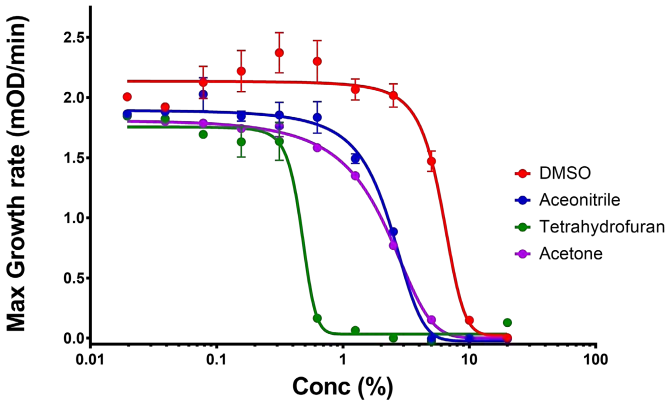

Figure 8. Effect of solvents on bacterial growth. Various concentrations of four different commonly used solvents were added to 2XYT media and the growth of *E. coli* monitored kinetically. The maximal growth rate of each well was calculated and plotted as a function of solvent concentration. Each data point represents the mean and standard deviation of eight determinations.

These data demonstrate the utility of the LogPhase 600 microbiology reader to characterize bacterial strains using light scatter as a means to quantitate growth rate. Using the JM109 strain of *E. coli*, the effects of several different media additives on bacterial growth have been investigated. They serve as examples of the proof of concept for testing bacterial growth under experimental conditions using the LogPhase 600.

The selection of bacterial strains that grow under defined conditions is used in a number of different scenarios. For example, soil remediation often uses bacteria that can use specific pollutants as an energy source. Areas with a long term industrial heritage have resulted in the formation of brownfields. Brownfields often contain petroleum hydrocarbons, persistent organic pollutants, such as PCBs, and toxic metal(oid)s. The identification of specific bacteria that can metabolize these compounds and use them as a carbon source has the potential to mitigate some of their toxicity. The strains used should ideally have: (a) superior ability to degrade the target contaminants; (b) be easy to cultivate; (c) provide fast growth; (d) have a tolerance to the high concentration of contaminant; and (e) ability to survive in a wide range of environmental conditions/stressors.[4] The identification of bacteria that best meets these criteria requires the testing of numerous strains of bacteria.

Conclusion

The Agilent BioTek LogPhase 600 microbiology reader is an ideal platform to undertake these types of experiments. Unlike other traditional microplate readers, the LogPhase 600 reader is capable of holding up to four 96-well plates simultaneously. Because all four plates are contained in the same reader environment for the duration of the experiment, true comparison of up to 384 data points can be made. The reader provides temperature control from 30° to 45 °C, in 1° increments, in conjunction with orbital shaking. The reader has been designed from the ground up for continuous shaking, with a robust counterbalance mechanism that reduces noise and vibration. Shaking speed can be varied from 500 to 800 rpm or turned off, if necessary. The LED light source provides wavelength-specific light for a lifetime without the need to change lamps. Assay runs as long as 72 hours can be programmed without intervention. During long runs, the assay can be temporarily interrupted and then restarted without loss of data integrity, if necessary.

A dedicated software application is used to set up the assay. Using the application, incubation temperature, assay duration, reading interval, and shaking speed are set. During the run, small icons representing each well are indicated. A mouse click on any well provides an enlarged OD_{600} plot. If desired, several plots can be overlaid for comparison. At the completion of the run, lagtime, maximal growth rate, and time to maximum OD are calculated. Raw and calculated data can then easily be exported as an Excel or text file, if further data analysis is desired.

References

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RA44232.0475347222

Printed in the USA, February 1, 2021
5994-2549EN
AN071520_03

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