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Comparing Two Functions for Optical Density and Cell Numbers in Bacterial Exponential Growth Phase

Xuelin Zhang, Yajuan Wang, Jun Guo, Yi YU, Jia Li, Yinghua Guo and Changting Liu*

Lab of Nanlou Respiratory Department, 301 Hospital, Beijing, 100853, China.

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Six strains of bacteria, comprising three Gram-negative and three Gram-positive strains, were utilised to reveal the functional relationship between optical density (OD) values and colony-forming units (CFU) during the exponential growth phase. CFU during the exponential growth phase were counted using the classic plate counting method. OD values were detected with both Multiskan Spectrum and N721 spectrophotometers at 600 nm at both 37°C and 4°C. Two functions (line function and power function) were simulated with Excel 2007 to reveal the relationship between OD values and the CFU. The two functions were compared with each other by using their coefficient of determination values (R^2), which represented degree of fitting for functions. For the ODs detected with the Multiskan Spectrum spectrophotometer at 37°C, the average R^2 values of the line function was 0.983±0.011 and the power function was 0.995±0.003; thus, the R^2 value of the power function was significantly higher than that of the line relationship ($p<0.05$). Similar results were obtained with the Multiskan Spectrum spectrophotometer at 4°C and with the N721 spectrophotometer at both temperatures. These results reveal that the power function is more suitable for modelling the relationship between OD600 and CFU during the exponential growth phase of bacteria.

Key words: Optical density; exponential growth phase; colony-forming units; power function; determination value.

When conducting experiments with bacteria, samples during the exponential growth phase are usually used[1, 2]; beside that, cell numbers are typically required[3]. Usually, to gain a relatively exact cell count, the classic plate counting method is used. However, this method takes at least two or three days, sometimes longer. Many experiments cannot be performed until colony-forming units (CFU) are determined, but by the time the values are obtained, the samples are no longer fresh. In addition, this method demands large amounts of labour, making it inefficient for microbiological research[4].

Various new methods have been used to measure or predict cell counts, such as turbidity detection, flow cytometry, microscopic methods or impedance detection[4]. Among them, the measurement of turbidity is the simplest and most feasible method. This technique is performed by detecting the optical density of a broth culture with a spectrophotometer. Several reports have revealed that CFU can be calculated from the optical density using a calibration curve. Commonly, the function used for calculation is a linear relationship[5, 6]. However, the data for this function are usually derived from only one strain of bacteria, and it was in lag phase. Thus, it's not clear that whether the linear function could explain the relationship between the optical density and cell count in one or more strains of bacteria in

* To whom all correspondence should be addressed.
Tel/Fax. +86-10-66876272;
E-mail: liuchangt@gmail.com

exponential growth phase. To answer this quetion, we utilised six strains of bacteria in exponential growth phase to produce two functions, a power correction and a linear function. Of the two functions, the power function was determined to be more suitable for representing the relationship between optical density and cell count in the exponential growth phase of bacteria.

MATERIALS AND METHODS

Strains and culture conditions

Three strains of Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) and three strains of Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecium*, and *Bacillus subtilis*) were all subcultured three times in Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK) before proceeding with the experimental protocol. All the bacteria were isolated from clinical samples.

Generation of the growth curves

The bacteria were cultured aerobically with shaking in 500 ml of BHI at 37°C. After 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, and 16 h, 1 ml of the suspension was removed to measure optical density at 600 nm using a Multiskan Spectrum spectrophotometer (Thermo Ramsey, Minnesota, USA). The measurements were corrected with a blank using pure BHI. All readings were repeated three times.

Cell counting and optical density detection

After 7 h of incubation, the bacteria were diluted in a 1/10 dilution series. One hundred microlitres of each dilution was added to a BHI plate with 1.5% agarose (Amresco, OH, USA) for cell counting; this procedure was repeated three times for each dilution.

Meanwhile, the primary suspension was diluted with pure BHI broth in a 1/2 dilution series for OD600 detection using both a Multiskan Spectrum and an N721 spectrophotometer (Jingke, Shanghai, China). OD600 detection was performed at both 4°C and 37°C to reveal the influence of temperature on the relationship between OD600 and CFU, resulting from the fact that OD values vary when temperature changes[7]. The measurements were corrected with a blank containing the same BHI utilised for dilutions. All measurements were repeated at least three times.

Statistical analysis

A model to simulate the relationship between OD600 and CFU measurement was generated using Microsoft Excel 2007 (Microsoft Corporation). Significant differences were analysed using the Wilcoxon signed-rank test. Differences were considered statistically significant when $P<0.05$.

RESULTS

Generation of the growth curves

To verify the exponential growth phase of each strain, we measured OD600 values of the bacterial strains over a series of time points while culturing with shaking. The data are shown in Figure 1. Although each bacterium own different

Table 1. Two Simulated Functional Relationships of the Data Detected with a Multiskan Spectrum Spectrophotometer at 37°C

Bacteria	Line Relationship		Power Correction	
	Line Relationship	R^2	Power Correction	R^2
Escherichia coli	y = 6.7671x - 0.1866	0.9836	$y = 5.1319x^{0.9027}$	0.989
Pseudomonas aeruginosa	y = 1.7802x - 0.0191	0.9924	$y = 1.7112x^{1.0159}$	0.9979
Klebsiella pnenmoniae	y = 9.6989x - 0.1701	0.9891	$y = 7.9378x^{0.9495}$	0.9965
Staphylococcus aureus	y = 4.5337x - 0.2078	0.9631	$y = 3.6153x^{1.0828}$	0.9963
Enterococcus faecium	y = 1.8696x - 0.049	0.9797	$y = 1.465x^{0.9341}$	0.9931
Bacillus subtilis	y = 1.3324x - 0.0319	0.9904	$y = 1.1558x^{1.0057}$	0.9976
Average		0.983±0.011		0.995±0.003*

OD600 was detected with the Multiskan Spectrum Spectrophotometer at 37°C. These formulas were generated automatically when simulating functions between OD600 and CFU in the exponential growth phase of bacteria with Microsoft Excel 2007. Y was cell count of bacteria. X was OD600 value at 37°C. R^2 represented degree of fitting for function. * $p<0.05$.

exponential growth phases, the period from 4 h to 9 h in their life times was the common exponential growth phase. Thus, we could choose the bacteria in this period to do further experiments.

Functional Relationships of the Data Detected with a Multiskan Spectrum Spectrophotometer at 37°C

To highlight the relationship between optical density and CFU in the exponential growth phase of these bacteria, we sampled bacterial

Table 2. Two Simulated Functional Relationships of the Data Detected with a Multiskan Spectrum Spectrophotometer at 4°C

Bacteria	Line Relationship		Power Correction	
	Line Relationship	R^2	Power Correction	R^2
Escherichia coli	y = 6.8353x - 0.1909	0.9852	$y = 5.8137x^{0.9969}$	0.9979
Pseudomonas aeruginosa	y = 1.7351x - 0.0193	0.988	$y = 1.3722x^{0.8601}$	0.9926
Klebsiella pnenmoniae	y = 9.7644x - 0.1955	0.9861	$y = 8.0504x^{0.9753}$	0.9961
Staphylococcus aureus	y = 4.424x - 0.1742	0.9711	$y = 3.4762x^{1.0309}$	0.9936
Enterococcus faecium	y = 1.8254x - 0.0556	0.9615	$y = 1.4384x^{0.9481}$	0.9946
Bacillus subtilis	y = 1.3433x - 0.0267	0.9892	$y = 1.149x^{0.9555}$	0.9964
Average		0.980±0.011		0.995±0.002*

OD600 was detected with the Multiskan Spectrum Spectrophotometer at 37°C. These formulas were generated automatically when simulating functions between OD600 and CFU in the exponential growth phase of bacteria with Microsoft Excel 2007. Y was cell count of bacteria. X was OD600 value at 37°C. R^2 represented degree of fitting for function. * $p<0.05$.

Table 3. Two Simulated Functional Relationships of the Data Detected with the N721 Spectrophotometer at 37°C

Bacteria	Line Relationship		Power Correction	
	Line Relationship	R^2	Power Correction	R^2
Escherichia coli	y = 6.0697x - 0.4641	0.9557	$y = 4.2245x^{1.0749}$	0.9896
Pseudomonas aeruginosa	y = 0.7337x - 0.0116	0.9935	$y = 0.6358x^{0.9414}$	0.9967
Klebsiella pnenmoniae	y = 7.7259x - 0.3569	0.9833	$y = 6.6094x^{1.1618}$	0.9961
Staphylococcus aureus	y = 3.7038x - 0.2194	0.9465	$y = 2.7535x^{1.0373}$	0.9929
Enterococcus faecium	y = 1.5203x - 0.0817	0.9517	$y = 1.1438x^{1.0187}$	0.9932
Bacillus subtilis	y = 1.1483x - 0.043	0.9857	$y = 0.9427x^{1.0324}$	0.9961
Average		0.969±0.02		0.994±0.003*

Y was cell count of bacteria. X was OD600 value at 37°C. R^2 represented degree of fitting for function. * $p<0.05$.

Table 4. Two Simulated Functional Relationships of the Data Detected with the N721 Spectrophotometer at 4°C

Bacteria	Line Relationship		Power Correction	
	Line Relationship	R^2	Power Correction	R^2
Escherichia coli	y = 6.0022x - 0.4452	0.9639	$y = 4.4305x^{1.1403}$	0.9932
Pseudomonas aeruginosa	y = 1.4992x - 0.0315	0.9941	$y = 1.2931x^{1.0035}$	0.9969
Klebsiella pnenmoniae	y = 7.8084x - 0.3641	0.978	$y = 6.5126x^{1.1267}$	0.9977
Staphylococcus aureus	y = 3.789x - 0.2082	0.9596	$y = 3.0614x^{1.1146}$	0.9943
Enterococcus faecium	y = 1.5505x - 0.1104	0.943	$y = 1.1061x^{1.1047}$	0.9907
Bacillus subtilis	y = 1.1689x - 0.0456	0.9796	$y = 1.0387x^{1.1231}$	0.9972
Average		0.97±0.018		0.995±0.003*

Y was cell count of bacteria. X was OD600 value at 37°C. R^2 represented degree of fitting for function. * $p<0.05$.

cultures after 7 h of shake-culturing for OD600 detection with a Multiskan Spectrum spectrophotometer (supporting information, Tables 1S-6S). Simultaneously, cell plate-counting was carried out. The CFU of the six bacterial strains were 10.5×10^{11} CFU/ml for *E. coli*, 2.13×10^{12} CFU/ml for *P. aeruginosa*, 11.5×10^{9} CFU/ml for *K. pneumoniae*, 6.2×10^{12} CFU/ml for *S. aureus*, 2.56×10^{10} CFU/ml for *E. faecium*, and 1.89×10^{11} CFU/ml for *B. subtilis*. Then, we used Excel 2007 to

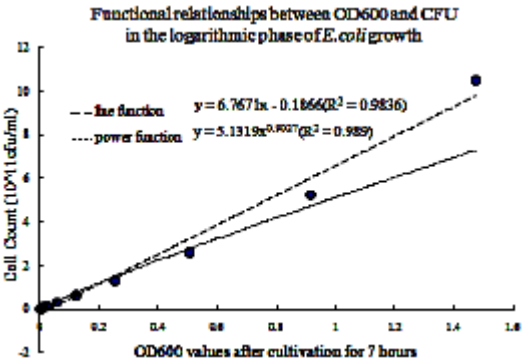

Fig. 1. Two Simulated Curves between OD600 and Cell Counts in *Escherichia Coli*

The two curves, line function and power function, were simulated by Excel 2003, using the data of cell count diluted serially by pure BHI at 37°C and OD600 values measured by Multiskan Spectrum. (—) line function; the equation was fitted as follows: y = 6.7671x - 0.1866, and the coefficient of correlation (r^2) was 0.9836. (%) power function; the equation was fitted as follows: y = $5.1319x^{0.9027}$, and r^2 was 0.989. The operation was repeated at least three times.

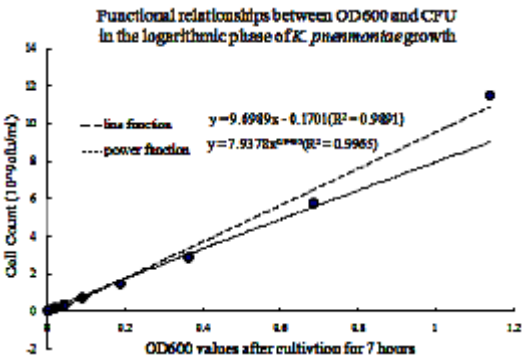

Fig. 2. Two Simulated Curves between OD600 and Cell Counts in *Klebsiella Pnenmoniae*

The two curves, line function and power function, were simulated by Excel 2003, using the data of cell count diluted serially by pure BHI at 37°C and OD600 values measured by Multiskan Spectrum. (—) line function; the equation was fitted as follows: y = 9.6989x - 0.1701, and the coefficient of correlation (r^2) was 0.9891. (%) power function; the equation was fitted as follows: y =$7.9378x^{0.9495}$, and r^2 was 0.9965. The operation was repeated at least three times.

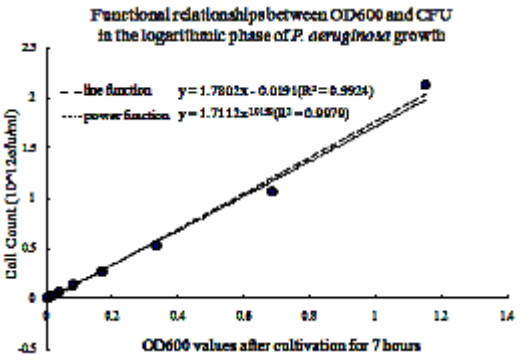

Fig. 3. Two Simulated Curves between OD600 and Cell Counts in *Pseudomonas Aeruginosa*

The two curves, line function and power function, were simulated by Excel 2003, using the data of cell count diluted serially by pure BHI at 37°C and OD600 values measured by Multiskan Spectrum. (—) line function; the equation was fitted as follows: y = 1.7802x - 0.0191, and the coefficient of correlation (r^2) was 0.9924. (%) power function; the equation was fitted as follows: y = $1.7112x^{1.0159}$, and r^2 was 0.9979. The operation was repeated at least three times.

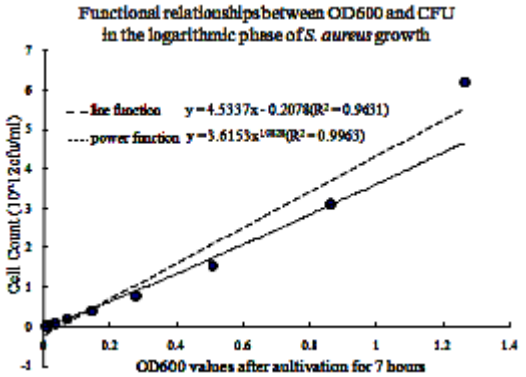

Fig. 4. Two Simulated Curves between OD600 and Cell Counts in *Staphylococcus Aureus*

The two curves, line function and power function, were simulated by Excel 2003, using the data of cell count diluted serially by pure BHI at 37°C and OD600 values measured by Multiskan Spectrum. (—) line function; the equation was fitted as follows: y = 4.5337x - 0.2078, and the coefficient of correlation (r^2) was 0.9631. (%) power function; the equation was fitted as follows: y = $3.6153x^{1.0828}$, and r^2 was 0.9963. The operation was repeated at least three times.

simulate two types of matching curves (supporting information, Figure 1S-6S): the line relationship and the power correction. Table 1 lists the simulated functions and their coefficients of determination (R^2) for all six bacteria. R^2 represents the degree of fit for the modelled function. The closer to 1 the R^2 value was, the better the function matches. The average R^2 values were 0.983±0.011 for the line relationship and 0.995±0.003 for the power correction; the average R^2 of the power correction was higher than that of the line relationship (P<0.05), indicating that the power function more closely modelled the relationship between OD600 values and bacterial cell counts.

Functional Relationships of the Data Detected with the Multiskan Spectrum Spectrophotometer at 4°C

As OD values vary when temperature changes, we measured OD600 values (supporting information, Tables 1S-6S) with the Multiskan Spectrum spectrophotometer at 4°C using the same broth that was measured at 37°C. Under different temperatures, OD600 values were different, but the differences were minor (Fig 2). Table 2 shows the simulated functions and their R^2 values for all six bacteria. The average R^2 value of the line relationship was 0.980±0.011, and the average R^2 value of the power correction was 0.995±0.002. The degree of fitting for power correction was significantly higher than that of the line relationship (p<0.05), indicating that temperature had no effect on the correction relationship between OD600 and cell counts in bacteria at exponential growth stage.

Functional Relationships of the Data Detected with the N721 Spectrophotometer

As different spectrophotometers detection may display different OD600 values for the same broth sample. To interpret the impact of spectrophotometers on the functional relationships, we also measured OD600 values with an N721 spectrophotometer at both 4°C and 37°C. Fig 2 manifests that though OD600 values detected by N721 spectrophotometer at 4°C were different from that at 37°C, the differences were minor. Curves generated at the two temperatures are almost the same. Both table 3 and table 4 showed that, at 4°C and 37°C, the power function more closely modelled the relationship between bacterial cell counts and OD600 values measured by the N721 Spectrophotometer. All the data indicating that, the power function remained the better match for the relationship between OD600 and bacterial

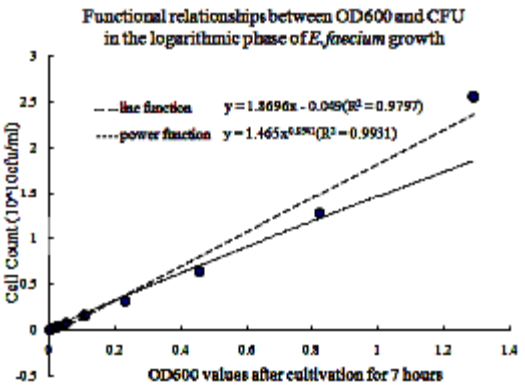

Fig. 5. Two Simulated Curves between OD600 and Cell Counts in *Enterococcus Faecium*

The two curves, line function and power function, were simulated by Excel 2003, using the data of cell count diluted serially by pure BHI at 37°C and OD600 values measured by Multiskan Spectrum. (—) line function; the equation was fitted as follows: y = 1.8696x - 0.049, and the coefficient of correlation (r^2) was 0.9797. (%) power function; the equation was fitted as follows: y = $1.465x^{0.9341}$, and r^2 was 0.9931. The operation was repeated at least three times.

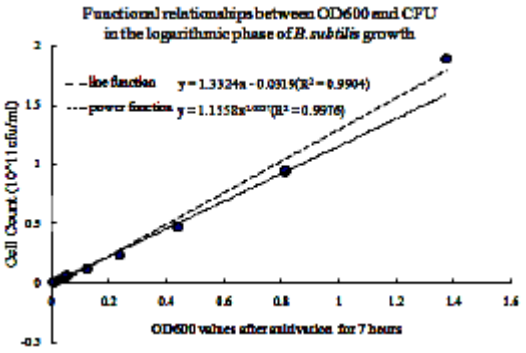

Fig. 6. Two Simulated Curves between OD600 and Cell Counts in *Bacillus Subtilis*

The two curves, line function and power function, were simulated by Excel 2003, using the data of cell count diluted serially by pure BHI at 37°C and OD600 values measured by Multiskan Spectrum. (—) line function; the equation was fitted as follows: y = 1.3324x - 0.0319, and the coefficient of correlation (r^2) was 0.9904. (%) power function; the equation was fitted as follows: y = $1.1558x^{1.0057}$, and r^2 was 0.9976. The operation was repeated at least three times.

cell counts, with no influence from different temperatures and spectrophotometers.

DISCUSSION

For experiments with bacteria, cell counts are routinely required [8, 9]. As optical density is related to the turbidity of a solution and is easy to detect, this method of predicting bacterial counts is commonly utilized in lab studies. Previous studies have shown that a linear function could predict the relationship between optical density and CFU. However, these observations were made during the lag phase of bacterial growth, when low cell viability and a large number of dead bacteria are present. Usually, the exponential phase of bacterial growth is the most desirable for experimentation, as bacteria are highly viable with little mortality under these conditions. Our data were collected during the exponential phase of six bacterial cultures, including Gram-positive and Gram-negative bacteria, cocci and bacilli. Additionally, the chosen strains represented organisms of different sizes, with the colonies of *P. aeruginosa* and *S. aureus* being visibly smaller than these of the other bacteria when cultured for the same time. Thus, the collected data are representative of typical laboratory needs and accurately reflect the relationship between optical density and cell count during the exponential growth phase of bacteria.

Optical density is influenced by environmental factors, such as temperature and detecting instrument. These factors often change while operation is handled by different people, at different time, or in different labs. As the detecting conditions change, OD600 values vary. This may influence the simulated relationships between OD600 and cell count. In our study, we investigated it with two spectrophotometers under two temperatures. Though different OD values gained when spectrophotometer and/or temperature varied, the fact remained consistent that the power functional relationship was more suitable than the line function. However, the coefficients of power function changed while the detecting conditions were different. Thus the common power functional relationship, not a common equation, could be availbale. But when the conditions are unchangeable, a rough equation could be gained to satisfy the experiment.

In short, based on these data, the power function relationship accurately illustrates the relationship between optical density and cell count during the exponential growth phase of bacteria.

ACKNOWLEDGEMENTS

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