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2D motility tracking of *Pseudomonas putida* KT2440 in growth phases using video microscopy

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Abstract

Pseudomonas putida KT2440 is a gram negative motile soil bacterium important in bioremediation and biotechnology. Thus, it is important to understand its motility characteristics as individuals and in populations. Population characteristics were determined using a modified Gompertz model. Video microscopy and imaging software were utilized to analyze two dimensional (2D) bacteria movement tracks to quantify individual bacteria behavior. It was determined that inoculum density increased the lag time as seeding densities decreased, and that the maximum specific growth rate decreased as seeding densities increased. Average bacterial velocity remained relatively similar throughout exponential growth phase (~20.9 $\mu\text{m}/\text{sec}$), while maximum velocities peak early in exponential growth phase at a velocity of 51.2 $\mu\text{m}/\text{sec}$. *Pseudomonas putida* KT2440 also favor smaller turn angles indicating they often continue in the same direction after a change in flagella rotation throughout the exponential growth phase.

Keywords

Pseudomonas putida KT2440; bacterial motility; cell growth; video microscopy

Pseudomonas putida KT2440 is a model saprophytic gram negative soil bacteria (1,2). The genome was sequenced in 2002, which provides insights into the pathways for metabolism and other cellular functions (2). Since KT2440 is well known for its ability to catabolize aromatic compounds such as toluene and benzoate, extensive research has investigated these metabolic pathways through genomic analysis (3). Toluene and benzoates are both toxins present in soil and water pollution and *P. putida*'s affinity to these toxins is useful in bioremediation applications. An understanding of these pathways and characteristics is a benefit for bioremediation and other biotechnologies (4).

Bacterial growth is also of interest when understanding the properties and characteristics of a bacterial population. Bacterial growth has been studied extensively, and each phase of the bacterial growth curve has been examined based on many parameters. There are many factors that influence the phases of a growth curve. The lag phase is effected by many variables and can vary greatly depending on the variables, which increases the difficulty of

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determining the lag phase parameters (5). However, the exponential phase of bacteria growth is known for its consistency, with a constant growth rate and metabolite uptake, which allows for the bacteria to approach a steady state system(6). The exponential phase is an important part of bioremediation, which often involves stimulating microbial growth to increase bioremediation efforts (7). Quantification of bacteria motility using 2D and 3D tracking has led to insights and modeling of chemotactic behavior in porous media, which will benefit the set up of bioremediation processes (8). For these reasons we investigated the motility of *P. putida* KT2440 during the exponential phase of growth.

Various assays exist to quantify motility and chemotactic behavior of bacterial populations such as: stab test, soft agar swarm plate, capillary, agarose plug, and densitometry (9–17). All of these assays help in the quantification of the bacteria affinity for a chemoattractant, but they don't allow for a quantifiable method for individual bacterial cells. Video microscopy allows for the quantification of bacteria in real time and for analysis of their motility. Three dimensional tracking of individual bacteria has been accomplished by microscopes that automatically track the moving cells (18,19). In this analysis, behavior is seen in the x, y, and z plane. Two dimensional tracking has also been used for quantifying bacterial movement (20). Video microscopy has an advantage over other methods when working on quantifying individual bacteria behaviors. In order to quantify the motility of KT2440, we used 2D video tracking with imaging analysis software capable of calculating individual bacteria characteristics. To increase understanding on the growth and motility of KT2440, we examined the growth parameters at varying inoculation densities, and quantified the motility of the bacteria in exponential growth phase.

Materials and Methods

Atomic force microscopy (AFM) and scanning electron microscopy (SEM) imaging

AFM image and analysis were performed with a PicoPlus AFM controlled by PicoScan 5.4 from Agilent Technologies (Chandler, AZ, USA). Samples were measured in air with a spring constant of the cantilever (Veeco, USA) calibrated to 0.06 N/m with tip length between 0.4 ~ 0.7 μm , and Si_3N_4 tip curvature radius of approximately 10 nm. SEM images were taken with a Hitachi S-4000 at 10 kV with 20,000 $\times$ magnification.

Bacteria culture and growth parameters

Pseudomonas putida KT2440 was acquired from ATCC (47054, Manassas, VA, USA). Bacteria from -80°C glycerol stocks were inoculated into 50 ml of LB broth (Fischer scientific, Fair Lawn, NJ, USA). The bacteria were then cultured to an OD_{600nm} of 2.0. New cultures in LB with initial OD_{600nm} of 0.01, 0.05, and 0.09 were used to measure growth curves over a 24 hour period.

The optical density growth curves allow for determination of the maximum specific growth rate (μ_m), the lag time (λ), and the maximum absorbance or asymptote (A). The maximum specific growth rate is defined as the slope of the line tangent to the inflection point of the growth curve. The y intercept of this line is considered the lag time. The lag phase is the time the bacteria take to adjust to their environment and enter into the exponential growth phase. The data sets were fit using a Gompertz equation provided in Origin Pro 8.0 from OriginLab (Northampton, MA, USA) as seen in equation 1, where a is the asymptote, x_c is the center of the inflection point, and k is a rate coefficient. The parameters of the Gompertz equation can be modified to include the growth parameters of bacterial growth (21). Following the methods of Zwietering et al.(21), we determined the growth parameters using the modified Gompertz model (equation 2), where, μ_m was determined using equation 3, λ was determined using equation 4, and a is equal to A .

$$y = a * e^{\{-e^{(-k*(x-x_c))}\}} \quad (1)$$

$$y = A * e^{\left\{-e^{\left(\frac{\mu_m * e}{A} (\lambda_G - t) + 1\right)}\right\}} \quad (2)$$

$$\mu_m = \frac{a * k}{e} \quad (3)$$

$$\lambda = \frac{k * x_c - 1}{k} \quad (4)$$

Bacteria samples were cultured on agar plates at various optical densities over a 72 hour period. This relates the measured optical densities to visual cell counts.

Bacterial mobility tracking

Motility during the exponential phase of *P. putida* KT2440 growth was quantified from 0.2–0.8 OD from 0.05 OD seeded cultures. An inverted microscope (Olympus IX71, Center Valley, PA, USA) with digital CCD camera (Olympus DP30BW) was used to collect video (15 frames/sec) for motility analysis. Once the bacterial population reached the desired optical density, an undiluted 5 μ L drop was placed on a cover slide and a 30 second video was recorded under an UPlanSApo 60X objective from Olympus.

Recorded videos were then transferred to Image-Pro Plus v6.1 (Media Cybernetics, Bethesda, MD, USA) for video enhancement and quantification of motility. Videos were enhanced first by using the difference of frames operation provided by Image-Pro Plus. The difference operation calculates the differences in the last frame from the first frame, which allows for the digital removal of bacteria that are stuck to the slide and bacteria that are motionless. This eliminates the possibility of some non motile bacteria from being tracked [21]. The fine detail of the bacterial motility videos was enhanced using a sharpen filter offered in Image-Pro Plus software. The sharpen filter enhances the edges of the bacteria allowing for more contrast with the background.

Video sequences, after video enhancement, were calibrated to the average size of *P. putida* KT2440. The spatial calibration of the images allows for various parameters of the bacterial tracks to be monitored, such as turn angle, accumulated distance, X and Y coordinates, and velocity. This is accomplished by relating the pixel size to physical distances. Once spatial calibration was completed, 2D bacterial tracks were measured automatically using the automatic track objects operation in Image-Pro Plus. Bacterial tracks are determined on image intensities with respect to the background. Contrast was adjusted to allow for the tracking of over 100 bacterial tracks for each sample. Each individual bacteria track was monitored over the entire video sequence as long as the bacterium remained in the field of view. Since the bacteria move in and out of the field of view during recording, multiple bacteria tracks were recorded to determine averages for the bacterial motility.

Using Image-Pro Plus automatic tracking feature velocity tracks were analyzed to reflect cell motility. Tracks with an average velocity slower than 17 μ m/sec. were eliminated before

analysis as to remove any non-motile bacteria or bacteria stuck to the slide (19,20). An average velocity was then recorded for all the tracks recorded in each OD of the growth curve, along with the average maximum velocity of the recorded tracks. The standard deviation (SD) was determined for the average maximum velocity and overall average velocity for each bacteria culture based on the number of motile tracks recorded.

Results and Discussion

Topography and morphology characterization of the cells

P. putida KT2440 are motile due to a polar tuft of flagella as shown in the AFM image in Figure S1A. It has been observed that when the flagella rotate in a counter clockwise direction the bacteria move in a smooth direction, while a reversal of the flagella results in a change of direction (20). The size and morphology of the bacteria was also determined from the AFM and SEM images (Figure S1B, S1C and S1D), which was measured to be approximately 2 μm in length and 1 μm in width.

Cell growth analysis

The seeding density had an effect on the specific growth rate and lag times of the bacterial cultures (Figure S2A). The lowest optical seeding density of 0.01 OD yielded the highest specific growth rate ($\mu_m = 1.28 \text{ hr}^{-1}$) and longest lag time ($\lambda = 3.42 \text{ hr}$) of the seeding densities. In contrast the highest seeding density of 0.09 OD had the slowest specific growth rate ($\mu_m = 0.67 \text{ hr}^{-1}$), and the shortest lag time $\lambda = 1.19 \text{ hr}$, while the seeding density of 0.05 OD had a specific growth rate and lag time similar to the 0.09 seeding density, as reported in Table 1. The decreased specific growth rate with increased seeding density has also been seen in a different strains of *Pseudomonas putida* (22). The differences in Table 1 of the asymptote values (A) are due to the different values of the absorbance at inoculation and also due to the calculation of the natural log of absorbance at time t over the inoculum absorbance. The recorded ODs at the asymptote for all three inoculum sizes are very similar (data not shown). Lag time is influenced by many factors including environmental and inoculum conditions (5); we only took into account the inoculum condition. To achieve an exponential curve with an adequate lag time and growth rate, cultures for video microscopy measurements were started with an inoculum density of 0.05 OD. Although for these studies we only observed individual motility of the bacteria at this inoculum density, we present a technique that involves the determination of population growth parameters while determining individual bacteria motility. This technique can be applied to future research in determining bacterial population and individual characteristics.

A relationship between the cell populations to the measured optical density for each of the measurement points of the early exponential phase, where motility was recorded using video microscopy from 0.05 OD seeded cultures was obtained (Figure S2B). It can be seen based on the linear fit of the logarithmic plot that the bacteria populations were indeed in the exponential phase of growth for each of the bacteria samples selected for video microscopy.

Video enhancement

In Figure 1, column one is an example image from the videos recorded at each OD. The bacterial tracks were added after the automatic tracking feature had been used on the enhanced videos. Column two of Figure 1 is an image of the bacteria after the video enhancements from the same frame as in Figure 1, column 1. It can be seen in the enhanced video images that the bacteria stuck to the slide were eliminated by the difference function. The enhancements allowed for better automatic tracking of bacterial motility. A surface plots for the corresponding images are shown in column three, giving an idea of the intensity differences between the bacteria and the background. The surface plot images were tilted to

allow for better viewing of the intensity differences and track labels were added. Figure 2 demonstrates the bacterial movement over a series of frames that represents a 5 second clip from the enhanced video of the 0.4 OD video sequence. Each circle on the linked tracks represents a frame as the bacteria moves through the field of view.

Tracking bacterial movement analysis

Figure 3 is an example from 0.2 OD measurements of each bacterial track recorded for accumulated distance (Figure 3A) and velocity (Figure 3B) versus the frame. Figure 3 includes all tracks prior to tracks being removed for non motility. Since Figure 3 includes all motile and non motile tracks, variations in the slope of Figure 3A can be seen. Bacteria tracks with a lesser slope represent bacteria with slower velocities and are deemed non motile if they fall below the 17 $\mu\text{m}/\text{sec}$ cut off, which excluded them from further investigation in this study. Bacterial tracks with a steeper slope represent bacteria with increased velocities and provided the data for further investigation into their motility. Each line represents a different bacterium as the bacteria move around in the field of view. The Image-Pro Plus software allows for multiple bacteria to be monitored simultaneously as demonstrated with the overlapping accumulated distances and velocities at various frames in the video sequence as the bacteria go through their runs and changes of direction (See Figure 3A and 3B). The ability to track multiple objects allows for quantification of larger bacterial populations as with our undiluted samples.

Figure 4 is a box plot for the maximum velocities recorded for each track of each OD step. The average maximum velocity peaked at 0.3 OD ($51.2 \pm 11.2 \mu\text{m}/\text{sec}$), which is early in the exponential phase. After 0.3 OD the average maximum velocities are very similar ($\sim 29 \sim 39 \pm 4.7 \mu\text{m}/\text{sec}$). It is also interesting to note that the average velocity for each OD is similar ($19 \sim 22 \pm 3.4 \mu\text{m}/\text{sec}$), but that the maximum velocities show more contrast (see Table 2). This variation in maximum velocity could indicate increase activity as the bacteria continue through exponential growth phase. In contrast, it has been reported that for *E. coli*, motility speed was low in early exponential phase and peaked in post exponential phase (23). It has also been previously reported by that *P. putida* PRS2000 has an average velocity between 27.5 and 44 $\mu\text{m}/\text{sec}$, with a maximum velocity between 53.8 and 75 $\mu\text{m}/\text{sec}$ (19,20) these measurements were made with 3D and 2D tracking system respectively. Our average reported velocities are similar to the 3D tracking system employed by Duffy and Ford (19). It is also noted that the average and maximum velocities for *P. putida* KT2440 measured in our experiment are slightly lower than previous data and most similar at the 0.3 OD velocity measurements. This decrease in velocity could be attributed to the inability of measuring bacteria movements in the orthogonal direction using a 2D tracking method. Since movement in this direction would take the bacteria out of focus, it is assumed that the Image-Pro Plus video enhancements removed measurements from bacteria moving in the orthogonal direction. Since this number can't be determined there will be an inherent error to the 2D tracking results. Future research will look into reducing this error, allowing for the increased accuracy of 2D tracking systems and the valuable information that is obtained. Even with the use of a 2D video tracking system, trends in the bacterial motility can be observed.

To examine the characteristics of direction for the *P. putida* KT2440 in exponential phase we looked at the displacement distance of the bacteria as they traveled through the medium. The bacterial tracks were normalized so that each bacterial track started at the coordinates (0, 0). This was accomplished by taking the difference between the coordinate at time t from the initial coordinate at the start of the video sequence. The displacement tracks were created for each of the bacterial tracks that were considered motile with average velocities greater than 17 $\mu\text{m}/\text{sec}$. The general trend, as seen in Figure 5, is that the bacteria tend to continue in the same direction. This coincides with what has previously been reported for

Pseudomonas putida PRS2000 (19,20), where after a reversal in flagella takes place the bacteria most likely continue in a straight line. We have demonstrated that this also holds true for *Pseudomonas putida* KT2440 and that as the bacterial population increases this behavior is not affected. This provides a good basis for future experiments determining chemotactic behavior during population growth.

To further investigate this behavior we also looked at turn angle distributions of the various cultures. The turn angle is the angle measured between the end of one run to the start of a new run for a cell (18). Turn angle is a factor also used for modeling bacterial movements in porous media (8). Image-Pro Plus was utilized to determine the angle between recorded frames for each bacterium. We used these angles to determine the angle distribution of the motile bacteria tracks. If the movement of bacteria was random a turn angle of 90° would dominate the maximum frequency (18). However, the distribution observed for a majority of the cultures is a bimodal distribution with peaks ranging from 16° to 66° for the first peak, and peaks ranging from 111° to 160° for the second peak of the distributions, as seen in Figure 6 where the data was fit with polynomials. There is a stronger tendency to the smaller angles. The lower peak angles indicate that the bacteria continue on in the same direction, while the higher peak angles indicate a change of direction, with a favor towards lower angles the bacteria tend to move in the same direction as seen with *Pseudomonas putida* PRS2000 (19,20).

We conclude that *P. putida* KT2440 are highly motile bacteria that grow quickly and are useful in the catabolism of aromatic compounds. The ability of KT2440 to degrade aromatic compounds makes them a primary candidate for bioremediation projects. The use of video microscopy allows for more insights in the behavior of motile bacteria, such as velocity measurements and angle distributions. Other methods to monitor motility provide valuable information, but do not provide insight into the movement of individual bacteria. Video microscopy, with high quality imaging software such as Image-Pro Plus, provides a more in depth analysis of the motility of *P. putida* KT2440 during the exponential phase of growth. Other methods of motility analysis make it difficult to pinpoint one stage of bacterial growth for analysis, making video microscopy a better choice. Due to the capability of obtaining quantitative information of bacterial motility through video microscopy, it makes it possible to quantify bacterial chemotaxis behavior in the presence of a chemoattractant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Bagdasarian M, Lurz R, Ruckert B, Franklin FCH, Bagdasarian MM, Frey J, Timmis KN. Specific-purpose plasmid cloning vectors II. Broad host range, high copy number, RSF1010-derived vectors, and a host-vector system for gene cloning in *Pseudomonas*. Gene. 1981; 16:237–247. [PubMed: 6282695]
2. Nelson KE, Weinl C, Paulsen IT, Dodson RJ, Hilbert H, Martins dos Santos VAP, Fouts DE, Gill SR, Pop M, Holmes M, et al. Complete genome sequence and comparative analysis of the

- metabolically versatile *Pseudomonas putida* KT2440. Environ. Microbiol. 2002; 4:799–808. 33 authors. [PubMed: 12534463]
3. Jimenez JI, Minambres B, Garcia J, Diaz E. Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440. Environ. Microbiol. 2002; 4:824–841. [PubMed: 12534466]
 4. Timmis KN. *Pseudomonas putida*: a cosmopolitan opportunist par excellence. Environ. Microbiol. 2002; 4(12):779–781. [PubMed: 12534460]
 5. Swinnen IAM, Bernaerts K, Dens EJJ, Geeraerd AH, Van Impe JF. Predictive modelling of the microbial lag phase: a review. Int. J. Food Microbiol. 2004; 94:137–159. [PubMed: 15193801]
 6. Monod J. The Growth of Bacterial Cultures. Annu. Rev. Microbiol. 1949; 3:371–394.
 7. Lee K, Park J-W, Ahn I-S. Effect of additional carbon source on naphthalene biodegradation by *Pseudomonas putida* G7. J. Hazard. Mater. 2003; 105:157–167. [PubMed: 14623425]
 8. Ford RM, Harvey RW. Role of chemotaxis in the transport of bacteria through saturated porous media. Adv. Water Resour. 2007; 30:1608–1617.
 9. Adler J. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. 1973; 98:77–91. [PubMed: 4632978]
 10. Childers SE, Ciuffo S, Lovley DR. *Geobacter metallireducens* accesses insoluble Fe(III) oxide by chemotaxis. Nature. 2002; 416:767–769. [PubMed: 11961561]
 11. Grimm AC, Harwood CS. Chemotaxis of *Pseudomonas* spp. to the polyaromatic hydrocarbon naphthalene. App. Environ. Microbiol. 1997; 63:4111–4115.
 12. Harwood CS, Nichols NN, Kim MK, Ditty JL, Parales RE. Identification of the *pcaRKF* gene cluster from *Pseudomonas putida*: involvement in chemotaxis, biodegradation, and transport of 4-hydroxybenzoate. J. Bacteriol. 1994; 176:6479–6488. [PubMed: 7961399]
 13. Ortega-Calvo JJ, AIM, Vorobyov AV, Borovick RV. Chemotaxis in polycyclic aromatic hydrocarbon-degrading bacteria isolated from coal-tar- and oil-polluted rhizospheres. FEMS Microbiol. Ecol. 2003; 44:373–381. [PubMed: 19719618]
 14. Marx RB, Aitken MD. Bacterial Chemotaxis Enhances Naphthalene Degradation in a Heterogeneous Aqueous System. Environ. Sci. Technol. 2000; 34:3379–3383.
 15. Parales RE, Ditty JL, Harwood CS. Toluene-Degrading Bacteria Are Chemotactic towards the Environmental Pollutants Benzene, Toluene, and Trichloroethylene. App. Environ. Microbiol. 2000; 66:4098–4104.
 16. Samanta SK, Jain RK. Evidence for plasmid-mediated chemotaxis of *Pseudomonas putida* towards naphthalene and salicylate. Can. J. Microbiol. 2000; 46:1–6. [PubMed: 10696467]
 17. Tittsler RP, Sandholzer LA. The Use of Semi-solid Agar for the Detection of Bacterial Motility. J. Bacteriol. 1936; 31:575–580. [PubMed: 16559914]
 18. Berg HC, Brown DA. Chemotaxis in *Escherichia coli* analysed by Three-dimensional Tracking. Nature. 1972; 239:500–504. [PubMed: 4563019]
 19. Duffy KJ, Ford RM. Turn angle and run time distributions characterize swimming behavior for *Pseudomonas putida*. J. Bacteriol. 1997; 179:1428–1430. [PubMed: 9023235]
 20. Harwood CS, Fosnaugh K, Dispensa M. Flagellation of *Pseudomonas putida* and analysis of its motile behavior. J. Bacteriol. 1989; 171:4063–4066. [PubMed: 2738028]
 21. Zwietering MH, Jongenburger I, Rombouts FM, Van 't Riet K. Modeling of the Bacterial Growth Curve. App. Environ. Microbiol. 1990; 56:1875–1881.
 22. Smets BF, Rittmann BE, Stahl DA. The specific growth rate of *Pseudomonas putida* PAW1 influences the conjugal transfer rate of the TOL plasmid. App. Environ. Microbiol. 1993; 59:3430–3437.
 23. Amsler CD, Cho M, Matsumura P. Multiple factors underlying the maximum motility of *Escherichia coli* as cultures enter post-exponential growth. J. Bacteriol. 1993; 175:6238–6244. [PubMed: 8407796]

Fig.1. Selected frames for each bacterial culture during 30 second video capture where column 1) is images before Image-Pro Plus enhancement with labeled bacteria, column 2) images after frame difference operation and sharpen filter of Image-Pro Plus with labeled bacteria, and column 3) surface plot of enhanced images to demonstrate intensity differences. Surface plots were angled and tilted to show 3D intensities, and labeled manually.

Fig.2.

Motility movements recorded and analyzed with automatic tracking tracks of bacteria for 0.4 OD over a 5 second period where A) 0.2 B) 1.1 C) 2.0 D) 2.4 E) 3.5 F) 5 seconds. Tails indicate locations where bacteria was in a previous frame (15 frames/sec).

Fig.3. Measured (A) accumulated distance and (B) velocity over recorded frames of 0.2 OD enhanced video from Image-Pro Plus automatic tracking feature.

Fig.4.
Box plots for bacterial cultures during the exponential phase of growth after the removal of non motile tracks.

Fig.5.

Distance displacement for each of bacterial cultures in the exponential phase. Each bacterial track was normalized to start at position (0,0), where A) 0.2 OD, B) 0.3 OD, C) 0.4 OD, D) 0.6 OD, E) 0.7 OD, and F) 0.8 OD. Each line indicates one individual cells' movement pathway.

Fig.6. Turn angle distributions for A) 0.2 OD, B) 0.3 OD, C) 0.4 OD, D) 0.6 OD, E) 0.7 OD, and F) 0.8 OD for motile tracks from enhanced video.

Table 1

Growth Parameters determined from Gompertz model for inoculation densities

Parameters	0.01 OD	0.05 OD	0.09 OD
μ_m (hr ⁻¹)	1.28	0.70	0.67
A	5.27	3.38	4.14
λ (hr)	3.42	1.24	1.19

Table 2

Average maximum velocity and average velocity for motile tracks of cultures with SD_n measured depending on number of motile tracks (n) of the cultures

OD	average max Velocity ($\mu\text{m}/\text{sec}$)	average Velocity ($\mu\text{m}/\text{sec}$)
0.2	29.4 ± 4.2	20.4 ± 3.1
0.3	51.2 ± 11.3	22.2 ± 3.5
0.4	35.0 ± 4.8	22.2 ± 4.5
0.6	29.3 ± 2.9	19.4 ± 3.5
0.7	31.6 ± 3.8	20.7 ± 2.7
0.8	39.0 ± 7.3	20.8 ± 2.8

n= 8, 135, 41,12,22,17 for 0.2, 0.3, 0.4, 0.6, 0.7, and 0.8 OD respectively