Rotational dynamics of *Bacillus subtilis* in an optical trap

Ashwini V Bhat\(^1\), Praveen Parthasarathi\(^1\), Shruthi S Iyengar\(^1\), Balaji Yendeti\(^2\), D C Mohana\(^1\), Ashok Vudayagiri\(^4\) and Sharath Ananthamurthy\(^{1,4}\)

\(^{1}\)Department of Physics, Bangalore University, Bangalore-560056, India  
\(^{2}\)Advance Center of Research in High Energy Materials, University of Hyderabad, Hyderabad-500046, India  
\(^{3}\)Department of Microbiology, Bangalore University, Bangalore-560056, India  
\(^{4}\)School of Physics, University of Hyderabad, Hyderabad-500046, India

Corresponding author’s mail id: asharath@gmail.com

Abstract. The swimming of a bacterium in fluids occurs in a low Reynolds number regime. The ability to confine the swimming motion by trapping a bacterium in laser light, can give information on the propulsion coefficients, which are important in explaining the efficiency of swimming of these bacteria. In this work, we report the results of an optically trapped *Bacillus subtilis* in an optical tweezer and the studies on the rotatory motion of the bacterium. The data is gathered and analysed using video microscopy. The propulsion coefficients of such swimming bacterium are determined through a power spectral analysis of the rotatory motion of the bacterium in the trap.

1. Introduction

Laser light can trap objects when it is passed through a high numerical aperture objective [1] and this trapping force arises due to interaction between focused laser and trapped object. This was first demonstrated by Arthur Ashkin in early 1970s [2]. The particle suspended in a medium whose relative refractive index (ratio of refractive index of the particle to refractive index of the medium) is greater than 1.2 acts as a lens and this results in change in momentum of light due to refraction. By conservation of momentum, the particle experiences a compensatory momentum pointed towards the laser focus and gets trapped near the focus of the lens. The total optical force experienced by the particle in a trap can be resolved into gradient forces (depends on gradient intensity of incident light) and scattering forces (describes the scattering of light by particle) [3]. A stable equilibrium trapping position is achieved when a strong gradient force dominates over the scattering force. For small displacement, the optical forces of a trap scales linearly with displacement and can be regarded as a simple harmonic force [4]. Later, trapping and manipulation of live cells (*Escherichia coli* and *Tobacco mosaic virus*) was demonstrated by Arthur Ashkin [5], which opened up a new avenue of using optical trap as a tool to probe biological cells [6, 7, 8]. In order to reduce the photodamage effect of the biological cells in a trap, near infrared laser source is used [8]. Several experiments have been carried out using an optical trap as a tool to understand the dynamics of asymmetric objects [9, 10, 11] and soft matter [12, 13]. Trapping a live bacterium is of interest as it entails both these aspects: asymmetry in shape of the bacterium due to presence of flagella and its active nature.

*Bacillus subtilis* is a gram positive, non-pathogenic bacterium which uses flagellar motion in run and tumble events to move in a liquid environment [14]. Flagella are thin hair like appendages that...
extend out from the cell body of the bacterium. A study of the cell biology of *Bacillus subtilis* indicates that it has a peritrichous flagella structure (synthesis of multiple flagella along the length of the rod shaped cells) which is not random [15]. A flagellum consists of three parts: the basal body, the hook and the filament [16]. The basal body is situated inside the cell membrane of the cell and encloses the flagella rotating motors which rotate due to the proton motive force [17]. The basal body is attached to the filament via hook and as a result the filament also rotates. Thereby, the rotation of the flagella helps in the motility of the bacterium. A detailed study on the efficiency of flagellar propulsion has been carried out [18, 19] and efficiency of flagellar motor of *Escherechia coli* (gram-negative and pathogenic bacterium) was studied [19] using an optical trap. The swimming propulsion of bacterium is based on the simple principle that in the absence of an external torque and presence of an applied force a translating propeller must rotate, and in the absence of an external force and presence of a rotating torque, a rotating propeller translates [18]. In this work we present studies on the flagellar rotation of *Bacillus subtilis* bacterium using an optical trap.

The study of bacterial movement in an aqueous medium is of importance as it is mediated by several different mechanisms and they play a key role in the virulence of pathogens. In this context, the flagellar movement has been studied extensively using two different methods: direct visualization of bacteria [20, 21] and investigations on rotation of tethered cells [22, 23, 24]. The study on a bacterium in an optical trap has been carried out to explain the aggregation using cell-cell approach [25], the synchronization between them [26], the flagellar motor switching rates under different chemical conditions [27] and to determine the swimming pattern of bacteria [28]. It has also been shown that a controlled alignment of bacterium can be achieved in a holographic optical trap [29]. Here we explain a method for determining the propulsion coefficients of *Bacillus subtilis* while swimming, by recording the rotational trajectory of the bacterium in an optical trap using video microscopy. The data obtained by video tracking method is processed by a Fourier transform to obtain the power spectrum and thus, the propulsion coefficients are determined. In this analysis we use the fact that the swimming is in a low Reynolds number regime.

2. Experimental details
The optical trap set up used in the experiment is described elsewhere [30]. We provide a brief summary of the same here. As shown in figure 1, laser light from the source is expanded using beam expanding lenses and is made to pass through a high numerical aperture (NA=1.4) objective with the help of a mirror (M1) and dichroic mirror (DM) to create an optical trap at the sample plane. The interaction of laser light with the particle (bacterium) gives rise to a change in momentum of the laser light, which results in a restoring force that can trap the particle [31]. Freshly cultured *Bacillus subtilis* strain is diluted [dilution of 1/300(μl)] in nutrient broth and loaded on the sample stage and trapped using a 1064 nm laser. The choice of this wavelength is so as to eliminate any structural damage to the trapped bacterium, as the absorption of laser light by the bacterial cell is minimal in this IR region. The trapped bacterium in the sample cell is viewed through the same microscope objective which is used to create trapping and is recorded using a high resolution camera with the help of illumination.

The growth rate (indicates the mean time for doubling of bacteria population) of these wild-type bacterium is 20 minutes. Therefore recordings are made within 15 minutes of loading the sample. The video of the trapped bacterium is recorded using a camera of 77 Hz frame rate. The video thus obtained, is further processed by windowing rendering the effective frame rate to 300 Hz.
Figure 1. Schematic of the optical tweezer set up used. L1, L2 are lenses used for beam expanding. M1, M2 are mirrors, DM – Dichroic mirror.

Figure 2: Schematic of rotation of the flagella and the bacterial body in the trap. The flagella starts bundling and each of the flagellum performs counter clockwise rotation to produce spirals and the bacterium cell will experience a torque in an opposite direction. The situation reverses as the flagella un-spirals by rotating in clockwise direction and the body rotates in anticlockwise direction.

The bacterium uses its flagella to swim in the liquid environment. The flagella rotate in one direction to produce a run sequence and opposite direction to produce a tumble sequence. A more detailed study of flagella rotation of a bacterium was carried out by fluorescence studies [15]. It is seen
that *Bacillus subtilis* flagella bundle together and move in counter clockwise direction to produce a run sequence. The flagella will perform clockwise rotation for tumble sequence. When confined in an optical trap the flagellar bundling and unbundling sequences result in rotation of the bacterium. A schematic of the rotation of bacterium trapped in an optical tweezer is drawn in figure 2. The bacterium flagella bundles together and each flagellum start rotating counterclockwise giving rise to spiraling of the bundle. The formation of one spiral wave represents a complete counterclockwise rotation of the flagellum [32]. The cell body will execute a rotation in opposite (clockwise) direction due to flagellar rotation to balance the torque, which we record as body roll frequency. In high frame rate video analysis, the change in position of the centroid of the bacterium due to flagellar motion can also be observed and tracked [33].

**Figure 3:** Rotation of a bacterium in an optical trap with interval of 13 ms between each image.

The bacterium shown in figure 3, is trapped in an optical trap and is observed to be rotating in a clockwise direction. However, the bacterium did not show same sense of rotation during entire trapping time. For a very short duration (~0.1s) of time it is observed that the cell body performs an anticlockwise rotation and later on resumes its clockwise rotation. Further, the clockwise rotation of the bacterium in the optical trap is tracked for 0.25 s and the rotational position data is decomposed into x and y motion. The schematic of the two dimensional trajectory is given in figure 4, the large circle indicates the body roll and the small circles indicate the flagella rotation as seen by change in position of centroid of the bacterium. The trajectory of x and y positions with time is given in figure 5. Since rotation is a symmetric one with respect to both axes, the one dimensional x-data is further used to obtain the power spectrum. The power spectrum consists of two prominent peaks (figure 6). The large peak at lower frequency (Ω) is due to the body roll motion of the bacterium (~12 Hz) and the short peak at a higher frequency (ω) is due to the rotation of the flagella (~63 Hz).
Figure 4: Schematic of the two dimensional projection of the centroid position of the bacterium trapped in an optical trap, the large circle indicates the body roll and the small circles indicate the flagella rotation.

Figure 5: Trajectory of the bacterium, $x(t)$ and $y(t)$ measured in the trap at 2.9 mW laser power near the sample stage.

The same procedure is repeated with several bacteria. Each bacterium has differing body roll and flagellar rotations. The frequency range in body roll measured is from 10-30 Hz and flagellar rotation is from 60-120 Hz. The bacterium showed both clockwise as well as anticlockwise rotation in the trap, with the rotation sense changing midway. This change in sense of rotation is seen as we use wild strain bacterium showing both run and tumble sequence.
3. Results and discussions

The *Bacillus subtilis* trapped in an optical tweezer using a 1064 nm laser will exhibit a rotational motion. This is due to flagellar rotation of the bacterium [28]. The bacterium shows both counter clockwise and clockwise rotation. This is due to run and tumble sequence respectively. During the run sequence the flagella will be rotating anticlockwise, so to balance the torque when the bacteria is rotating clockwise and during tumble sequence, the flagella moves clockwise to unbundle, therefore the body of the bacteria shows antclockwise rotation [32]. Using the power spectrum of the position data of this rotating bacterium in the trap one can easily find the propulsion matrix.

3.1. Theory

The fluid flow at low Reynolds number is described by Navier - Stokes equation as

\[ \eta \nabla^2 \mathbf{v} = \nabla p \]  

(1)

Since (1) is independent of time, the force \( \mathbf{F} \) and torque, \( \mathbf{N} \), on the propeller are linearly related to propeller’s velocity \( \mathbf{v} \) and angular velocity \( \omega \) as [18]

\[ \mathbf{F} = A \mathbf{v} + B \omega \]

\[ \mathbf{N} = C \mathbf{v} + D \omega \]  

(2)

where \( A, B, C, D \) are propulsion matrix \( P \) coefficients of the flagella given by

\[ P = \begin{pmatrix} A & B \\ C & D \end{pmatrix} \]  

(3)

These coefficients depend on viscosity \( \eta \), size and shape of the propeller. Here \( B=C \), which indicates that every propulsion matrix must be symmetrical. To complete the description of swimming bacterium, propulsion matrix \( P_0 \) of the cell body is also needed. This is defined as [19]
where $A$, $B$, $D$, $A_0$ and $D_0$ are propulsion coefficients. The non-diagonal elements of equation (2) are zero as the bacteria cell cannot propel on its own [18].

Using the body roll frequency and flagellar rotation frequency of the bacterium we can find the propulsion coefficients. If we treat the cell as a prolate sphere with minor semi axis ‘$a$’ and major semi axis ‘$b$’, then $A_0 = 4\pi \eta b / (\ln(2b/a)-(1/2))$ is the linear drag co-efficient and $D_0 = 16\pi \eta a^2 b/3$ is the rotational drag co-efficient. In the absence of any external flow in fluid, the other propulsion coefficients are given by, $A = A_0$, $B = F_{\text{thrust}} / \omega$ and $D = D_0 \left( \Omega / \omega \right)$ [19]. Here, $F_{\text{thrust}}$ is the thrust force generated by the flagella rotation. The trapping force along with the thrust force balances the linear viscous force acting on the body [5]. However, since the bacterium is trapped, the linear viscous force is zero and $F_{\text{thrust}}$ will be the same as trapping force. Thus, we are able to characterize the bacterial motion using an optical trap.

3.2. Calculations

For the bacterium whose trajectory is given in figure 5, the propulsion co-efficients are calculated as $A_0 = 1.38 \times 10^{-8}$ N.s/m, $D_0 = 6.87 \times 10^{-21}$ N.s.m, $B = 1.596 \times 10^{-14}$ N.s and $D = 13.1 \times 10^{-22}$ N.s.m. Similar study on *Escherichia coli* using resistive force theory in an optical trap [19] reveals values that are in the range of what we calculate.

4. Conclusions

The bacterial propulsion is investigated using an optical tweezer. We have been able to obtain the propulsion matrix elements using Purcell’s theory [18] with a simple optical trap. This method can further be used to determine the force and torque involved in propulsion of the bacterium [19]. The method of using an optical trap to characterize a single bacterium and determining the propulsion co-efficients is important as this can be extended in developing an understanding of the interactions involving multiple flagellar motors [32, 34]. This study may also help in understanding biofilm formation process, where the flagellum becomes inactive due to various external conditions [35] to form the film. Such studies on natural swimmers are useful in developing designs of artificial self-driven swimmers in future [36]. Further, we propose to employ this method in characterizing the changes of swimming patterns of different bacterial strains.

Acknowledgement

Ashwini V Bhat acknowledges a DST-INSPIRE fellowship. The experimental setup was enabled through a previous DST (Nanoscience and Technology Initiative) project.

References

[1] Ashkin A, Dziedzic J M, Bjorkholm J E and Chu S 1986 *Opt. Lett.* 11 (5): 288-90.
[2] Ashkin A 1970 *Phys. Rev. Lett.* 24 156-59
[3] Ashkin A 1992 *Biophys. J.* 61 569-82
[4] Koch M D and Shaeveitz J W 2017 Introduction to optical tweezers. *Optical Tweezers Methods and protocols* (Methods in Molecular Biology 1486- Humana press, Springer protocols) ed Arne Gennerich chapter 1 pp 3-24
[5] Ashkin A and Dziedzic J M 1987 *Science* 235 1517-20
[6] Fazal F M and Block S M 2011 *Nat.Photonics* 5 318-21
[7] Svoboda K, Schmidt C F , Block S M 1993 *Nature* 365 721-27
[8] Praveen P, Nagesha B V, Yogesha L,Iyengar S S,Ananthamurthy S and Bhattacharya S 2013 *J. of Biomedical optics* 18(2) 025001
[9] Gauthier R C, Mike Ashman and Chander Grover P 1999 *Applied Optics* **38** No.22
[10] Peter Galajda and Pal Ormos 2001 *Appl. Phys. Lett.* **78** 249
[11] Higurashi E, Ukita H, Tanaka H and Ohguchi O 1994 *Appl. Phys Lett.* **68** 2209
[12] Rusciano G 2009 *Optical Society of America OMB1*
[13] Martinez I A, Roldan E, Dinis L, and Rica R A 2017 *Soft Matter* **13** 22-36
[14] Paoluzzi M, Di Leonardo R, Angelani L 2013 Effective run and tumble events of bacteria baths arXiv:1305.6475v2
[15] Guttenplan S B, Shaw S, and Kearns D B 2013 *Mol. Microbiology* **87**(1) 211-29
[16] DePamphilis M L and Adler J 1971 *J. of Bacteriology* **105** 384-95
[17] Berg H C 2003 *Annu. Rev. Biochem.* **72** 19-54
[18] Purcell E M 1997 *Proc. Natl. Aca. Sci. USA* **94** 11307
[19] Chattopadhyay S, Moldovan R, Yeung C and Wu X L 2006 *Proc. Natl. Aca. Sci. USA* **103** 13712-13717
[20] Mendelson N H, Bourque A, Kathryn W, Anderson K R and Watkins J C 1999 *J. of Bacteriol.* **181**(2) 600-09
[21] Copeland M F and Weibel D B 2009 *Soft Matter* **5** 1174-87
[22] Diethmier C, Chawla R, Canzoneri A, Kearns Daniel B, Lele P P and Dubnau D 2017 *Molecular Microbiology* **106** 367-80
[23] Berg H C and Brown D A 1972 *Nature* **239** 500-04
[24] Terahara N, Noguchi Y, Nakamura S, Kami-ike N, Ito M , Namba K and Minamino T 2017 *Nature Scientific Reports* **7** 46081
[25] Dienerowitz M, Cowan L V, Gibson G N,Hay R, Padgett M J and Phoenix V R 2014 *Curr Microbiol* **69**(5) 669-74
[26] Rouger V, Bordet G, Couillault C, Monneret S, Mailfert S, Eubank J J, Pujol N and Marguet D 2014 *Biophys. Journal* **106**(10) 2096-3104
[27] Altindal T, Chattopadhyay S and Wu X L 2011 *PLOS ONE* **6** e18231
[28] Ignacio A M, Susana C, Meritxell T, Montserrat L and Dimitri Petrov 2013 *PLOS ONE* **8** e61630
[29] Horner F, Woerdemann M, Muller S, Maier B and Denz C 2010 *J. of Biophotonics* **3** 468-75
[30] Yogesha, Raghu A, Nagesh B V, Bhattacharya S, Mohana D C and Ananthamurthy S 2011 *International Journal of Nanoscience* **10**(1-2) 181
[31] Ashkin A 1992 *Biophysics Journal* **61** 569-82
[32] Darnton N C, Turner L, Rojesky S and Berg H C 2007 *Journal of Bacteriology* **189** 1756-64
[33] Dewenter L, Alpmann C, Woerdemann M and Denz C 2012 *Proc. Of SPIE* **8427** 84270 N
[34] Chen X and Berg H C 2000 *Biophysical Journal* **78**(2) 1036-41
[35] Blair K M, Turner L, Winkelman J T, Berg H C, Kearns D B (2008) *Science* **320** 1636–38
[36] Wan M B, Olson Reichhardt C J, Nussinov Z and Reichhardt C 2008 *Phys. Rev. Lett.* **101** 018102