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Spatio-Temporal Patterns and Dynamics in Motion of Pathogenic Spirochetes: Implications toward Virulence and Treatment of Leptospirosis

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***Abstract*—**We apply a particle tracking technique to track the motion of individual pathogenic Leptospira. We observe and capture images of motile Leptospira by means of CCD and darkfield microscope. Image processing, statistical theories and simulations are used for data analysis. Based on trajectory patterns, mean square displacement, and power spectral density characteristics, we found that the motion modes are most likely to be directed motion mode (70%) and the rest are either normal diffusion or unidentified mode. Our findings may support the fact that why leptospires are very well efficient toward targeting internal tissues as a result of increase in virulence factor.

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I. Introduction

LEPTOSPIRA is one of interesting spirochetes including some highly pathogenic species and extremely virulent in humans [1]. Leptospira is thin helical-shaped bacteria with a diameter of 0.1-0.2 μm and a length of 6-20 μm. The structure of Leptospira is a common morphological feature of spirochete. Outermost is an outer membrane sheath (OS), and within this sheath is a helically shaped protoplasmic cylinder (PC). Between the OS and PC, attached subterminally to the cell ends, are periplasmic flagella (PFs). Leptospira has fast movement with spinning or flexing the cell end. It is well known fact that spirochetes are a unique group of bacteria that the flagella are internalized. These flagella are important cellular structures that cause a twisting motion which allows the spirochaete to move about [2,3]. Since the spirochetes have internal flagella, they may offer protection from possibly disruptive extremes in the environment such as pH or salt concentration [2]. In consequence, these spirochetes differ from other bacteria in their ability to movement. They not only swim in low viscosity, but they also can swim in high-viscosity media [4, 5, 6]. The mobility of Leptospira is the important factors to the pathogenesis of leptospirosis [7]. This implies a chance of success in movement toward targeting tissues and also leads to the understanding of virulence factor [7].

The study of the mobility of pathogenic Leptospira is of central significant in the comprehension of the pathogenesis of leptospirosis. According to previous researches, only a few studies of the pathogenic Leptospira have been done because of the small size of the bacteria and the lack of advanced apparatus. Most of the investigations take an interest in protein expressions and immune systems. Although there are a great number of studies of the motility of Leptospira, main researches have been focused on the non-pathogenic type. Moreover, they pay attention to morphology and model of the motility of Leptospira [8, 9, 10]. Nevertheless, the researches in experiment about movement of Leptospira have been done with the latex bead experiments [9 10]. In these researches, the antibody-coated latex beads (Ab-beads) were used for attachment to antigens of the outer membrane sheath of Leptospira and tracking the motion of the cell. The movement of Ab-beads relative to the motion of cells was observed by darkfield microscope and was recorded on videotape or video cassettes. This work of Charon *et al* was to test the motility model of Leptospira [9]. They tracked the rotational movement of the Ab-beads as the cells swam in a given direction. The results obtained provided information on the nature of the outer membrane sheath of pathogenic Leptospira, the basis for certain movements of spirochetes, and gave insight into how spirochetes attach to eukaryotic cells and tissues. Later the same research group [10] tested the model of translation configuration of non pathogenic Leptospira by analyzing cells tethered to a glass surface. The experimental results indicated that the shape of the cell ends rotate in the directions predicted by the model.

Evidently the research that focuses on the mobility of pathogenic Leptospira will yield the enhanced understanding of the Leptospira motility in response to the media condition and external stresses. It thus is also our aim regarding to our work. Here we observe and capture images of motile Leptospira by means of CCD and dark field microscope. We

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then apply a particle tracking technique to track the motion of these bacteria. Transport properties of the particle are investigated through a statistical analysis of the trajectory, which includes the mean square displacement (MSD) and power spectral density (PSD). We hope that our findings could lead to the great benefit in medical and public health community.

II. Materials and Methods

A. Strain and culture condition of Leptospira

Pathogenic Leptospira interrogans serovar Copenhakeni were obtained from the National Leptospirosis Reference Center, National Institute of Health (NIH), Thailand and maintained in the Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok. They were cultured in liquid DifcoTM Leptospira Medium based on EMJH (Ellinghausen and McCullough, Modified by Johnson and Harris) at 27-30 °C in the dark room. The samples were sub-cultured on a weekly interval.

B. Image acquisition

The dark field image sequences were performed with the Zeiss Axioskop2 and with 20x objective lenses. Images were collected at 10 frame/second (for about 50 seconds) using a charge-coupled device (CCD) camera (Model RevolutionTM QEI Camera Monochrome) and an InVivo software support in exposure times of 300 ms. The 2-3 of samples were dropped in a ground glass slide at room temperature (25 °C) and images of motile bacteria were retained as two dimensional moving objects. Bacteria were captured as two dimensional moving objects.

C. Image processing and the particle tracking method

The dark field image sequences from InVivo software were converted to movie. Next, we used the particle tracking technique together with implemented in ImagePro™ software, to follow the region of interest (ROI) of Leptospira images. This region is represented by the center of mass obtained from the average of the intensity of selected pixels (ROI: region of interesting). Now, bacteria positions are represented by point like particles or pixels of image which are in the form of the coordinate at each time as shown in Fig. 1. These positioning coordinates were then read into Matlab™ software to visualize the patterns of trajectory paths and calculate the physical quantities including mean square displacement, velocity, and power spectral density. In fact, other dynamic quantities can also be calculated via this method. More details of this tracking in time algorithm can be found in [11-14].

D. Data Analysis

1) Trajectory patterns of motion

Generally the diffusion has a random like pattern in nature. This is a "fingerprint" of Brownian like or normal diffusion driven by thermal fluctuations. In contrast other motion modes like directed motion tends to move toward in one specific direction unless encountering the obstacle causing changing in direction.

2) Mean Square Displacement

The mean square displacement (MSD) of the trajectory is a convenient quantitative characteristic of the motion. A major

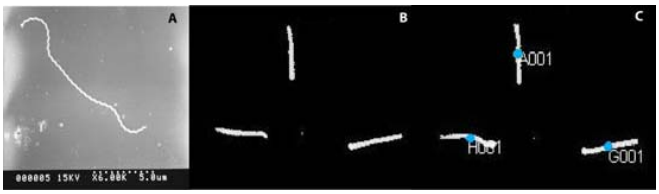

Fig. 1 shows *Leptospira interrogans* serovar copenhageni (A) The high resolution scanning electron micrograph of Leptospira, (B) The darkfield image of Leptospira from experimental, (C) The blue points are the representation of *Leptospira* used in tracking process.

advantage of MSD is the ability to resolve modes of motion of particle which is the quantity to explain the transport behavior and mobility of particle. For the analytical MSD, there are two ways to calculate MSD for a given timescale. One is averaging over independent pairs of points. This method is a check the time correlation in each step. For each trajectory, The MSD for every time interval was calculate according to the formula

$$\mathrm{MSD}(n\Delta t) = \frac{1}{N-1-n}\sum_{j=1}^{N-1-n}\left\{\left[x(j\Delta t + n\Delta t) - x(j\Delta t)\right]^2 + \left[y(j\Delta t + n\Delta t) - y(j\Delta t)\right]^2\right\} \quad (1)$$

Where $\mathrm{V}t$ is the time between steps to observe a moving particle

n is the number of step

N is the total number of step in a track

$n\mathrm{V}t$ is unit of time over which particle displacement is calculated. The minimum and maximum is 0 and $(N/2)\mathrm{V}t$, respectively.

3) Power spectral density

In the stationary stochastic process, the explanation of data perspective on frequency is consideration the correlation for each step of particle. Mean square displacement, the autocorrelation function of position in time domain, is transformed from time domain into frequency domain by using Discrete-time Fourier transforms. The quantity is called power spectral density (PSD) that is described by

$$S(f) = \sum_{t=-\infty}^{\infty} R_{\mathrm{rr}}(t)\exp(-i2\pi f t) \quad (2)$$

$$R_{\mathrm{rr}}(t) = \left\langle \mathrm{V}r(t) \times \mathrm{V}r(t + nt) \right\rangle \quad (3)$$

where

$S(f)$ represents the power spectral density

$R_{\mathrm{rr}}(t)$ is the autocorrelation function of position in time

domain or MSD.

III. Results and Discussion

In this paper we have developed an experimental approach for moving leptospira in a media. From experimental data that the Leptospira's positions were measured by using particle tracking technique, we have firstly focused our analysis on trajectories of a point-like marker attached to an individual moving object. Then the mean square displacement (MSD) and power spectral density (PSD) were performed to classify the mode of motion.

Using the tracking process, it yields the trajectory r(t)=[x(t),y(t)], i.e., the coordinates at each time t, of a leptospire's center of mass undergoing two-dimensional diffusion and/or systematic transport. We consider the characteristic of trajectory path to find the mode of motion. As shown in Fig. 2 and Fig. 3, they depict the path of a leptospira cell monitored for about 1 min behaving directed motion and normal diffusion, respectively. Based on the basic principle of normal diffusion and diffusion with drift (or flow), the cell's trajectory resembles those of directed motion mode are 70% and are either normal Brownian motion or un-identifiable motion mode are 30% (see Fig. 3 and Fig. 4). For the directed motion each Leptospira cell tries to keep its direction until it detects the obstacles then it will change the direction (see fig. 2). This situation is similar to the event of moving ball towards goal on macroscopic level. This ball is kicked by many players in any direction around the yard, but the target of the ball is still going into the goal. In very short time scale, leptospires randomly interact with their environmental around them driven by thermal fluctuation. However the natural behaviors of Leptospira attempt to preserve the way to go and have high mobility of movement.

From MSD results, The MSD versus time plots are used to describe modes of motions. Anticipating that the MSD versus time should obey diffusive phenomena and according to take the form, $\left\langle \left|\Delta\vec{r}\right|^2 \right\rangle \propto \tau^{\alpha}$. If α = 1 (in terms of the Ornstein–Uhlenbeck process [13]), this case indicates the normal diffusion or Brownian motion, while $0<\alpha<1$ performs sub-diffusive motion and $\alpha > 1$ indicates superdiffusion and $\alpha \approx 2$ is directed motion. Our MSD results were found to be consistent with those of trajectory patterns, namely about 70% corresponding directed or ballistic motion and the rest are either designating normal diffusion or un-identifiable. Lastly, we calculated the power spectral density (PSD) that focuses attention on frequency domain from the experimental data. It should be pointed out the fact that for the power law form of the power spectral density according to $S(f): \frac{1}{f^b}$.

β = 0 White noise. [Uncorrelated]
β = 1 Pink (1/*f*) noise. [Medium strength long-range persistence]
β = 2 Brownian motion. [Strong long-range persistence, e.g. topography]

Hence we can use this characteristic of PSD to distinguish among diffusive modes as well as those between a normal mode and directed motion. In fact, we aim to use the characteristic of power spectral density to confirm or double-check if this quantity obtained is consistent with the pattern and MSD results. It was found that the characteristic of PSD are very well consistent with other two quantities. Furthermore, to gain further insight into the origin of the dynamics and to accurate determination of data characteristics, we then performed the computer simulations as a supplement evident to find the modes of motion (data not shown. We designed the simulations of moving point-like particle and use the same criteria to classify transport modes as done with experiment. We found that the simulation results are also consistent with the experimental results.

IV. Conclusion

We applied the particle tracking method to study the motility of pathogenic Leptospira. Using combination of both experimental approaches via image processing, particle tracking and statistical analysis, we were able to identify modes of motion using motion trajectory, mean square displacement, and power spectral density. It was found that leptopspiral bacteria are most likely to undergo directed motion and the rest execute normal free diffusion. From our findings, it may be inferred that most of Leptospira living in vitro with abundant food and free boundary will behave in directed motion and/or diffusion mode. In vivo, we speculate that they can move toward targeting tissues efficiently (with directed motion mode) which lead to increase in virulence factor because of the high mobility of them and ability to avoid treated drug which expect to be in diffusion mode. Further researches concerning this problem could lead to the great benefit in medical and public health community.

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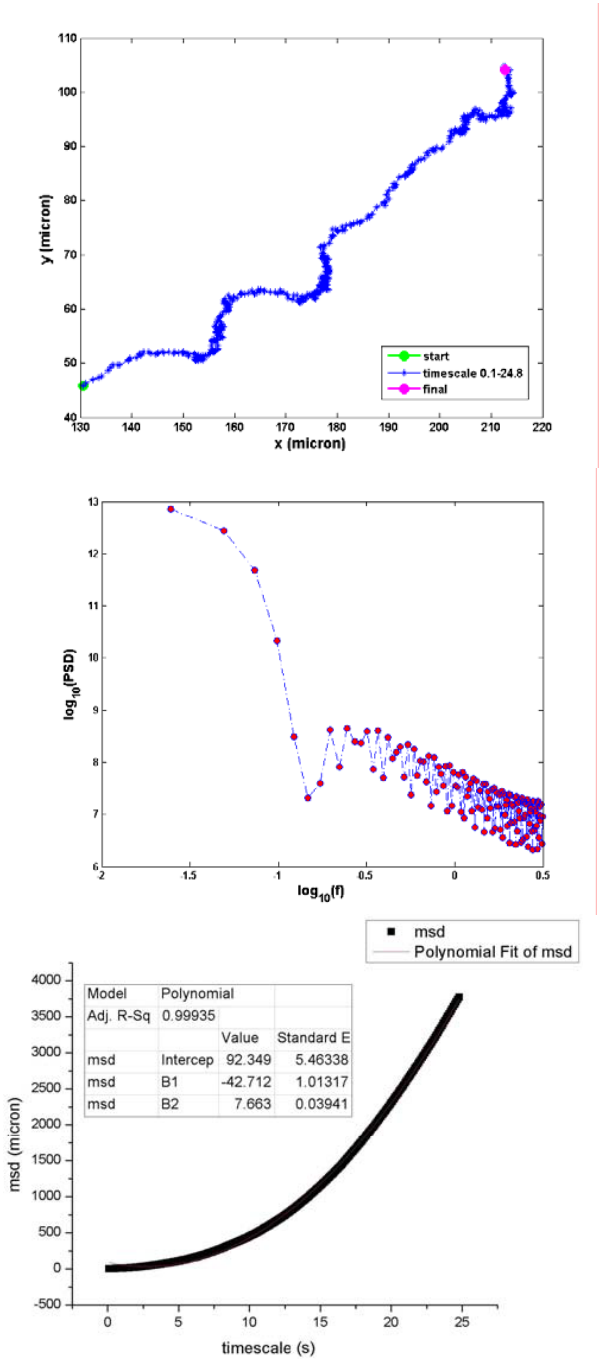

Fig. 2 The typical characteristic of directed motion obtained from experiments: (TOP) the positional trajectory at each time, (MIDDLE) MSD vs. time, (BOTTOM) PSD vs. frequency.

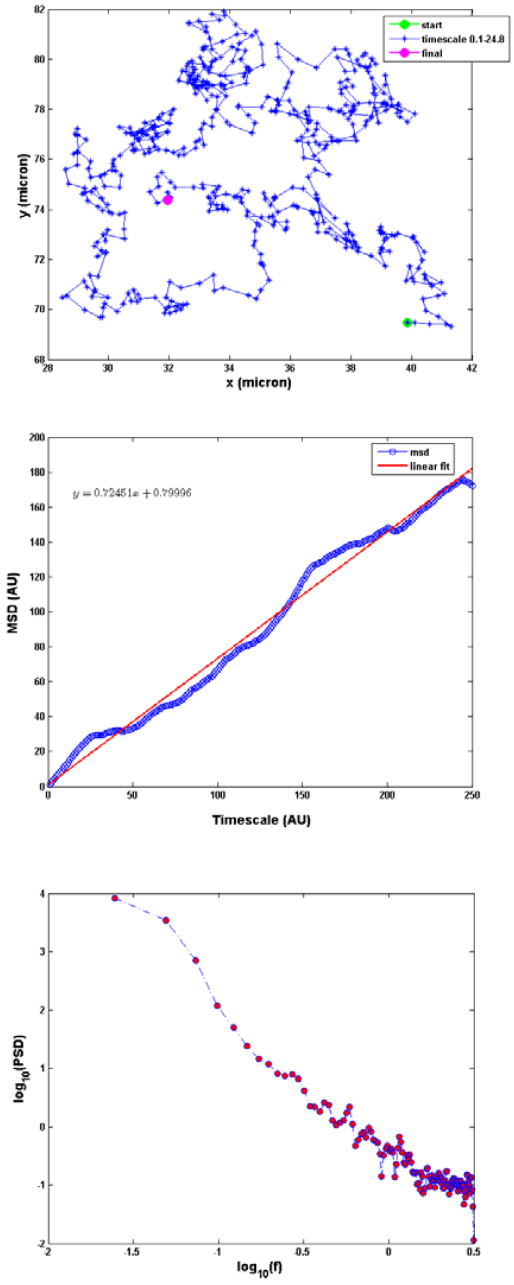

Fig. 3 The typical characteristic of Brownian motion obtained from experiments: (TOP) the positional trajectory at each time, (MIDDLE) MSD vs. time, (BOTTOM) PSD vs. frequency.

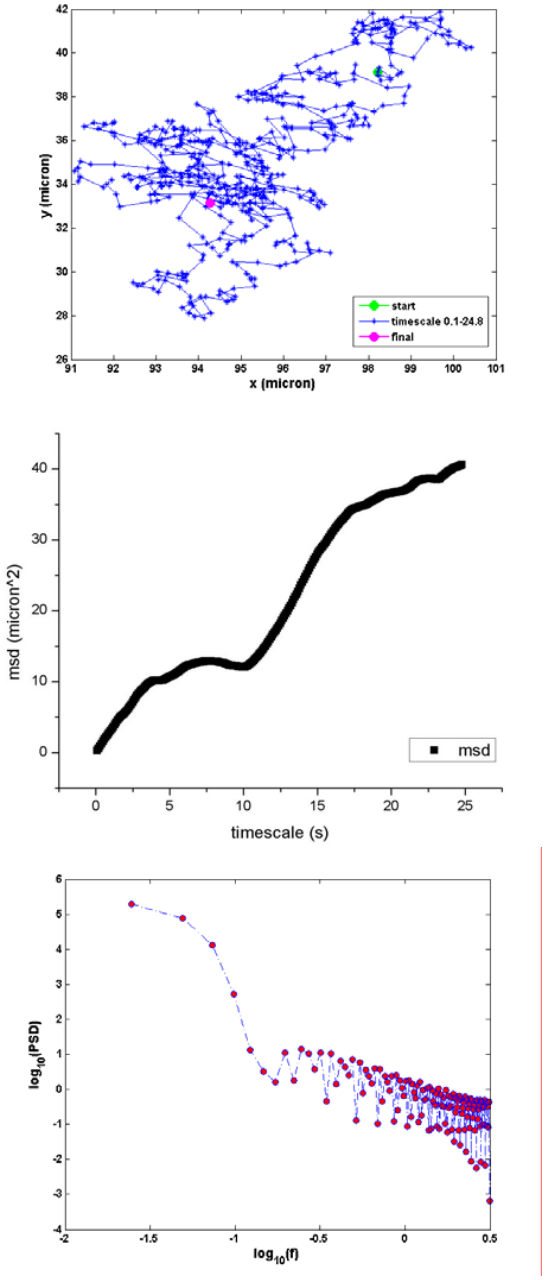

Fig. 4 The possible characteristic of un-identifiable motion mode obtained from experiments: (TOP) the positional trajectory at each time, (MIDDLE) MSD vs. time, (BOTTOM) PSD vs. frequency.