Bio-Micropump by Using A Flagellate Propulsive Force of *Escherichia Coli*

K. Kikuchi, R. Matsuura, H.Ueno, Y. Imai, N. Matsuki, T. Yamaguchi, and T. Ishikawa

***Abstract*—This paper has demonstrated that a novel bio-pump is realized using a propulsive bacterium, which is *Escherichia coli*, in PDMS microchannel. The propulsive force of *E. coli* is applied successfully to generate a pump pressure in the microchannel. *E. coli* is retained in a position by using double optical laser tweezers for driving surrounding fluids to backward using a propulsive force. The optical tweezers stabilize a bacterium body horizontally and vertically in the center of channel for keeping a propulsive direction hence the microchannel with a bacterium has a pump effect geometrically. The generated flow ratio in the microchannel is measured by tracking of tracer particles suspended in surrounding fluids. The bacterial pump pressure is estimated from the measuring flow rate according with the theoretical analysis. As results present bio-pump utilized more than 6 times bacterium propulsive force with the energy efficiency of about 0.2% by trapping their body horizontally. This is the first report on a bio-micropump with a more effective way to utilize bacteriological propulsion. This finding would be useful for a bio-inspired design of micro devices, especially a flagellate bio-micropump.**

***Index Terms*—bio-micropump, *Escheichia coli*, flagellate swimming, laser tweezers, micro devices**

I. Introduction

MICRO devices using microorganisms have been reported by many researchers for a few decades, which is well called as Bio-MEMS. The advantages of utilizing microorganisms are (i) culturing stable and low cost, (ii) their high-energy cost and (iii) biological and theoretical understanding for their locomotion. The bacterium culture method has already been constructed for over 60 years because of the development of genetic molecular biology [1]. The propulsive bacteria swim randomly in aqueous solutions using flagella attached to their surfaces. The flagella are rotated by flagella motor using protons as generating rotational force with remarkable efficiency, especially *E. coli* [2]. The bundled flagella push the bacterium cell to move forward. Recently the locomotion of bacteria and other microorganisms have been applied to micro mixing and pumping devices [3,4]. The single way for generating flow by bacterium propulsion was employed to a micro fluidics to realize a bio-pump. Recently, the optic trap techniques using a laser tweezers have been reported [5-7]. These techniques controlled the bacterium posture under a microscope without loss of their swimming ability. This paper has performed challengingly to realize a bacterium bio-pump, however, is obviously difficult to realize because their random swimming could control a bacterium bio-pump using a captured *E. coli* in the microchannel. Finally we discuss an efficiency of present bio-pump evaluated an experimental results and previous numerical simulation.

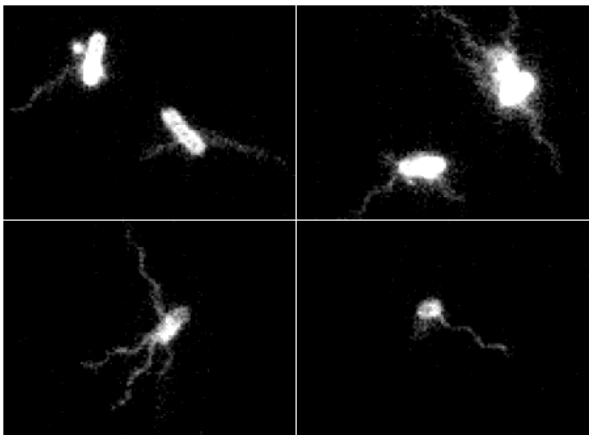

Fig.1 Flagellate bacterium of *E. coli*. The cylindrical body was ~4μm in length and ~0.5 μm in width. The flagella bundle generated the swim speed with ~ 20 μm/s.

II. Experimental Setup and Methods

A. Escherichia coli

A flagellate bacterium (*E. coli*, wild type, MG1655) was employed because they swim by using its bendable flagella bundle to generate a thrust force in our experiments. A body length is about 4 μm, a width of body is about 0.5 μm, and a swimming speed is about 20 μm/s in our culturing condition as shown in Fig.1. A nutritionally rich medium for the growth of bacteria, TB medium, was used for culturing *E. coli* according to our former experiments [8]. The bacteria suspension in the medium was initially incubated at 33 ˚C in a shaking incubator with 200 rpm for overnight till saturation of cell growth. After saturated culturing, the diluted bacteria suspension (50μl: 1:100 dilution) was cultured under the same condition for 4.5 hours. After second culturing, the bacteria were rinsed by MB solution (Motility-Broth: 10mM KPO4, 6.7mM NaCl, 0.1mM EDTA [pH7.0]), and centrifuged with 1400 rpm for 5 minutes. The supernatants

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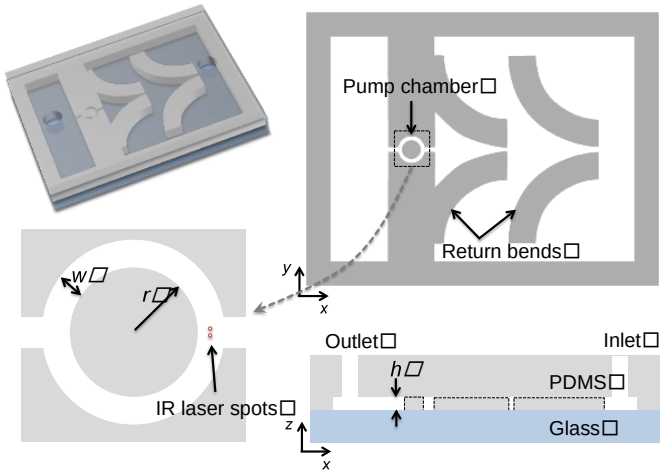

Fig. 1 Schematics of Bio-pump assembled with a PDMS channel and glass plate. Pump chamber has a rectangular cross section with 5 μm in width, *w*, and 10 μm in height, *h*; it has a circular channel length with 10 μm in inner diameter, r_i and 15 μm in outer dimeter, r_o. IR laser spots are focused on the center microchannel for trapping a bacterium. The inlet and outlet were closed in the experiments.

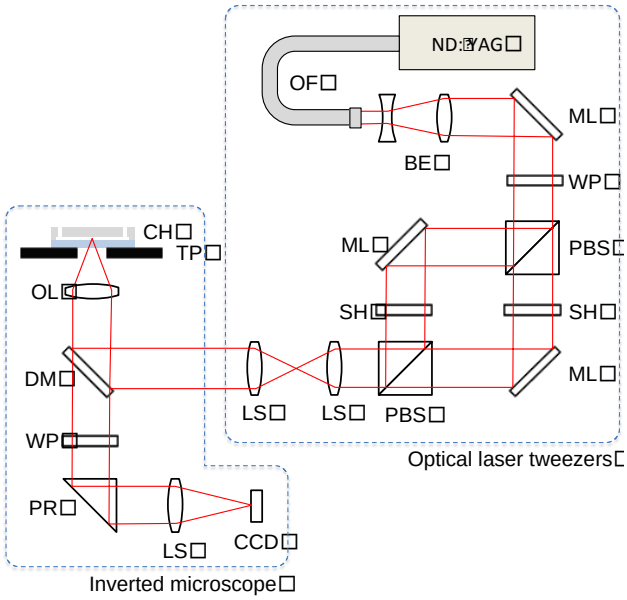

Fig. 3 Experimental setup for micro PTV (particle tracking velocimetry) system with optical laser tweezers. OF; optical fiber, BE; beam expander, ML; mirror, WP; wavelength plate, PBS; Polarization beam splitter, SH; shutter, LS; lens, DM; dichroic mirror, OL, objective lens, TP; thermo plate, CH: channel, PR; prism, CCD; CCD camera.

were rid and diluted again by the medium solution. Then the tracer particles (φ1μm, Thermo Fisher Scientific, USA) were put into the suspension.

B. PDMS Pump Chamber with Laser Tweezers

The pump chamber was designed simply as a loop square channel manufactured by a PDMS microchannel and glass plate shown in Fig. 2. The channel walls were coated by 1% BSA solution (Bovine Saline Albumin) in the heat dried-oven with 40˚C for 30 minutes due to prevention from adherences of the bacteria and particles to the channel surface in advance. The microchannel has an inlet for pouring the bacteria suspension, which were included *E. coli* and tracer particles with kept 33˚C on a thermo plate (Tokai Hit, Japan). Hence the channel has two return bends for a geometrical

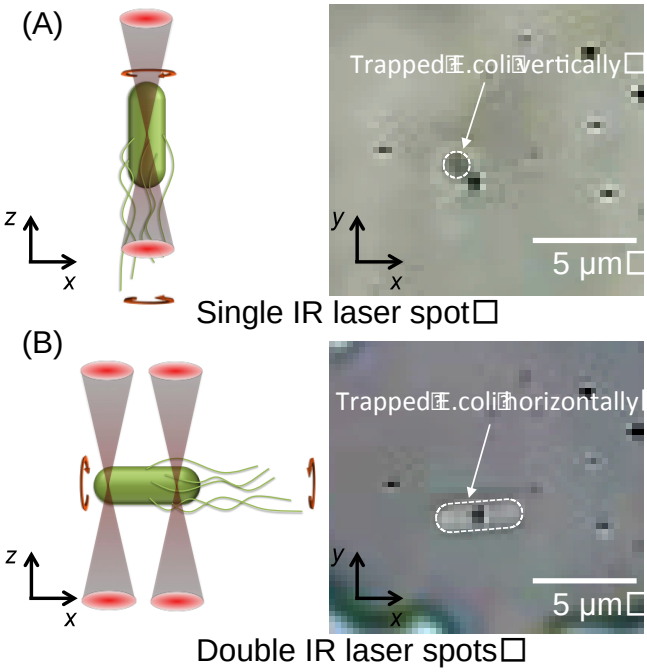

Fig. 4 Trapping of a bacterium, E. coli., using optical laser tweezers. Laser spots were illuminated from the bottom of the pump chamber. The bacterium was trapped at the middle depth of the channel. (A) Single laser spot traps the bacterium vertically. The bacterium was postured the up-right or head-down positions, and rotated along with the IR beam axis. (B)Double laser spots trap the bacterium horizontally. The bacterium rotated along with perpendicular axis of the beam.

selection of bacterium, a few bacterium was able to swim randomly through narrow gaps and channels with 5 μm in both widths. When the bacterium came into the loop chamber inlet, the bacterium was trapped by an optical laser tweezers (SKDCE-2EX, Shigma Koki, Japan) at the center and middle depth of the channel as shown in Fig.2. The laser tweezes was installed on an inverted microscope (IX71, Olympus, Japan). The Nd: YAG laser (infrared (IR) 1064 nm in wavelength, (IPG LASER, YLM-2-1064-LP, Japan) was split two polarized paths, which were individually controlled by the precise angle manipulators of mirrors. The IR laser spots were focused on the middle of channel with Gaussian intensity distribution. The transparent objects, especially the bacterium in our case, in the Gaussian spot was forced to accumulation power toward the center of spot as seen as the optical tweezers effect [9]. The trapped bacterium and free tracer particles were observed by a differential interference contrast microscopy with a objective lens (Olympus, UPlanSApo 100x/1.40 oil, Japan), and recorded a CCD video camera. The obtained images were analyzed using an imaging analysis software (Image J, NIH, USA) for a tracing the particles to measure a driven flow in the pump chamber.

III. Experimental Results

A. Trapped E. coli by Laser Tweezers

The bacterium was trapped by the IR spot as shown in Fig.4. The trapped posture angle, however, was changed by the number of spots. When single IR laser spot was used for it, the bacterium was postured vertically as the up-right or dead-down positions. Although the bacterium tried to swim

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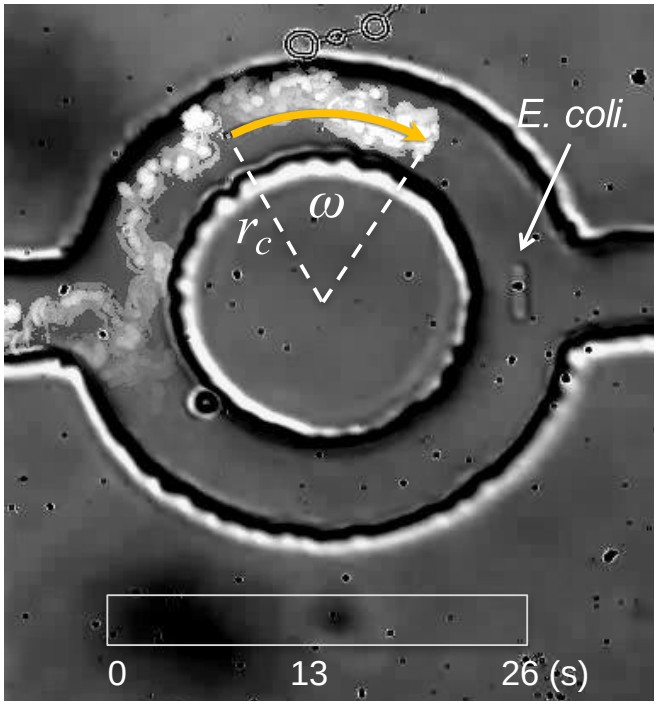

Fig. 5 The particle trajectory in a circular flow driven by a bacterium propulsive force. Particle colors show a time history. The particles were transported in the circular channel by the generated circular flow of bacterium.

Table 1 Properties of generated flow and *E. coli.*

Samples(N=7)	Average ± S.D.
Number of particles n	3 ± 0.6
Average angular velocity of particles $\omega 10^{-2}$ rad/s	3.1 ± 1.0
Free-swimming speed of bacterium V μm/s	13.5 ± 4.5
Length axis of bacterium L μm	3.1 ± 0.8
Width of bacterium W μm	0.8 ± 0.2

away from the entrapment, it was kept the same position but it rotated along with the IR laser beam axis as shown in Fig.4(A). This vertical axis fixation is affected to elongated objects because the vertical posture is the most stable angle in the optical condition of laser tweezers [10]. Fig4(B) shows the bottom view of trapped bottom wall, even if the bacterium swam horizontally. The trapped posture angles were eventually tilted toward the beam axis, which was a vertical angle against the the other hand, when double IR laser spots were applied for trapping the bacterium on near the ends of body, the posture was kept horizontally as shown in Fig4. The bacterium body rotated along with the perpendicular axis straddling the both beam focuses, which were similar result with the recent report by Mears et al (2014) [7].

B. Flow Generation by Flagellate Swimming of Trapped E. coli

When the double laser spots were applied to a bacterium body, the flow in the microchannel was produced constantly as seen as the motion of tracer particles suspended in the channel. The tracer particles were transported toward posterior bacterium body along with the circulate channel as shown in Fig. 5. We measured the averaged angular velocity ω of particles in the channel by 7 samples in totally 21 particles, which were presumed to flow at the center of channel at r_c = 12.5 μm as shown in Table 1. Since the averaged angular velocity ω was approximately 3.1×10^{-2} [rad/sec], the particle would turn around the circular channel with about 200 seconds. The free-swimming speed U and the size of length and width of bacterium were almost the same as the former researches [8,11,12].

IV. Discussions

We evaluate the pump characteristics of present bio-pump driven by the flagella motion of bacterium in terms of the flow rate Q and pump head Δp. The average flow velocity in the channel U was estimated from the averaged angular velocity ω as a following equation,

$$U = r_c\omega,$$

as being 0.39 ±0.12 μm/s. This is about 28 times slower than its swimming speed. The flow rate in the circular channel Q is calculated from U and the area of cross sectional area as $A = (r_o\text{-}r_i)\ h$. Thus,

$$Q = UA = (r_o\text{-}r_i)\ U\ h,$$

as being 19.6 μm^3/s. Moreover, the pressure head of this bio-pump is estimated by following equation based on laminar square channel flow distribution [13];

$$Q = \frac{h^3 w \Delta p}{12 \mu L}\left[1 - \sum_{n:odd}^{\infty} \frac{192h}{n^5 \pi^5 w} \tanh\left(n\pi \frac{w}{2h}\right)\right].$$

Here, n is an iteration number of infinite series of right hand side of second term, L is a length of channel, μ is the viscosity of working fluid at 33˚C as 0.92×10^{-3} Pa·s, which was measured by a cone-plate viscometer in advance. We solved analytically this equation, and obtained the pressure head as being Δp = 23.7 mPa.

In addition, the output energy of the bio-pump E_p is estimated by following equation;

$$E_p = \Delta p Q.$$

The E_p was being 3.87×10^{-19} J/s, which is namely regarded as the energy used by bacteria for flow generation.

We discuss how the bacteria utilize energy for free-swimming to that for flow generation; i.e., how much energy of regular swimming contributes to the flow generation. Mathematically, the energy input from bacteria to the fluid E_b can be defined as an integration of traction force multiplied by the velocity at the surface of bacterium, as following;

$$E_b = \int \mathbf{q} \cdot \mathbf{u}\ dA_{cell}$$

Here, $\mathbf{q}$ is a thrust force, which is proportional to a viscosity and velocity at boundary elements $\mathbf{u}$, is a velocity at the

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boundary elements, and A_{cell} is a cell surface of the bacterium in our previous study, we employed a boundary element method to numerically calculate E_b [8]. The numerical bacterium model was employed an ellipsoidal body connected a spiral-rotational flagellum at its tail position. The cell body was assumed to be 1 μm in width and 2 μm in length. Flagellum length 6μm, which rotates with the rotational frequency of 100 Hz. The viscosity of fluid was assumed to be 9.8×10^{-4} Pa. The boundary elements of 754 triangles were generated in the simulation. In the case of the previous study, the swimming speed of bacterium *V*[8] was estimated to be about 19 μm/s. The energy of swimming bacterium E_b[8] was estimated as 3.9×10^{-16} J/s. When the bacterium did not show motility, the drag force was found as being 0.36 pN for a translational velocity with 1 μm/s. The dissipate energy of viscous drag on the bacterium model surface E_b [8] was 6.8×10^{-18} J/s, multiplying the thrust force of 0.36 pN by the swimming velocity of 19 μm/s for the towing energy of the bacterium translation. Eventually the swimming efficiency of the bacterium η was evaluated as follow;

$$\eta = \frac{E_{tow}}{E_b}.$$

Thus, η[8] was calculated as being 2% in the case of previous conditions. In present condition, since the swimming velocity *V* was slightly different from the previous condition as being 13.5 μm/s, we should modify the total energy input E_b, the towing energy E_{tow}, and the swimming efficiency η. Since **q** is in proportion to **u**, E_b must be changed in proportion to the square of the velocity ratio, $(V/V[8])^2$, which leads to 1.97×10^{-16} J/s. In addition, E_{tow} is modified in proportion to a velocity ratio as being 4.8×10^{-18} J/s. Consequently, η was estimated as being 2.5% in present bacteria.

Finally, we discuss that the pump efficiency of present bio-pump η_{pump} as well. η_{pump} can be define as the ratio of the energy of the swimming bacterium E_b to the output energy of the bio-pump E_p as follow;

$$\eta_{pump} = \frac{E_p}{E_b}$$

As a result, η_{pump} was estimated as being 0.2%. In general, the efficiency of impeller pumps, which are saved loss of friction at actuator; especially motor, would be 40–60% under the inertial regime. While the efficiency of present bio-pump using *E. coli* was significantly low. Namely such a small pump in the viscous regime of Stokes flow would lost the almost energy to dissipative thermal energy (>99%). Basically we should need to design a small pump, which is well taken account of the energy loss, especially dissipative thermal energy. Therefore such a high viscous dominant field, we might be better to utilize one of wisdom of small organisms as like a generation of thrust force.

V. Conclusion

We had been successfully achieved that the bacterium bio-pump realized by using their propulsive force with *E. coli* and PDMS microchannel mounted on laser tweezers.

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References

[1] Subhash Chandra Prija, "*Textbook of Microbiology & Immunology*," (Elsevier Health Sciences, 2014, pp. 3445
[2] Li G, Tang JX. "Low Flagellar Motor Torque and High Swimming Efficiency of *Caulobacter crescentus* Swarmer Cells". *Biophysical Journal*. 2006, 91(7), pp. 27262734. oi:10.1529/biophysj.106.080697.
[3] Nagai M, Hayasaka, Y, Kawashima T. and Shibata T., "Active Mixing in Microchamber using Cilia of *Vorticella convallaria"*. IEEJ Trans Elec Electron Eng 2014, 9, pp. 575–576. doi: 10.1002/tee.22010
[4] Rajveer S and Mira S O, "Transverse Mixing Enhancement due to Bacterial Random Motility in Porous Microfluidic Devices", *Environmental Science & Technology* 2011, *45*, 20, pp. 8780–8787, DOI: 10.1021/es201706w
[5] Chattopadhyay S, Moldovan R, Yeung C, and Wu, X L, "Swimming efficiency of bacterium Escherichia coli", PNAS, 103 (37), 2006, pp.13712–13717.
[6] Carmon G and Feingold M, "Controlled alignment of bacterial cells with oscillating optical tweezers", Journal of Nanophotonics, 5, 2011pp. 1–9, DOI: 10.1117/1.3590242.
[7] Mears P J, Loirala S, Rao C.V., Golding I., Chemia Y. R., "Escherichia coli swimming is robust against variation in flagella number", Elife, 2014, 11(3), e01916, DOI: 10.7554/elife.01916.
[8] Ishikawa T, Yoshida N, Ueno H, Wiedeman M, Imai Y, Yamaguchi T, "Energy transport in a concentrated suspension of bacteria", Physical Review Letters, 2011, 107, 028102, pp. 1–4, DOI: 10.1103/PhysRevLett.107.028102
[9] Ashkin A. "Acceleration and Trapping of Particles by Radiation Pressure". Physical Review Letter. 1970. **24** (4) pp.156–159. DOI:10.1103/PhysRevLett.24.156.
[10] Callegari A, Mijalkov M, Gököz A B, and Volpe G, " Computational toolbox for optical tweezers in geometrical optics", Journal of the optical Society of America B, 32(5), 2015, pp. B11–19, DOI: 10.1364/JOSAB.32.000B11.
[11] Darnton N C, Turner L, Rojevsky S, and Berg H C, "On torque and tumbling in swimming Escherichia coli", J Bacteriol 2007, 189(5), pp. 1756-1764, DOI: 10.1128/JB.01501–06
[12] Lauga E, DiLuzio W R, Whitesides G M, and Stone H A, "Swimming I ncircles: Motion of bacteria near solid boundaries", Biophysical Journal, 2006, 90, pp. 400–412
[13] Lima R, Wada S, Tsubota K, and Yamaguchi T, "Confocal micro-PIV measurements of three dimensional profiles of cell suspension flow in a square microchannel", Measurement Science and Technology, 17, 2006, pp.797–808.