CHARACTERIZATION OF TORQUE AND ROTATIONAL BIAS OF BACTERIAL FLAGELLAR MOTOR

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CHARACTERIZATION OF TORQUE AND ROTATIONAL BIAS OF BACTERIAL FLAGELLAR MOTOR

by

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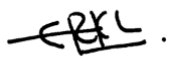
Dedicated to

My Family, Friends, and Teachers

CERTIFICATE

This is to certify that the thesis titled **"Characterization of Torque and Rotational bias of Bacterial Flagellar Motor"**, being submitted by **Mr. Vidhu S** to the Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by him, which has been prepared under the supervision and guidance of conformity with the rule and regulations of "Indian Institute of Technology Delhi". The research reported and the results presented in this thesis have not been submitted in part or full to any other University/Institute for the award of any degree or diploma.



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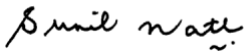

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Vidhu S

ABSTRACT

Bacteria are present everywhere. Some are deadly pathogens, and some live symbiotically with us. Many species of bacteria use flagella to navigate in their environment. The flagellum is a 7-10 μm long helical filament with a rotary motor at its base, embedded in the cell membrane, and almost a dozen stator complexes. Proton motive force across the cell membrane powers the flagellar motors of *E. coli* and *Salmonella*. The motor stochastically switches between the clockwise (CW) and counterclockwise (CCW) direction. A chemotaxis system causes the motor to change its direction, but the process is more complex as the switch is sensitive to load and proton motive force. This thesis presents my work on characterising the output of the bacterial flagellar motor, namely, the torque, rotate rate, and rotational bias, by changing the experimental conditions. First, I changed the proton motive force of the bacteria while keeping the load on the motor constant. The speed and directionality of swimming *Salmonella enteritidis* were quantified as well. Second, I changed the load on the motor while keeping the proton motive force constant. In both cases, I quantified the output of the motor in those conditions and analysed them.

NaCl is significant regarding the flagellar motor as it affects the stator dynamics, proton motive force, and osmotaxis at higher concentrations. Chemotaxis helps the bacteria for its growth and survival. *E. coli*'s natural habitat has high osmolarity, and the organism uses various mechanisms for osmoregulation. However, the flagellar motor's role in adapting to the changes in osmolarity or osmotaxis is not well studied. Some studies have shown bi-directional osmotaxis. The motor shows a repellent-like response in high and low osmolarity solutions, and they elicit an attractant like response in medium osmolarity solutions. This suggests the role of the flagellar motor in finding ideal water conditions in *E. coli*'s environment. In this work, the membrane potential of bacteria in pH 7 was dissipated using NaCl and studied the output of *E. coli'*s flagellar motor and swimming *Salmonella enteritidis* cells in response to the stepwise increase in the concentration of NaCl in motility buffer. A 1.1 μm polystyrene bead was tethered to the flagellar filament of *E. coli* KAF 84 strain, and the rotation rate was analysed using a custom made Matlab program. Changes in motor speed and switching with a stepwise increase in NaCl concentration in the motility buffer was observed. The mean speed of the motors decreased with NaCl concentration. At the single-cell level, the motors were biased to CCW rotation. The population of swimming cells tumbled more with an increase in the concentration of NaCl. NaCl was found to affect the various aspects of the bacterial flagellar motor.

I used tethered cell assay to study the output by changing the load on the motor while maintaining a constant proton motive force. In the tethered cell assay, a mutant strain such as *E. coli* KAF 84 with sticky flagellar filaments was stochastically tethered to a glass surface, making the cell body rotate. The change in position of the rotational axis changes the load on the motor, which would affect the output of the flagellar motor. However, quantifying the location of the rotational axis and the cell size is technically challenging. *Cell Tethering Analysis Program (CTAP)* was developed that simultaneously measures the tethering geometry (position of rotational axis), cell size, and rotation rate of the cells to overcome this challenge.

The experiments were performed in controlled conditions to avoid any factors that may affect the output of the flagellar motors. The cell shape was approximated as ellipsoid objects rotating around the axis of rotation, and torque was estimated using the frictional drag coefficient of the ellipsoids. The cell size and the tethering geometry changed the motors' rotation rate, and a bias of cell tethering with size was observed. The estimated torque was 951 pN nm, and 1390 pN nm as the rotational axis changed from the middle of the cell body to the tip, respectively. The high torque motors rotated exclusively in the CCW direction. The study provides a method to measure the position of the rotational axis and the cell size and discusses their significance to characterize the output of the flagellar motor.

सार

बैक्टीरिया हर जगह मौजूद हैं। कुछ घातक रोगजनक हैं, और कुछ हमारे साथ सहजीवी रूप से रहते हैं। बैक्टीरिया की कई प्रजातियां अपने वातावरण में नेविगेट करने के लिए फ्लैगेला का उपयोग करती हैं। फ्लैगेलम एक 7-10 माइक्रोन लंबा पेचदार फिलामेंट है जिसके आधार पर एक रोटरी मोटर होती है, जो कोशिका झिल्ली में एम्बेडेड होती है, और लगभग एक दर्जन स्टेटर कॉम्प्लेक्स होते हैं। कोशिका झिल्ली में प्रोटॉन प्रेरक बल ई. कोलाई और साल्मोनेला के फ्लैगेलर मोटर्स को शक्ति प्रदान करता है। मोटर स्टोकेस्टिक रूप से दक्षिणावर्त (CW) और वामावर्त (CCW) दिशा के बीच स्विच करता है। एक केमोटैक्सिस प्रणाली मोटर को अपनी दिशा बदलने का कारण बनती है, लेकिन प्रक्रिया अधिक जटिल है क्योंकि स्विच लोड और प्रोटॉन मकसद बल के प्रति संवेदनशील है। यह थीसिस प्रायोगिक स्थितियों को बदलकर बैक्टीरियल फ्लैगेलर मोटर के आउटपुट को चिह्नित करने पर मेरा काम प्रस्तुत करता है, अर्थात् टोक़, घुमाने की दर और घूर्णी पूर्वाग्रह। सबसे पहले, मैंने मोटर पर लोड को स्थिर रखते हुए बैक्टीरिया के प्रोटॉन प्रेरक बल को बदल दिया। साल्मोनेला एंटरिटिडिस तैरने की गति और दिशा भी निर्धारित की गई थी। दूसरा, मैंने प्रोटॉन प्रेरक बल को स्थिर रखते हुए मोटर पर भार को बदल दिया। दोनों ही मामलों में, मैंने उन स्थितियों में मोटर के आउटपुट की मात्रा निर्धारित की और उनका विश्लेषण किया।

फ्लैगेलर मोटर के संबंध में NaCl महत्वपूर्ण है क्योंकि यह उच्च सांद्रता पर स्टेटर डायनामिक्स, प्रोटॉन मोटिव फोर्स और ऑस्मोटैक्सिस को प्रभावित करता है। केमोटैक्सिस बैक्टीरिया को उसके विकास और जीवित रहने में मदद करता है। ई. कोलाई के प्राकृतिक आवास में उच्च परासरणता है, और जीव परासरण के लिए विभिन्न तंत्रों का उपयोग करता है। हालांकि, ऑस्मोलैरिटी या ऑस्मोटैक्सिस में परिवर्तन के अनुकूल होने में फ्लैगेलर मोटर की भूमिका का अच्छी तरह से अध्ययन नहीं किया गया है। कुछ अध्ययनों ने द्वि-दिशात्मक ऑस्मोटैक्सिस दिखाया है। मोटर उच्च और निम्न ऑस्मोलैरिटी समाधानों में एक विकर्षक जैसी प्रतिक्रिया दिखाता है, और वे मध्यम ऑस्मोलैरिटी समाधानों में प्रतिक्रिया की तरह एक आकर्षक प्रतिक्रिया प्राप्त करते हैं। यह ई. कोलाई के वातावरण में आदर्श जल स्थितियों का पता लगाने में फ्लैगेलर मोटर की भूमिका का सुझाव देता है। इस काम में, पीएच 7 में बैक्टीरिया की झिल्ली क्षमता को NaCl का उपयोग करके नष्ट कर दिया गया था और गतिशीलता बफर में NaCl की एकाग्रता में चरणबद्ध वृद्धि के जवाब में ई. कोलाई के फ्लैगेलर मोटर और तैराकी साल्मोनेला एंटरिटिडिस कोशिकाओं के उत्पादन का अध्ययन किया गया था। ई. कोलाई केएएफ ८४ स्ट्रेन के फ्लैगेलर फिलामेंट के लिए १.१ माइक्रोन पॉलीस्टाइनिन मनका टेदर किया गया था, और एक कस्टम मेड मैटलैब प्रोग्राम का उपयोग करके रोटेशन दर का विश्लेषण किया गया था। मोटर गति में परिवर्तन और गतिशीलता बफर में NaCl एकाग्रता में चरणबद्ध वृद्धि के साथ स्विचिंग देखी गई। NaCl सांद्रता के साथ मोटर्स की औसत गति कम हो गई। एकल-कोशिका स्तर पर, मोटर्स CCW रोटेशन के पक्षपाती थे। NaCl की सांद्रता में वृद्धि के साथ तैराकी कोशिकाओं की आबादी और अधिक घट गई। NaCl को बैक्टीरियल फ्लैगेलर मोटर के विभिन्न पहलुओं को प्रभावित करने के लिए पाया गया था।

मैंने निरंतर प्रोटॉन मोटिव फोर्स को बनाए रखते हुए मोटर पर लोड को बदलकर आउटपुट का अध्ययन करने के लिए टीथर्ड सेल परख का उपयोग किया। टेथर्ड सेल परख में, चिपचिपे फ्लैगेलर फिलामेंट्स के साथ ई. कोलाई केएएफ ८४ जैसे उत्परिवर्ती तनाव को एक कांच की सतह पर स्थिर रूप से बांधा गया था, जिससे कोशिका का शरीर घूमता है। घूर्णी अक्ष की स्थिति में परिवर्तन से मोटर पर भार बदल जाता है, जो फ्लैगेलर मोटर के उत्पादन को प्रभावित करेगा। हालांकि, घूर्णी अक्ष और सेल आकार के स्थान की मात्रा निर्धारित करना तकनीकी रूप से चुनौतीपूर्ण है। सेल टेथरिंग एनालिसिस प्रोग्राम (सीटीएपी) विकसित किया गया था जो इस चुनौती को दूर करने के लिए कोशिकाओं के टेदरिंग ज्योमेट्री (घूर्णी अक्ष की स्थिति), सेल आकार और रोटेशन दर को एक साथ मापता है। फ्लैगेलर मोटर्स के उत्पादन को प्रभावित करने वाले किसी भी कारक से बचने के लिए प्रयोग नियंत्रित परिस्थितियों में किए गए थे। सेल आकार को रोटेशन की धुरी के चारों ओर घूमने वाली दीर्घवृत्त वस्तुओं के रूप में अनुमानित किया गया था, और दीर्घवृत्त के घर्षण ड्रैग गुणांक का उपयोग करके टोक़ का अनुमान लगाया गया था। सेल आकार और टेदरिंग ज्यामिति ने मोटर्स की रोटेशन दर को बदल दिया, और आकार के साथ सेल टेदरिंग का पूर्वाग्रह देखा गया। अनुमानित टोक़ ९५१ पीएन एनएम, और १३९० पीएन एनएम था क्योंकि घूर्णी अक्ष क्रमशः सेल बॉडी के मध्य से टिप में बदल गया था। उच्च टोक़ मोटर्स विशेष रूप से सीसीडब्ल्यू दिशा में घुमाए गए। अध्ययन घूर्णी अक्ष और सेल आकार की स्थिति को मापने के लिए एक विधि प्रदान करता है और फ्लैगेलर मोटर के आउटपुट को चिह्नित करने के लिए उनके महत्व पर चर्चा करता है।

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ABBREVIATIONS

μ	-	micron
ACR	-	Arc-to-Chord Ratio
ATP	-	Adenosine triphosphate
ATPase	-	Adenosine triphosphatase
CCCP	-	Carbonyl cyanide m-chlorophenylhydrazone
CCW	-	Counterclockwise
CheY	-	Chemotaxis Protein Y
C_i	-	Intracellular concentration of Ions
C_o	-	Extracellular concentration of Ions
CTAP	-	Cell Tethering Analysis Program
CW	-	Clockwise
EDTA	-	Ethylene Diamine Tetra Acetic Acid
FRAP	-	Fluorescence Recovery After Photobleaching
H^+	-	Proton
Hz	-	Hertz
IMF	-	Ion Motive force
K_B	-	Boltzmann Constant
KPi	-	Potassium Phosphate
MB	-	Motility Buffer
MCP	-	Methyl Accepting Proteins
MDa	-	Mega Dalton
NA	-	Numerical Aperture
Na^+	-	Sodium Ion

NaCl	-	Sodium Chloride
NADH	-	Nicotinamide Adenine diphosphate
OD	-	Optical Density
PMF	-	Proton Motive Force
q	-	Charge of ions
R	-	Reynolds Number
SMF	-	Sodium Motive Force
TIRF	-	Total Internal Reflection Microscopy
V_m	-	Membrane Voltage
Δp	-	Proton Motive Force
$\Delta\Psi$	-	Electric Potential
ω	-	Angular Velocity
η	-	Viscosity of media