様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Elucidating the mechanism of bacteria-mediated oil degradation by imaging biofilm formation on oil drops in microfluidic traps

研究課題名（英文）Elucidating the mechanism of bacteria-mediated oil degradation by imaging biofilm formation on oil drops in microfluidic traps

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　13,400,000円

研究成果の概要（和文）：私たちは、Alcanivorax borkumensis (Ab)による油分解の長期観察のために油滴を捕捉する革新的な装置を開発しました。研究を通じて、このプロセスの物理的メカニズムを解明しました。また、理論物理学者との協力により、包括的な理論物理モデルを構築しました。さらに、Abの界面特性を詳細に測定し、油との相互作用について重要な洞察を提供しました。私たちの研究は、化学分散剤がバイオフィルムに与える破壊的な影響も明らかにしました。この成果はScienceに発表され、バクテリアによる油分解メカニズムと分散剤が油流出管理に果たす役割を解明することで、生物浄化の進展に貢献しました。

研究成果の学術的意義や社会的意義
Our research advances bioremediation by revealing how Alcanivorax borkumensis degrades oil and how dispersants impact biofilms. Published in Science, our findings enhance understanding of oil spill management, promoting more effective environmental cleanup and reducing ecological harm.

研究成果の概要（英文）：We developed an innovative device capable of trapping oil droplets for the long-term imaging of Alcanivorax borkumensis-mediated oil degradation. Through our research, we determined the physical mechanism underlying this process. In collaboration with theoretical physicists, we constructed a comprehensive theoretical physical model. Furthermore, we meticulously measured the interfacial properties of Alcanivorax borkumensis, providing critical insights into its interactions with oil. Our study also revealed the disruptive effects of chemical dispersants on biofilms. Our findings were published in Science, advancing bioremediation by elucidating bacterial oil degradation mechanisms and the role of dispersants in managing oil spills.

研究分野：Biophysics of bacteria

キーワード：bioremediation　biofilm　active matter　microfluidics

１．研究開始当初の背景

Bacterial **biofilms** are active, non-equilibrium, social systems. This property underlies their behavior, modulating their benefits (bioremediation) and problems (pathogenicity). Bioremediation of spilled petroleum is globally mediated by oil-degrading bacteria; however, the microscopic process is poorly understood. We aimed to: (*i*) elucidate the forces biofilms exert on the drop surface to achieve efficient petroleum degradation and (*ii*) develop a mechanistic model for this behavior; these results provide insight into improving existing bioremediation technology. We take an interdisciplinary approach combining an active-matter framework with microbiology, utilizing a microfluidic platform to enable precise environmental control.

２．研究の目的

The primary goal of this proposal was to uncover the biophysical mechanisms underlying biofilm-mediated oil degradation by *Alcanivorax borkumensis*, an area still lacking clarity despite extensive biochemical and genetic studies on oil decomposition. We had the following aims to achieve: 1) Establish a microfluidic platform capable of generating, trapping, and storing oil drops for prolonged observation of biofilm-mediated oil degradation; 2) Analyze high spatio-temporal image data using adapted cell-tracking algorithms to quantify cell-surface and cell-cell interaction energies; 3) Engineer fluorescent, reporter, and deletion mutant strains to enhance tracking and elucidate the genetic basis of observed biophysical mechanisms; and 4) Develop a predictive model of bacterial behavior and biofilm development influencing oil degradation, utilizing experimental measures such as surface tension and cell hydrophobicity from the proposed experiments.

３．研究の方法

We utilized our prototype devices to complete the initial phase and advanced our understanding of bacterial surface accumulation and defect motion. Preliminary data on the nematic surface order on trapped drops guided our progress. We focused on constructing mutant strains to explore genetic influences on bacterial behavior. We also generated a set of deletion mutants.

An optimized microfluidic platform with enhanced tracking algorithms enabled continuous monitoring of biofilm formation. This setup facilitated the investigation of how nematic defects correlated with early-stage drop deformation. We imaged groups of individual cells, with single-cell level resolution.

In collaboration with French partners (initiated from a JSPS-SAKURA project), we developed a comprehensive model of biofilm formation. This model synthesized findings from our research on surface ordering and resource sharing concepts. Initial model development commenced based on previously measured input forces, with iterative refinement anticipated to achieve a robust representation of biofilm dynamics.

４．研究成果

We developed an innovative device capable of trapping oil droplets for the long-term imaging of *Alcanivorax borkumensis*-mediated oil degradation. Through our research, we determined the physical mechanism underlying this degradation process. In collaboration with theoretical physicists, we constructed a comprehensive theoretical physical model to better understand the dynamics at play. Furthermore, we meticulously measured the interfacial properties of *Alcanivorax borkumensis*, providing critical insights into its interactions with oil droplets. Our study also revealed the disruptive effects of chemical dispersants on biofilms, showcasing the delicate balance required for effective bioremediation. The significance and impact of our findings were recognized through publication in the journal *Science*. Furthermore, I have given seminars around the world on the results reporter here. This work advances the field of bioremediation by elucidating the mechanisms of bacterial oil degradation and highlighting the potential and limitations of chemical dispersants in managing oil spills.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　1件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 Prasad M.、Obana N.、Lin S.-Z.、Zhao S.、Sakai K.、Blanch-Mercader C.、Prost J.、Nomura N.、Rupprecht J.-F.、Fattaccioli J.、Utada A. S.	４．巻 381
２．論文標題 Alcanivorax borkumensis biofilms enhance oil degradation by interfacial tubulation	５．発行年 2023年
３．雑誌名 Science	６．最初と最後の頁 748～753
掲載論文のDOI（デジタルオブジェクト識別子） 10.1126/science.adf3345	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 M. Prasad, N. Obana, S.-Z. Lin, S. Zhao, K. Sakai, C. Blanch-Mercader, J. Prost, N. Nomura, J.-F. Rupprecht*, J. Fattaccioli*, A. S. Utada*	４．巻 0
２．論文標題 Alcanivorax borkumensis Biofilms Enhance Oil Degradation By Interfacial Tubulation	５．発行年 2022年
３．雑誌名 bioRxiv	６．最初と最後の頁 0
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/2022.08.06.503017	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　3件／うち国際学会　2件）

１．発表者名 Andrew S. Utada
２．発表標題 Bacteria on High-Fat Diets Cooperate to Stay Fit
３．学会等名 World Biomaterials Congress（招待講演）（国際学会）
４．発表年 2024年

１．発表者名 Andrew S. Utada
２．発表標題 Biofilms on High Fat Diets Cooperate to Stay Fit
３．学会等名 日本細菌学会（招待講演）
４．発表年 2023年

１．発表者名
Andrew S. Utada

２．発表標題
Bacteria on High-Fat Diets Cooperate to Stay Fit

３．学会等名
ACS Surfaces and Colloids（招待講演）（国際学会）

４．発表年
2024年

〔図書〕　計0件

〔産業財産権〕

〔その他〕
Our work was highlighted in Science Magazine in an accompany Perspectives article, on Science's Youtube channel (https://www.youtube.com/watch?v=UwPVeiMYi-M), in Nature Microbiology Reviews (https://www.nature.com/articles/s41579-023-00971-6), in Physics Today (https://pubs.aip.org/physicstoday/online/42663/How-bacteria-help-clean-up-oil-spills?searchresult=1), it was highlighted on the university website and in a press release by JST (https://www.jst.go.jp/pr/announce/20230822-3/index.html).

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関