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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）A multidisciplinary research program to enable the discovery of inhibitors of colanic acid excretion in bacteria

研究課題名（英文）A multidisciplinary research program to enable the discovery of inhibitors of colanic acid excretion in bacteria

研究代表者

Ｋｏｎｇ　Ｌｉｎｇｂｉｎｇ（KONG, Lingbing）

香川大学・農学部・助教

研究者番号：２０７９８３８４

交付決定額（研究期間全体）：（直接経費）　　13,300,000円

研究成果の概要（和文）：提案された研究プロジェクトのすべての分野で重要な成果が得られました。特に、分子生物学的実験では、目的の膜輸送体の発現を可能にする必須の細菌プラスミドを作成し、これらのタンパク質を用いた単一分子研究、続いてこれらのタンパク質チャネルに対する潜在的なブロッカーのスクリーニングを行うことができました。詳細は英語版をご覧ください。

研究成果の学術的意義や社会的意義
Antibacterial resistance is a global challenge. Novel antibacterial strategies and targets are urgently needed. Colanic acid (CA) is one of outermost and most essential outer bacterial matrices in the formation of most biofilms. Inhibition of CA export will help eradicate biofilms.

研究成果の概要（英文）：Three pathogenic bacterial strains have been cultured and stored. WzaCA is transporter of colonic acid. Genomic DNA extraction followed by polymerase chain reaction and cloning onto desired vectors has enabled the expression of WzaCA. Meanwhile, chemical strategies have been developed for efficient production of potential blockers. A LC-MS system equipped with autosampler, fraction collector, temperature controller and multiple detectors which include UV/Vis, 3D-field, refractive index, high-resolution mass, etc. has been established for fast and accurate access to various potential blockers. Lastly, the proposed microscopic system equipped with an ideal EMCCD camera has been set up. Further screening of blockers is currently ongoing. Once a desired blocker is identified in single-molecule electrical recordings, all sorts of biochemical, biophysical and biological tests that have been established will be carried out. Results will be published in due course.

研究分野：Chemical Biology

キーワード：Antibacterial　Colanic acid　Wza　Single-molecule

１．研究開始当初の背景

I am the pioneer in Wza-based drug discovery and have achieved success in all closely related disciplines. I have also set up initial solid foundations for the proposed research project at Kagawa. The following are some representative projects that I have created from 2007 to 2016.

1.1 Establishment of a novel and multidisciplinary program to enable the discovery of inhibitors against the transporter of K30 capsular polysaccharides Wza_{K30}.

1.2 An antibacterial strategy that combines the Wza_{K30} inhibitor and a novel glycoconjugate vaccine candidate based on the conserved base of lipopolysaccharides.

1.3 Recapitulation of K30 capsular polysaccharide export at the single-molecule level.

In 2017, I initiated independent research work on Wza at Kagawa University

In summary, my previous work at Oxford and recent findings at Kagawa have provided very solid foundations for the proposed research.

２．研究の目的

How to eradicate biofilms that are already formed and prevent new ones from formation is **the biggest question in antibacterial research**. Exopolysaccharides such as colanic acid (CA) are loosely associated with the outer membrane and/or other outer matrices. This results in a greater volume in the outer cell envelope for every single bacterial cell, which provides better protection and enables the interweaving of these matrices between bacterial cells. CA is one of the first and the most essential outer bacterial matrices in the formation of most biofilms. Therefore, inhibition of the excretion of colanic acid will be of great signification in bacterial research. T**he "key scientific question" here is whether the biofilm production could be significantly reduced by inhibiting the colanic acid transporter Wza_{CA}.** If it is proven to be true, Wza_{CA} will be validated as a novel antibacterial target and the Wza_{CA} inhibitors will be potential novel antibacterials.

３．研究の方法

In order to address the key scientific question above, **the proposed project dedicates to establishing a novel and multidisciplinary program to enable the discovery of inhibitors against the conserved transporter of colanic acid Wza_{CA}.** The success of it requires a multidisciplinary program that includes molecular biology, microbiology, single-molecule biophysics, computation, chemical synthesis, imaging, cell biology, etc.

４．研究成果

The outermost protective layer of most pathogenic bacteria is colanic acid (CA), instead of capsular polysaccharides (CPS). The proposed project is designed to establish a novel multidisciplinary program to enable rapid discovery of inhibitors

against the transporter of colanic acid Wza_{CA} (**Fig.1a-b**). After three years, significant progress has been made in all the principles for the proposed research project. A brief summary of each of the key disciplines is as follows.

4.1. Molecular biology and protein expression and purification

The pathogenic bacterial strains have been cultured and stored. Genomic DNA extraction followed by polymerase chain reaction and cloning onto desired vectors has enabled the expression of Wza_{CA} (**Fig.1c-d**).

4.2. Chemical strategies

Creation of diverse chemical scaffolds is essential for the project. Chemical strategies have therefore been developed for efficient production of potential blockers. A HPLC-MS system equipped with autosampler, fraction collector, temperature controller and multiple detectors which include UV/Vis, 3D-field, refractive index, high-resolution

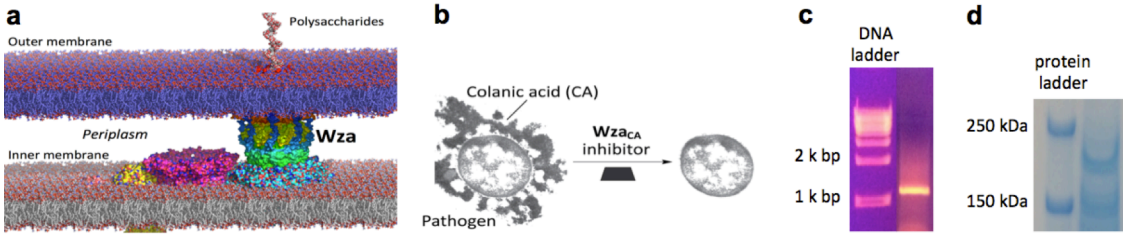
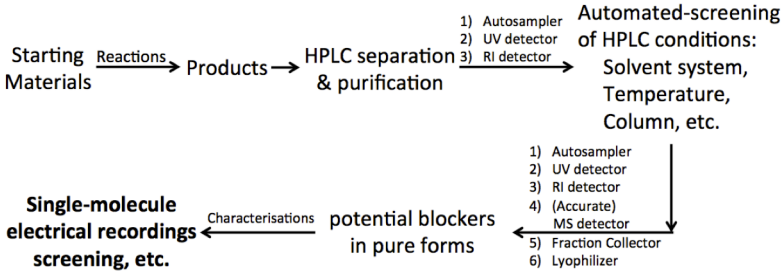


Figure 1. Inhibition against polysaccharide exporter Wza_{CA} for novel antibacterials. a) General machinery for polysaccharide synthesis and export [Kong, Methods in Mol. Bio., 2021, cover image]**. b) Expected function of Wza_{CA} inhibitor. c) Gene of Wza_{CA}. d) Expression of otameric Wza_{CA}.**

mass, etc. has been established for fast and accurate access to various potential blockers (**Scheme 1**).

4.3. Single-molecule techniques for blocker screening

Single-molecule electrical recording systems have been established and used for the study of the Wza_{CA} octamers. Promising results have been obtained. In addition, the



Scheme 1. Established chemical strategies for efficient production and purification of potential Wza_{CA} channel blockers.

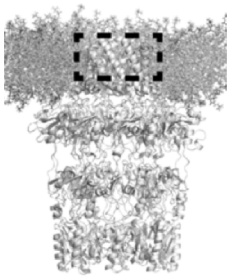


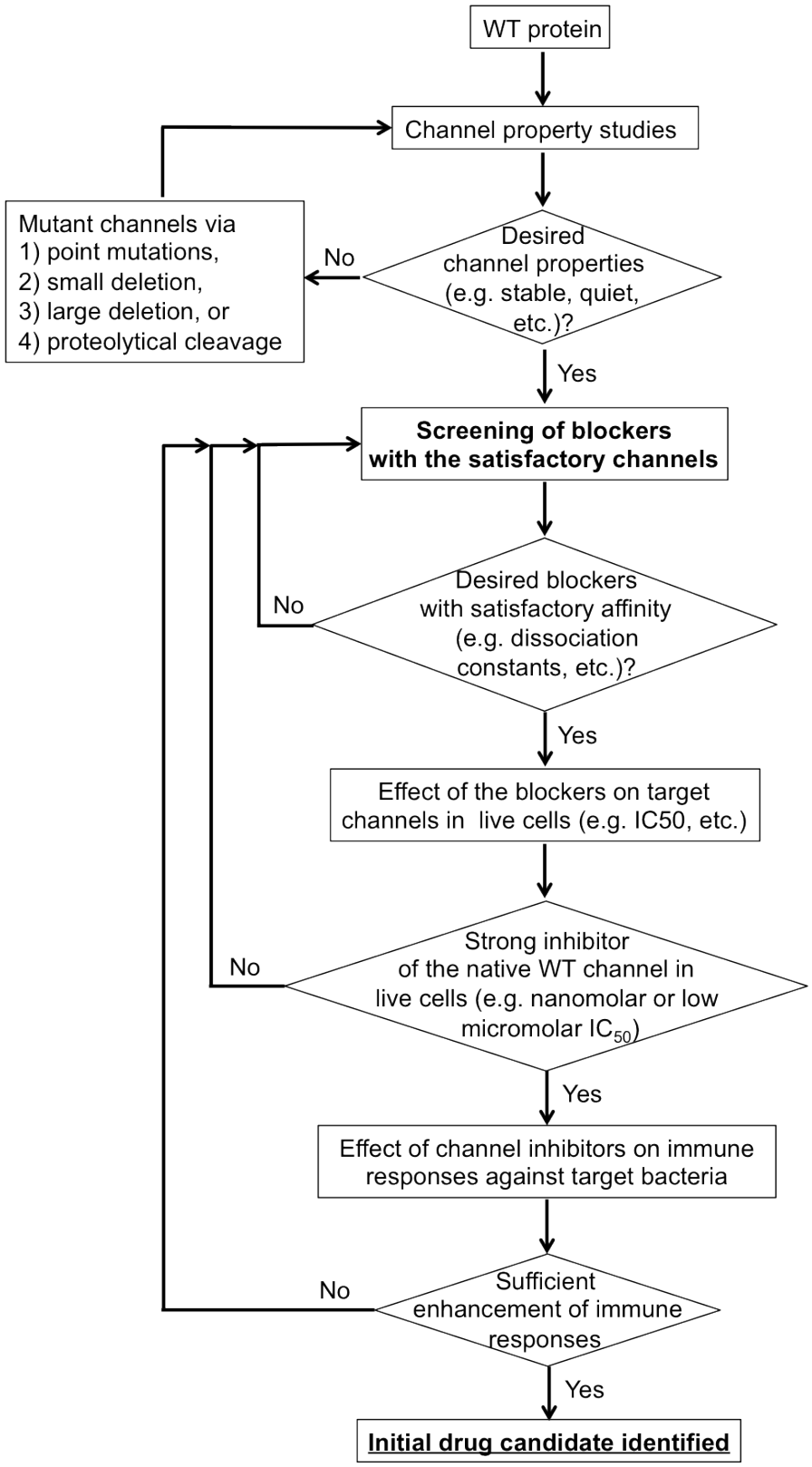
Figure 2. Molecular model of Wza_{CA} in a lipid bilayer for single-molecule studies.

proposed TIRF microscopic system equipped with an ideal EMCCD camera has been set up. Currently, further screening of blockers is currently ongoing (**Fig.2**).

4.4. Summary and perspectives

Most of the proposed experiments have been completed and exciting results have been achieved. All disciplines are still ongoing. Once a desired blocker is identified in single-molecule electrical recordings, all sorts of biochemical, biophysical and

biological tests that have been established will be carried out (**Flowchart 1**). Results will be published in due course.



Flowchart 1. Flowchart to discover initial drug candidates against membrane protein Wza channels. This flowchart is from [Kong, Methods in Mol. Bio., 2021].

4.5. References

1. ***Kong L.***, **Revelation of function and inhibition of Wza through single channel studies,** *Methods Mol. Biol.*, **2021**; 2186:63-76. (Highlighted on the Cover; credit: *Kong L.*)
2. ***Kong L.***, Harrington L., Li Q., Cheley S., Davis B. G., Bayley H., **Single-molecule interrogation of a bacterial sugar transporter allows the discovery of an**

extracellular inhibitor, *Nat. Chem.*, **2013**, *5*, 651-659. (Highlighted on the Cover; credit: Harrington L. and *Kong L.*)

5．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　0件／うちオープンアクセス　0件）

1．著者名 Kong Lingbing	4．巻 2186
2．論文標題 Revelation of Function and Inhibition of Wza Through Single-Channel Studies	5．発行年 2021年
3．雑誌名 Methods in Molecular Biology	6．最初と最後の頁 63～76
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/978-1-0716-0806-7_6	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 －

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　0件）

1．発表者名 Lingbing Kong
2．発表標題 Interrogation of a bacterial sugar transporter for novel biomedicines and biotechnologies
3．学会等名 Annual Meeting of the Biophysical Society of Japan
4．発表年 2018年～2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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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

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