Pursuing the Origin of Pathogenic Bacterial Species

Pursuing the Origin of Pathogenic Bacterial Species

By

Shu-Lin Liu

Cambridge
Scholars
Publishing

Pursuing the Origin of Pathogenic Bacterial Species

By Shu-Lin Liu

This book first published 2022

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-8752-5
ISBN (13): 978-1-5275-8752-6

To my PhD supervisors Profs. Eiko Yabuuchi and Takayuki Ezaki and to all my students

Professor Eiko Yabuuchi

Professor Takayuki Ezaki (left) and me

学海无涯 逆水行舟乃是常态

书山有路 披荆斩棘方能成蹊

Flying higher after bachelor graduation

Marching to diverse professional fields after PhD graduation

TABLE OF CONTENTS

Part III: Genomic divergence of the *Salmonella* lineages: saltatory or gradual?

PREFACE BY THE AUTHOR

I have been haunted for over half century by mental illusions of a transformation fiction in which benign bacteria were turned into deadly pathogens. In reality, I have pursued the origins of pathogenic bacterial species for my entire career. I was curious about why some bacteria are pathogenic but many others are not. Or, more specifically, why closely related pathogenic bacteria may cause radically different diseases; how pathogenic bacteria branch out from commensal ancestors; and how we should control infections more appropriately.

Thousands of investigators in the fields of microbiology and infectious diseases have worked on these questions for hundreds of years and have, to date, built up solid foundations for continual and sustainable research toward answers to these questions. They have made great efforts to uncover the mysteries about pathogenic bacteria and have contributed enormously to human health. In ancient times, thousands of years ago, people had already felt the existence of live infectious agents that caused diseases and guessed that they might be too tiny to be seen by the naked eye. The first sight of live bacteria by Antony van Leeuwenhoek under the 200x lens of his microscope in the mid-1670s opened the window to the microbial world. Then Louis Pasteur started a new epoch in Microbiology, well-known as “the golden age of microbiology”, from 1857. Beginning with Pasteur, scientists including Robert Koch and many others made a series of great discoveries, such as the association of microbes with diseases, immunity,

vaccines, antibiotics, etc. The use of antibiotics has saved countless lives and vaccination has brought so many epidemics under control.

Therefore, human beings have fought with pathogenic microbes with great success across the centuries, especially over the past couple of centuries. However, deadly infections and disastrous pandemics are still threatening human health and taking numerous human lives. Penicillin was highly effective to kill cocci like *Neisseria* spp., but we do not have so many effective antibiotics against the large diversity of pathogenic bacteria. Additionally, initially effective antibiotics may soon become useless due to resistance arising in bacteria. In the mid-20th century, streptomycin and rimifon brought *Mycobacterium tuberculosis* under control. As a result, many people overoptimistically believed that tuberculosis would be eradicated. However, unfortunately, *M. tuberculosis* soon produced resistance to streptomycin and rimifon. Therefore, currently *M. tuberculosis* remains a huge problem worldwide.

Vaccines are extremely effective for prevention or treatment against many infectious diseases, such as measles and rabies, leading even to the eradication of some devastating diseases, such as smallpox and (almost) poliomyelitis. However, vaccination has not been sufficiently effective against certain diseases, such as tuberculosis or typhoid. Furthermore, tremendous efforts in developing vaccines against some microbial pathogens have ended in total failure, such as those against HIV. All the difficulties or failures in developing effective and sustainable antimicrobials and vaccines have resulted from insufficient knowledge about the pathogenic microbes.

Therefore, knowing how pathogenic bacteria have originated from their amiable ancestors is a prerequisite for the effective control of infectious

diseases. This is truly urgent. An in-depth understanding of pathogenic microbes and novel insights into their evolution and biology will greatly facilitate the development of effective control strategies, in addition to antimicrobials and vaccines. New strategies would be based on non-killing measures, as killing will inevitably induce resistance in the microbes and stimulate cycles of an arms race between mankind and pathogens. The key idea is "change", as changes based on novel insights into the microbes or their hosts may lead to radical differences in host-pathogen relationships. Certainly, we cannot make changes on the pathogenic microbes themselves living in nature, but we may make changes to the way they approach their human host, such as by altering the environment by which the pathogenic microbes could contact human beings, or on the way they interact with the human host, such as by enhancing human resistance to microbial infections. Indeed, human society has greatly changed the environment that the pathogens use as the route to infecting human beings. For example, changes in environmental sanitation have remarkably reduced new annual cases of many epidemics, such as typhoid fever, and similar changes are continuing for better human health. Changes for enhancing the natural resistance of humans to microbial infections are the current topics of my research team. This is based on a concept of *elevating relative infectious doses* of pathogenic microbes by enhancing the human defensive functions. All pathogens have a natural infectious dose, below which a pathogen may hardly be able to establish infections in the host. In addition to normal functionalities of all anatomic and physiologic systems such as hemopoietic and immune systems, the intestinal microbiota plays key roles in defense against malignancies and infections. Our preliminary work has demonstrated that the population structure and biological functions of the intestinal microbiota can be dramatically improved for stronger defensive

functions by several measures including the intake of dietary lignans. In animal experiments, the natural infectious dose of *Salmonella* could be increased by two orders of magnitude after ingestion of dietary lignans and we call the new and much higher infectious dose of *Salmonella* the “relative infectious dose” for mice.

The long journey of pursuing the origins of pathogenic bacterial species brings me a general picture of a bacterial cell budding from a benign ancestral tree to form a new and pathogenic branch by acquiring laterally transferred pathogenic traits and streamlining the genome through structural rebalancing and nucleotide amelioration. Once in a human host as a nascent pathogen, this new bacterial lineage may have the chance to co-evolve with the host through countless confronting interactions to become an “established” pathogen with a natural infectious dose. But scientific human beings may at a point of time repel them by enhancing resistance against infections, so that the pathogen may have to increase the infectious dose a hundredfold, or more, to possibly infect the host. At the community scale, such enhanced resistance may become a strong *herd resistance* against pandemics.

PREFACE BY DR. RANDAL N. JOHNSTON

I am deeply honored that my dear friend and valued colleague Professor Liu has invited me to provide a second preface for this volume. When he told me that he was finally ready to begin working on "Pursuing The Origins of Pathogenic Bacterial Species", which he had been planning for many years, I anticipated that the result of this effort would be wonderfully satisfying – and in truth my high expectations have been greatly exceeded. Over the past several decades, I have held many long and enjoyable discussions with him about this book and the questions or theories behind it, so I had understood its general contents even before its completion. He first began working with me in the Southern Alberta Cancer Research Institute, University of Calgary, in the late 1990s, when I was the Director of the Institute. He was the first of the microbiology field to investigate bacterial evolution based on

physical genomic parameters, employing the chromosome mapping strategy that he developed specifically for the comparative genomics of bacteria. These studies complemented very well my own interests in the genomics and rapid evolutionary changes observed in cancer cells as they become malignant and spread to multiple locations during metastasis. Even when I later assumed demanding positions as the Associate Vice President (Research) of the University of Calgary, and then as the President of Genome Prairie, and later as the Director of the Biomedical Technology Graduate Program, I frequently found opportunities to discuss bacterial evolution with him, because this research topic was so important and fascinating. His studies progressed swiftly during his time in Calgary and intensified even further after his move to Beijing and finally to Harbin, where he rejoined Harbin Medical University, the same institution where he had enjoyed some of his early formative education. It was my great pleasure to visit him and present research seminars (and continue our many discussions) once at Peking University and twice in Harbin, and to observe first-hand the rapid progress being made in China.

Professor Liu's discovery of genetic boundaries among discrete bacterial species will not only place bacterial taxonomy on a solid molecular foundation but may also greatly help research on the elucidation of structural genomic mechanisms involved in the emergence of pathogenic bacterial species from their benign ancestors. In fact, it has never in the past been clear precisely how bacteria diverge enough to become different species and how different they must be if they are to be defined as different. Thus, his discovery of genetic boundaries that circumscribe bacteria into discrete (rather than continual) phylogenetic clusters enables him to describe natural rather than arbitrary bacterial species.

In this book, Dr. Liu narrates his long educational and scientific march that begins with his initial surprise about the radical difference in pathogenic features among very closely related bacteria: he wondered whether they belonged to the same, or different, species and how one can rigorously establish that. Then he describes his stepwise decadal progress in searching for genomic parameters that can be used as common yardsticks to universally define natural species of bacteria. Finally, he describes the technical details of the genetic boundaries he proposes, interpreting these boundaries as the consequences of the independent accumulation of adaptive genomic rearrangements and variations plus the eventual formation of semi-closed gene pools once threshold differences have been reached. This book will be a key, and unique, contribution to the field of bacterial evolution and will be eagerly consulted by experts in the field worldwide. But in addition, it also provides a very touching and personal history of how one individual, through sheer hard work in difficult circumstances over many years, coupled with creative thought and critical analysis, has been able to achieve a conceptual breakthrough that will have ramifications and applications that are potentially far beyond bacterial evolution and include many other aspects of the evolution of life and the manifestation of disease. Finally, it should also be clear that Professor Liu has been able to set a shining example for his many students and colleagues, locally and internationally, and thus, to secure an enduring legacy for his life and his work.

ACKNOWLEDGMENTS

This long journey of pursuing the origins of pathogenic bacterial species would not have been possible without the support and encouragement of my family, mentors, friends, colleagues and students. More than sixty years ago, my parents sent me to elementary school and did their best to support my education at what was an extremely difficult time for them. My wife and son accompanied me all the way from my hometown Fujin, China, to Calgary, Canada, and provided all the assistance I needed for the forthcoming journey and for this book.

During my studies as a medical student, I had a burning enthusiasm for research in the field of microbiology and had the chance to join the research activities of Dr. Shao-Xian Li, who brought me from a virtually zero research background to several journal publications by the time of my graduation from medical school, showing me how to read literature, how to design research projects, how to conduct experiments, and how to analyze and summarize the results for publication. As a result, when I began my graduate study for the MSc degree, supervised by Drs. Zhen-Zhou Long and Wei-Feng Chen in Beijing, China, I quickly adapted to the pure research environment, compared to my medical school routines when I spent most of the time attending lectures or seeing patients.

My special thanks go to my PhD supervisors Profs. EikoYabuuchi and Takayuki Ezaki, who showed me new scientific horizons and brought me to the research of bacterial pathogenesis. After graduation with a PhD degree, I stayed for a few more months in Japan to focus on my research design for

comparative genomic studies of *Salmonella* and sent the research plan to Dr. Kenneth Sanderson in Calgary, Canada, for him to make the necessary preparations for the research projects. Ken provided me with all the research conditions and materials needed, so the work went quite well.

I joined Dr. Randy Johnston in late 1996 to work on a cancer therapeutics project using genomics methods. Therefore, my research was then extended from bacterial genomics to human genomics. We had a common interest in molecular evolution, with a focus on the phylogenetic and pathogenic evolution of bacteria. We used to have long discussions together on the definition of bacterial species and the origins of pathogenic bacteria. His support and encouragement were key factors to bringing my research ideas to experimental tests and then to publication, including this book.

The latter parts of the research were conducted at Peking University and Harbin Medical University, where I received great support from Dr. Qi-De Han and Dr. Bao-Feng Yang and many colleagues of the two institutions, respectively. Finally, I thank all my students in Calgary, Beijing and Harbin – they did the experiments and collected the data. Many of them left my team decades ago but they have kept contact with me, mostly on Teachers' Day. My current students, including eight MSc and nine PhD students and eight postdoctoral fellows, are working mostly on genomics-related projects, with a focus on the phylogenetics of bacteria by genomic sequence analyses and microbial pathogenesis using both *in vitro* and *in vivo* experiments. One of the research groups in my lab is challenging lignan-treated animals with pathogenic bacteria to test the "*relative infectious dose*" concept and evaluate its role in enhancing the host resistance against infection. They all work hard and hope that such studies may lead to better control of microbial infections, including pandemics caused by viruses like SARS-CoV-2.

People of Liu lab, Teachers' Day of 2021 (Sept. 10, 2021)

My research has been supported by funding from the Natural Sciences and Engineering Research Council of Canada, the Medical Research Council of Canada, the Canadian Institutes of Health Research of Canada, the University of Calgary, Peking University, Harbin Medical University, and the National Natural Science Foundation of China (NSFC). This book was financed by NSFC 81871623 and 82020108022 and by a special funding from the College of Pharmacy, Harbin Medical University.

INTRODUCTION

Pathogenic bacteria originate from non-pathogenic ancestors, which has been very well documented by many generations of scientists, but whether the pathogens have become separate species from their ancestors or siblings was largely unclear. Elucidation of the phylogenetic status of a pathogen and the evolutionary scenarios leading to the transformation from benign to pathogenic bacteria helps in developing novel strategies for the control of infections. For this, the definition of bacterial species will have to be made unambiguous based on natural parameters without arbitrary judgements, which has been a serious challenge with many hardly resolvable questions. For example, man and chimpanzee belong to distinct species delineated by multiple decisive parameters, even though these two species share as much as 99% genetic similarity, whereas bacteria are classified by different standards with arbitrary cut-offs into species so that bacteria sharing as little as 70% genomic similarity may be categorized into the same species. As a result, bacterial species so defined often contain diverse bacteria with radically different biological or pathogenic traits. In this book, the author narrates the long journey towards finding natural parameters to define bacterial species, pursuing the origins of pathogenic bacterial species, and proposing a novel strategy, which is not antibiotics- or vaccine-based, for the control of pathogenic microbes.

PART I

BACTERIA AND HUMAN HEALTH: NON-PATHOGENIC, MILD PATHOGENIC OR DEADLY SPECIES

I tell my students to drop in any time to my office when they pass by to discuss research issues and they are encouraged to enter the office directly without having to knock at the door, even when they hear me meeting with any kind of VIPs – my students are more important than any other Very Important Persons.

Meeting and discussing research issues with students.

CHAPTER 1

MANY BACTERIA ARE DEADLY PATHOGENS BUT MOST ARE FRIENDLY TO US

Parents pay great attention to the dirty hands of their babies – worrying about the bacteria carried by the dust and stains on the skin. They all hear so much about gastroenteritis, diarrhea, dysentery, enteric fever, cholera, plagues, and tuberculosis, so they wash their baby's hands before the child grabs the food to eat, or urge their three or four year olds to wash their hands by themselves before meals. It is the right thing to do, of course. I remember how stern I, like many parents and grandparents, was when urging children to wash their hands at eating times, and I am still stern. Many parents believe that it is the countless bacteria on unwashed hands that may cause infectious illnesses. It is true, in a way. However, most adults may not know the fact that only a tiny portion of the bacteria that scientists have so far documented may cause diseases and the rest (>99% of all known bacteria) are essentially harmless or, rather, even beneficial to human health, directly or indirectly. Bacteria are virtually everywhere around us – in nearly all kinds of environments and on the surface of us (skin and mucous membrane), except in the lava from erupting volcanoes. Despite some being notorious pathogens such as *Yersinia pestis* or *Mycobacterium tuberculosis*, most bacteria support our normal life, essentially by harnessing solar energy, converting inorganic substance to life compounds, recycling elements, producing oxygen, making nutrients, suppressing pathogens, stimulating our immune functions, and too many others to list. What readers are expected to know from this book are the general distinctions between pathogenic and non-pathogenic bacteria and how pathogens might have

arisen from their non-pathogenic ancestors in evolution. As a result, readers may have a clearer understanding about how to protect endangered bacterial species in order to protect ourselves and how to live and co-evolve in the best possible harmony with our bacterial companions in and around us.

Bacteria in and around us

Quite often, in the Microbiology class, students yell or scream, all at once, when I show micrographs or plate cultures of the beautiful (but they call them awful or horrifying) bacteria on our skin or the mucous membrane of the tongue. “Don’t worry”, I then comfort them, “they usually do not hurt you but, rather, they protect you”. I show them a picture of *Staphylococcus aureus* colonies on a blood agar plate and say: “See the hemolysis zone around the *S. aureus* colonies?” I tell them that many hemolytic bacteria such as *S. aureus* have the excellent ability to cause infections. They are horrifying pathogens and may cause local skin or tissue infections, such as furuncles, but they may also elicit systemic illnesses, such as meningitis or sepsis. In the former cases, I continue, “you do not need any treatment, just let them go hot and mature, making you look beautiful in another way if they are on your face or neck, or torturing you by pain if they are on your waist or buttocks when you lie down to sleep”. Students usually laugh. “However”, I change to a serious face, “in the latter cases, you really need treatment as soon as possible, since most systemic infections are potentially fatal if not treated properly and timely enough”. People may think that such bacteria dwelling on your body surface may be extremely dangerous. They are, indeed, in a way. However, they are ordinary members of the normal human microbiota.

At this point, a small wave of low surprised noises often comes from the audience again. Students do not find it easy to relate *S. aureus* to “ordinary members” of their normal microbiota. *S. aureus* is a too familiar bacterial pathogen name for the students to accept as a close daily companion.

To this, I would continue: “they do not invade you unless you invite them by heavy smoking, staying up all night with your web games, or eating too many sweet or oily foods. Or you have compromised immune functions, such as in cases where you are under enormous stress before an exam for which you are not really prepared…”

Students laugh again. The course I teach is Systematic Bacteriology, not Medical Microbiology, which I also teach but for other classes. Whereas I also have a focus on pathogenic bacteria in the Systematic Bacteriology course as in Medical Microbiology, the difference is that I deliberate on how pathogenic bacteria cause infections in Medical Microbiology but on how new bacterial species, pathogenic or benign, arise in Systematic Bacteriology. Traditionally, medical students learn Medical Microbiology to deal with disease-related microbes in their medical career. However, I believe that they do also need to have at least a very brief introduction to other microorganisms in nature, such as Cyanobacteria producing oxygen and Rhizobia fixing nitrogen. Such non-medical bacteria do not cause infections but they nurture human health and even safety, by nourishing an optimal environmental setting suitable for life and thus, making the otherwise inorganic globe livable for humans and other creatures.

While encouraging the medical students to read General Bacteriology for more detailed information about such deemed “disease-irrelevant bacteria”, I show a genealogical graph of known bacteria at the phylum level (Figure 1-1).

Among the documented phyla, three contain most of the known pathogenic bacteria, including Proteobacteria (e.g., *Salmonella typhi*), Firmicutes (e.g., *Clostridium tetani*), and Actinobacteria (e.g., *M. tuberculosis*).

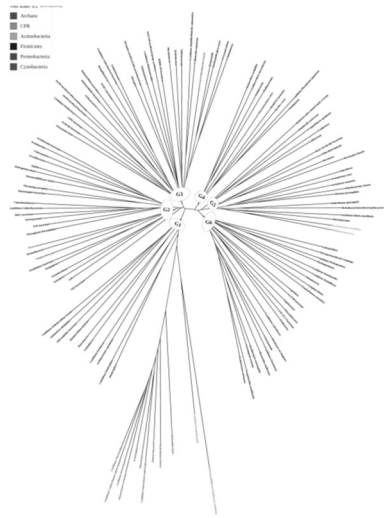

Figure 1-1. A phylogenetic tree showing the main phylogenetic lineages of bacteria at the phylum level. Branches represent phyla. Archaea lineages are included on the tree for a reference of genetic distances among the microbes. All phyla are clustered into six phylogenetic groups, G1-G6, which we call the six Kingdoms.

Pathogens: primary or opportunistic, causing mild or deadly diseases

Many textbooks divide pathogens into primary (or "true") and opportunistic pathogens. Primary or true pathogens are "professional" infectious agents, causing diseases in both vulnerable and healthy hosts by direct interaction using specialized "weapons" such as exotoxins or other virulence effectors. Illnesses caused by primary pathogens have specific disease names, specific symptoms and signs, and specific and mostly typical clinical patterns, such as typhoid fever caused by *S. typhi*, tetanus caused by *Clostridium tetani*, and tuberculosis caused by *M. tuberculosis*. Such diseases are generally called infectious diseases and have natural courses. In contrast,

opportunistic pathogens infect individuals with compromised immune functions. Infections caused by opportunistic pathogens do not have specific disease names, specific symptoms or signs, nor typical courses, such as nosocomial infections by *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *etc*. In the lectures, I tell my students "please do not think that primary pathogens are deadlier than opportunistic pathogens in general". I usually use *Salmonella* gastroenteritis as an example, which is often caused by the primary pathogen *Salmonella typhimurium*. *Salmonella* gastroenteritis may, clinically, appear quite serious, with horrible vomiting and diarrhea, but usually does not require hospitalization or any medication. On the other hand, nosocomial infections caused by opportunistic pathogens could be fatal. I assign the students homework to make brief lists of primary and opportunistic pathogens causing mild or deadly diseases, telling them "I will compare a pair of closely related primary pathogens in the next lecture for you to have a better understanding about host-pathogen interactions and bacterial pathogenesis, one causing local infections without the need of medical treatment and the other eliciting systemic infections that are potentially fatal if not treated timely and properly". Then, in the next lecture, I usually make detailed comparisons between *S. typhimurium* and *S. typhi* for the students.

My first sight of real bacteria: some looking lovely but causing serious illnesses

My first glance at pathogenic bacteria was on October 22, 1971, when I began my career with bacteria in the clinical laboratory of Fujin Central Hospital in the small town of Fujin 富锦, in Heilongjiang Province, northeast China. I graduated from Fujin Second Middle School Class 1970 and was assigned to work in the Fujin Central Hospital. From the mid-1960s to late 1970s, most schools, including elementary and middle schools (which had junior high and senior high classes together), colleges and

universities, stopped teaching. In the spring of 1970, when the schools had stopped producing graduates for a straight four-year period, the whole of society had an urgent workforce shortage. As a result, the hospital requested, and it was approved by the upper institution, the urgent recruitment from Senior High Class 1970 pre-graduates (dismissed from the school before the graduation time) and regular Class 1970 graduates (graduated according to the regular schedule; I was a graduate and so was in the latter group). Altogether, the hospital recruited about 60 youngsters from Fujin First, Second and Third Middle Schools and many of the young newcomers were assigned to the Departments of Internal Medicine, Pediatrics, Infectious Diseases, Surgery, Gynecology and Obstetrics, or Ophthalmology and Otorhinolaryngology, as assistants of nurses (many were promoted to nurses three years later). A few were assigned to Pharmacy as assistants of pharmacists, to Radiology as assistants of the technicians, or the Clinical Laboratory as junior staff. In general, the 60 of us were collectively referred to as "trainees" and we carried this label for about three years, as we had not received any medical education previously. We, as the Class 1970 Senior High graduates, received the diploma of senior high school education but the "pre-graduates" did not, as they did not have the chance to stay in the school according to the regular schedule and their school time was terminated prematurely. More striking is the fact that, being Senior High Class 1970 pre-graduates or even regular graduates, we all had only about five years of elementary school education. As a result, all 60 of us had to receive medical training on an educational background of Grade 5 or 6 elementary schooling. The training subjects included Anatomy, Histology, Physiology, Pathology, Pharmacology, Microbiology, Biochemistry, Internal Medicine, Pediatrics, Infectious Diseases, Surgery, Gynecology and Obstetrics, etc. The trainers were the best physicians in the hospital and the training was unbelievably successful. In another book, I provide moving details about this legendary history (Liu 2015).

I began my career at the hospital on March 1, 1971, and was assigned to work in the Clinical Laboratory on October 22, 1971. I met with the staff in the Laboratory on that day. I was very enthusiastically welcomed, because their workload had been so heavy that, unlike in a normal Clinical Laboratory where a person works either in Hematology, Biochemistry, Microbiology, or the Blood Bank, one had to work in all of these sections for daily routines and for night and weekend turns. They had night turns once in every two or three days and they did look pretty tired.

It was in the late afternoon when I was brought to the Clinical Laboratory to meet with the staff. They had just completed their daily work and Dr. Shu-You Wang (second from left, Fig. 1-2), Chair of the Clinical Laboratory, was the first to shake my hand. After asking my name and other general information, such as my father's profession (most mothers did not have a profession at that time) or my graduation middle school, he encouraged me to learn hard and work hard. A senior lab scientist, Dr. Song-Xue Wang (fifth from left, Fig 1-2), became my main mentor.

Figure 1-2. The staff of the Clinical Laboratory at Fujin Central Hospital. I am sixth from left. This photo was taken in the early winter of 1972.