A career working on bacteria, which now may be poised to revolutionize medicine

Richard J. Roberts

New England Biolabs

Geneva October 19th - 2015

RJR - a brief history

1969-1972 Harvard - tRNAs involved in bacterial cell wall biosynthesis

1972-1992 Cold Spring Harbor – restriction enzymes

Cold Spring Harbor – discovery of RNA splicing

Cold Spring Harbor – discovery of base flipping

1993-today New England Biolabs – genomics and bacterial epigenetics

Cold Spring Harbor – 1972-1992 restriction enzymes

1972-75	30 restriction enzymes discovered
1975	Scientific Advisor to New England Biolabs
1975	Restriction enzyme list, the precursor of REBASE
1976-	Many RM system genes cloned
1978	BIOinformatics methods started

Cold Spring Harbor – 1972-1992

1977 discovery of RNA splicing

Cell, Vol. 12, 1–8, September 1977, Copyright © 1977 by MIT

An Amazing Sequence Arrangement at the 5′ Ends of Adenovirus 2 Messenger RNA

Louise T. Chow, Richard E. Gelinas, Thomas R. Broker and Richard J. Roberts
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

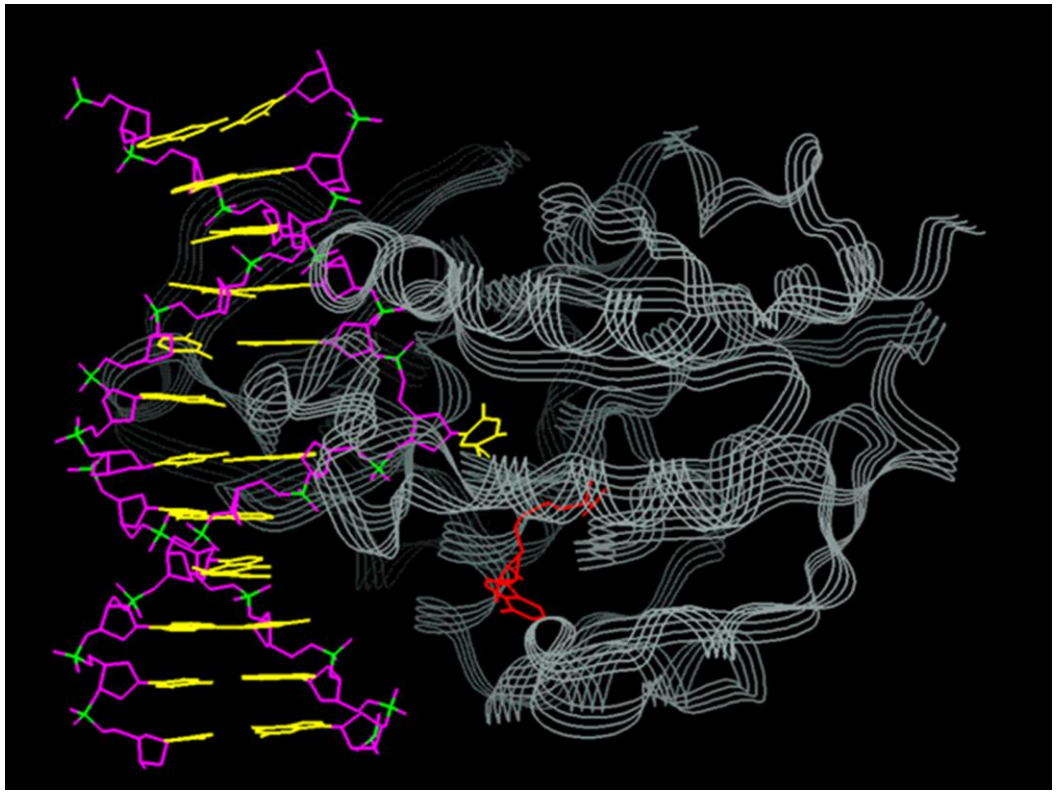
Summary

The 5′ terminal sequences of several adenovirus 2 (Ad2) mRNAs, isolated late in infection, are complementary to sequences within the Ad2 genome which are remote from the DNA from which the main coding sequence of each mRNA is transcribed. This has been observed by forming RNA displacement loops (R loops) between Ad2 DNA and unfractionated polysomal RNA from infected

teristic of that of the host genome (Lewin, 1975a, 1975b). For example, long polyadenylated transcripts appear in the nucleus, but only a small percentage of this nuclear RNA appears as polyadenylated mRNA on cytoplasmic polysomes (Philipson et al., 1971). These mRNAs are "capped" at their 5′ ends (Moss and Koczot, 1976; Sommer et al., 1976). Gelinas and Roberts (1977) found that most Ad2 mRNAs isolated at late times during infection contain the same "capped" 11 nucleotide sequence at their 5′ ends. This sequence was sensitive to ribonuclease cleavage in mRNA:DNA hybrids (Gelinas and Roberts, 1977; Klessig, 1977) and led to the suggestion that this 5′ terminal sequence might not be coded immediately adjacent to the main body of the mRNA.

Cold Spring Harbor/NEB – 1991-1993

With X. Cheng - discovery of base flipping in M.HhaI



New England Biolabs – 1992-present

Cloning, BIOinformatics and genomics

1. Cloning restriction enzyme genes for commercial production

2. Exploring the limits of bioinformatics

3. Genome sequencing to find restriction enzyme genes

New England Biolabs – 1992-present

Bacterial epigenetics

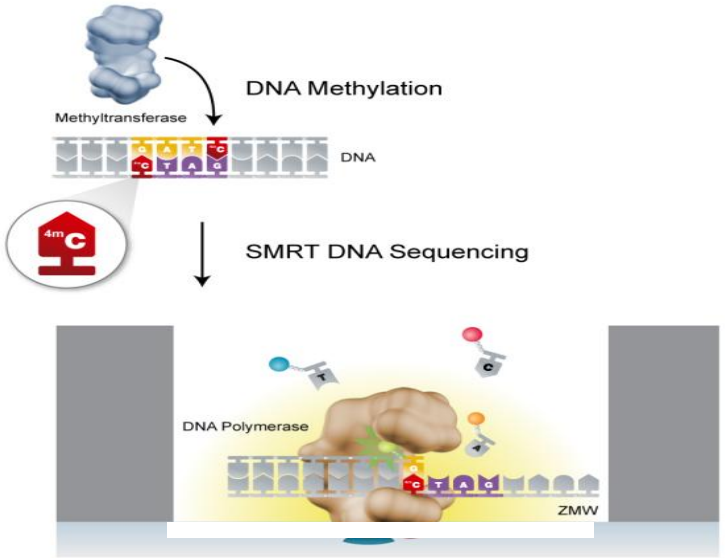
Pacific Biosciences RSII sequencing

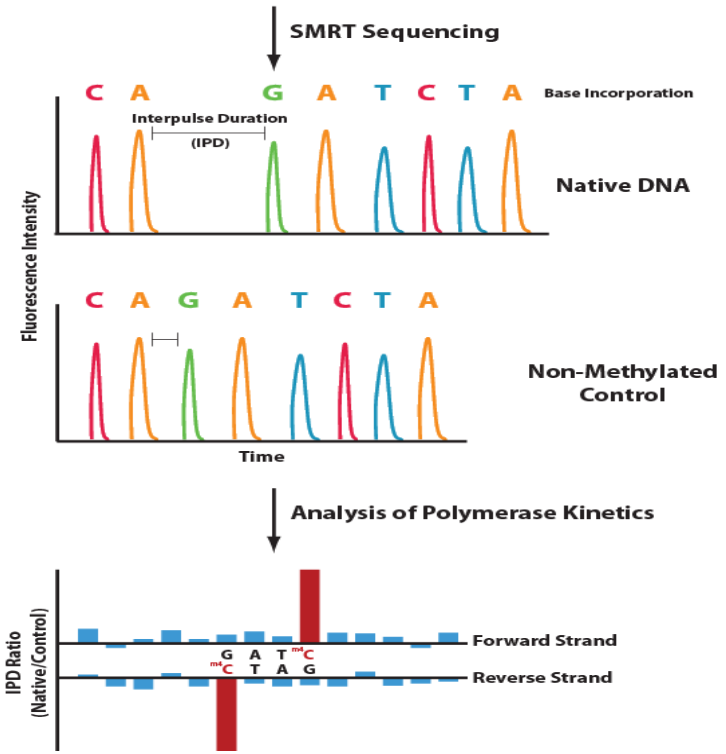
Humans and Bacteria

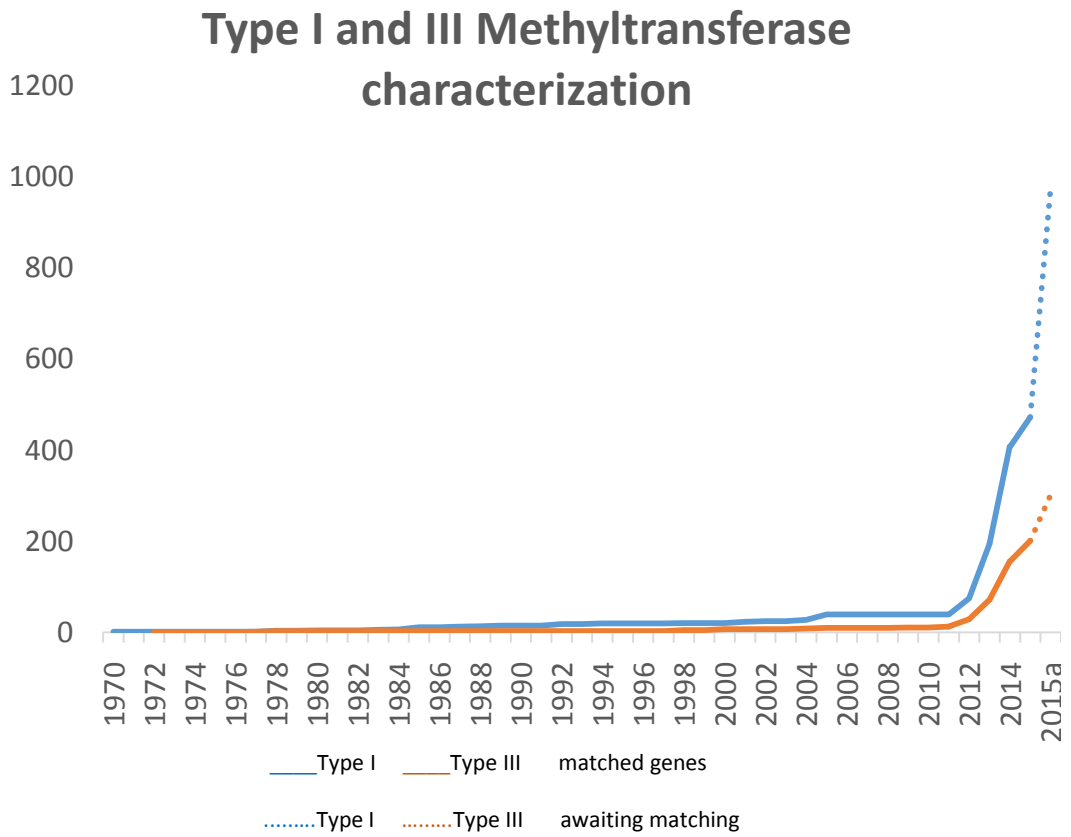
	Humans	Bacteria	Bacteriophages
# Cells	10^{13}	10^{14} (100 trillion)	?
# Strains	1	>20,000	?
# DNA Bases	3×10^{9}	$>6 \times 10^{9}$	?
# Genes	25,000	>1,000,000	?

Why study bacterial DNA methyltransferases?

1. As part of Restriction-Modification (RM) systems they mitigate phage infection
2. They are easily found by bioinformatics and are good markers for RM systems
3. Several have been shown to have regulatory activity in bacteria
4. They support epigenetic phenomena
5. They are a good example of proteins that recognize specific DNA sequences
6. They probably do some very interesting new things


DNA Methylation
Methyltransferase
DNA
4mC
SMRT DNA Sequencing
DNA Polymerase
ZMW


SMRT Sequencing
C A G A T C T A
Base Incorporation
Interpulse Duration
(IPD)
Native DNA
Fluorescence Intensity
C A G A T C T A
Non-Methylated
Control
Time
Analysis of Polymerase Kinetics
IPD Ratio
(Native/Control)
Forward Strand
Reverse Strand


Type I and III Methyltransferase characterization
1200
1000
800
600
400
200
0
1970
1972
1974
1976
1978
1980
1982
1984
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
2010
2012
2014
2015a
Type I
Type III
matched genes
Type I
Type III
awaiting matching

Probiotics – Our Friends!

Lactobacilli

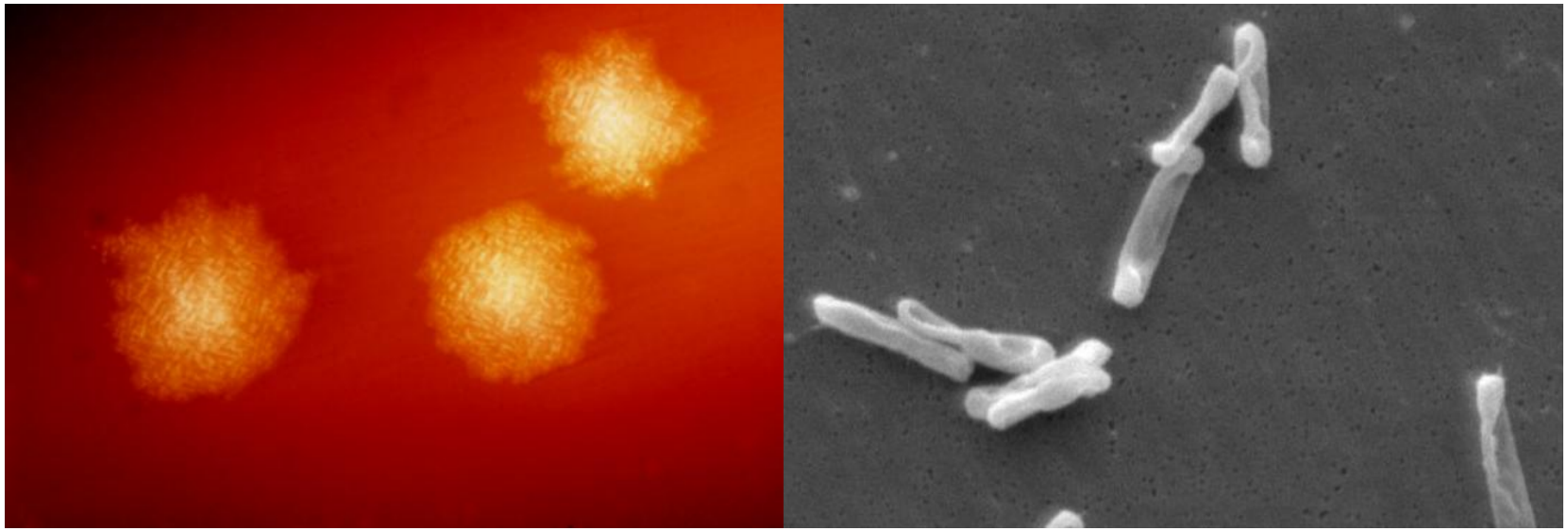
Bifidobacteria

Helicobacter

?????


Helps Boost
Calcium
Absorption*
nonfat
yogurt
GRADE A

Clostridium difficile (colitis)



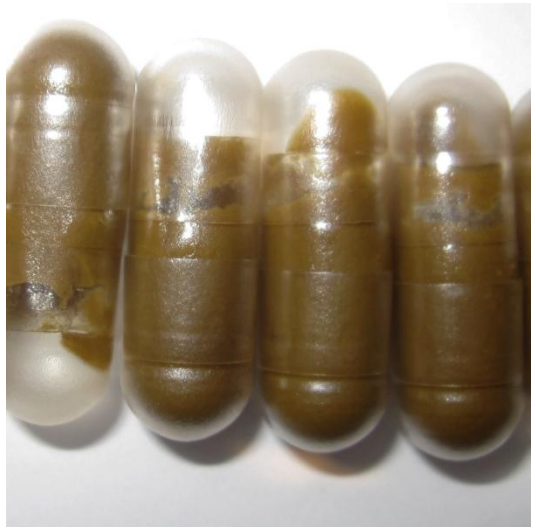
Increasingly resistant to vancomycin and fidaxomicin

Casugel ™ Capsules

DRcaps acid resistant hypromellose capsules

Balance between protection and targeted release

Unfortunately, they are translucent….



Key Performance Claims

- ✓ New & Improved
- ✓ Acid Resistant
- ✓ Slower to Dissolve Than Gelatin or Standard HPMC
- ✓ Minimize the risk of bad aftertaste

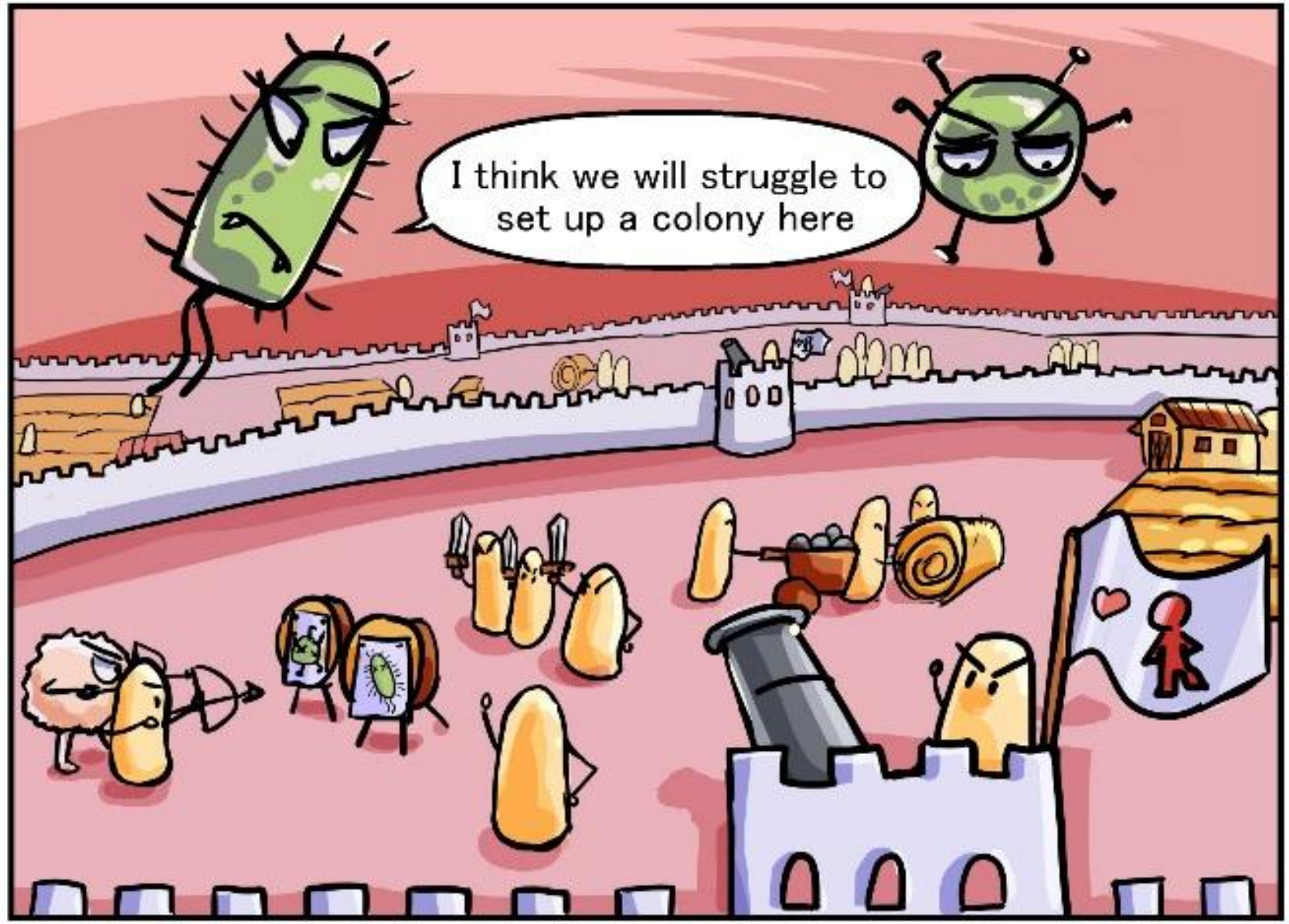
Courtesy of E. Hohmann

Referrals: ehohmann@partners.org; jsauk@partners.org

Bacteria get a lot of bad publicity

Year	Disease	Organism	Discoverer
1877	Anthrax	*Bacillus anthracis*	Koch, R.
1878	Suppuration	*Staphylococcus*	Koch, R.
1879	Gonorrhea	*Neisseria gonorrhoeae*	Neisser, A.L.S.
1880	Typhoid fever	*Salmonella typhi*	Eberth, C.J.
1881	Suppuration	*Streptococcus*	Ogston, A.
1882	Tuberculosis	*Mycobacterium tuberculosis*	Koch, R.
1883	Cholera	*Vibrio cholerae*	Koch, R.
1883	Diptheria	*Corynebacterium diphtheriae*	Klebs, T.A.E.
1884	Tetanus	*Clostridium tetani*	Nicolaier, A.
1885	Diarrhoea	*Escherichia coli*	Escherich, T.
1886	Pneumonia	*Streptococcus pneumoniae*	Fraenkel, A.
1887	Meningitis	*Neisseria meningitidis*	Weichselbaum, A.
1888	Food poisoning	*Salmonella enteritidis*	Gaertner, A.A.H.
1892	Gas gangrene	*Clostridium perfringens*	Welch, W.H.
1894	Plague	*Yersinia pestis*	Kitasato, S., Yersin, A.J.E. (independently)
1896	Botulism	*Clostridium botulinum*	van Ermengem, E.M.P.
1898	Dysentery	*Shigella dysenteriae*	Shiga, K.
1900	Paratyphoid	*Salmonella paratyphi*	Schottmüller, H.
1903	Syphilis	*Treponema pallidum*	Schaudinn, F.R. and Hoffman, E.
1906	Whooping cough	*Bordetella pertussis*	Bordet, J. and Gengou, O.

From "Brock Biology of Microorganisms"


I think we will struggle to set up a colony here
RunawayLabBook.com