Recent Developments in Microbiology

Claire Cummins, BSc

Infectious Diseases and One Health (Erasmus Mundus Master's: Université de Tours, Universitat Autònoma de Barcelona and Hannover Medical School)

https://doi.org/10.57098/SciRevs.Biology.1.1.4
Received June 09, 2022. Accepted August 03, 2022.

Abstract: Research in microbiology is a dynamic and exciting field, and this review article outlines some recent significant developments within microbiology including using shotgun metagenomics to uncover the connection between diet and lifestyle and superbug infections, the current state of Ebola virus diagnostic tests, and the potential of treating superbug infections via innovative phage virus therapy as an alternative to antibiotics. This review also explores two studies using the CRISPR gene editing technique, which has been a revolution within microbiology. The first study undergoes genetic engineering of potatoes to improve their nutritional and industrial applications, which has important implications in improving food stability as well as bolstering production. The second study utilizes CRISPR to engineer bacteria to kill themselves on command, which is important to prevent the excessive spread bacteria that are used for a variety of applications from eating plastic waste in the environment to treating the diseased human gut. Engineering these so-called 'kill switch' allows these bacteria to be utilized for their intended purpose without growing uncontrollably in the environment or in the gut.

Keywords: microbiology, CRISPR, gene editing, kill switch

Diet and lifestyle determine our vulnerability to resistant bugs

Antimicrobial resistance (AMR), that is viruses, bacteria and fungi that are resistant to antibiotics, is a significant cause of morbidity and mortality worldwide and it is projected that the consequences of AMR will continue to worsen in the coming decades. Scientists estimate that in 2019 there were 4.95 million deaths worldwide in which AMR was a causative factor, thus investigating ways to control and combat AMR is an extremely important field within microbiology research. The gut microbiome has garnered attention for researching AMR circulation within the population, since microbes present in the gut carry antibiotic resistant genes (ARGs), of which the total composition is called the human resistome. The human resistome is affect by many factors, such as the living environment, climate change, sanitation, and diet. Recent research by Oliver et al aimed to better understand which dietary and lifestyle factors are predictive of AMR in health U.S. adults. They found that those who had a higher fiber, more diverse diet had lower rates of ARGs, indicating that diet may be a potential method for reducing the future global burden of AMR (Oliver et al., 2022). This study utilized a technique called shotgun metagenomic sequencing, which is an extremely valuable method that has transformed the field of microbiology, as it allows researchers to exhaustively sample all genes in all organisms present in a given diverse sample, such as fecal samples in this case, without the need for traditional culturing (Quince et al., 2017).

Gene editing potatoes for nutritional and industrial applications

Clustered regularly interspaced short palindromic repeat (CRISPR)-Cas systems are well-characterized forms of acquired immunity systems that are found in bacteria and archaea. They were first described in 1987, when it was found that bacteria species Escherichia coli had an unusual repetitive DNA sequence in its genome. Since then, CRISPR-Cas-based tools are widely regarded as the most reliable tools for genome editing and engineering and has created a revolution within the field of microbiology (Ishino et al., 2018), with applications in a wide range of topics such as gene editing to cure cancer (Tiruneh G/Medhin et al., 2021), gene editing crops to improve their resilience and growth (Mallapaty, 2022), to learning more about the functions

of unknown genes (Kwon et al., 2015). A recent study by Toinga-Villafuerte et al utilized CRISPR/Cas9-mediated mutagenesis to modify starches in potatoes. The potato is the third most important food crop in the world after rice and wheat, and is energy-dense, yielding four times more calories per hectare compared to grain crops, thus for many countries it is considered a food security crop. In addition to its nutritional value, potato starch is utilized in producing processed foods, adhesives, paper, and textiles. Thus, the team identified the importance of the potato as a target crop for genome editing for modifications to improve either its nutritional qualities or for industrial applications. Starch is composed of two types of polysaccharides: amylose and amylopectin, and the ratio of these will affect the physical and chemical properties of the starch as well as the nutritional properties. Although amylose starches are currently used more in products that target health-related nutritional applications due to their lower glycemic index, starches higher in amylopectin have more applications in relation to processed food and other industrial applications, and in fact amylopectin-based starches show much more promise for ethanol production than those starches found in normal potatoes. Thus, this team used the CRISPR-Cas9 system to generate an amylose-free potato by targeting the GBSS alleles in the potato to eliminate amylose. They found that the Yukon Gold variety of potato yielded the best results and they successfully created them amylose-free. The CRISPR-Cas system has been a revolution in crop breeding, as conventional breeding processes could take 10 to 15 years, and gene editing has made that process much shorter. It can prove to be indispensable in improving industry and securing the global food chain in a world with a growing population (Toinga-Villafuerte et al., 2022).

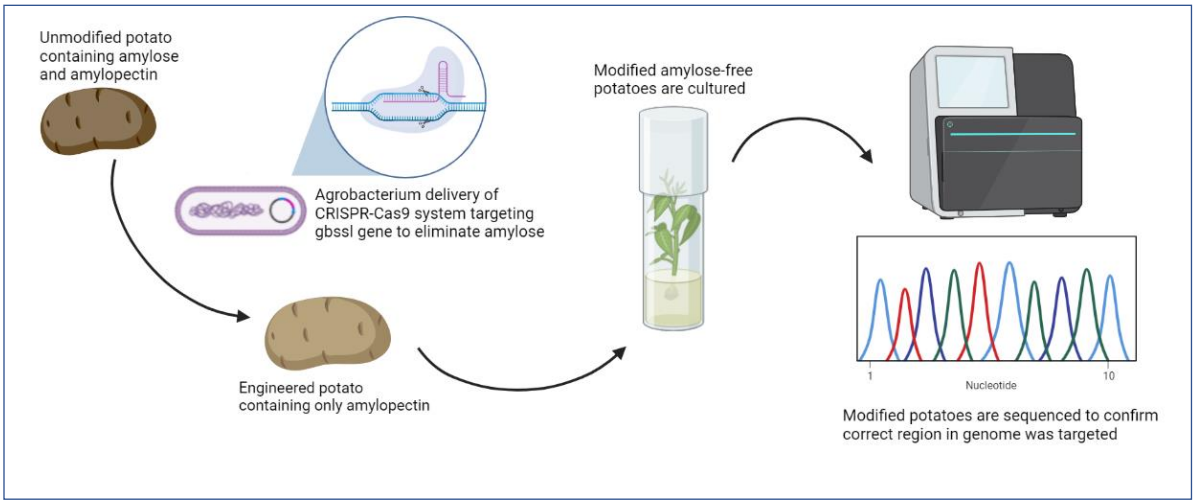

Figure 1: Schematic of the overall process of genetic engineering to create amylose-free potatoes. (Created with BioRender.com)

Treating superbug infections with viruses

As mentioned previously, antimicrobial resistance (AMR) represents a major public health challenge for the future and is associated with high mortality rates and combined with the fact that there has been a significant reduction in the discovery of new antibiotics to treat multi-drug resistant (MDR) bacterial infections, researchers are examining various alternatives to combat the problem. One of such alternatives being explored are bacteriophages, or phages for short, which are viruses that infect bacteria (Delattre et al., 2022). Phage therapy is the use of phages to treat bacterial infections and has been receiving growing support especially over the past 15 years, and although they are not as popular as traditional antibiotics, they are used in Europe in cases of therapeutic failure (i.e.: when there are no other antibiotic options left to treat a bacterial infection. Phages must attach to the bacterium, inject its genetic material which gets replicated, virions assemble and the bacterium is lysed, releasing new phages which can again attach to new bacteria and continue the cycle (Brives and Pourraz, 2020).

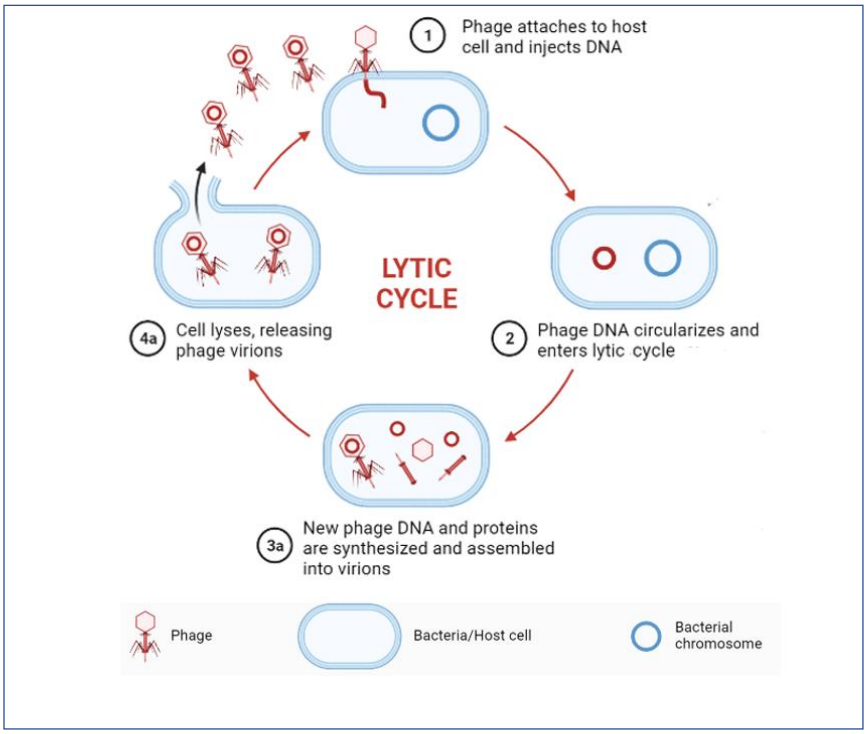

Figure 2: Schematic of the bactericidal lytic phage cycle that is utilized in phage therapy.
(Adapted from "Lytic and Lysogenic Cycle", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.)

The attachment stage of the phage is highly specific, which is great news in considering the therapeutic uses of phage therapy for AMR cases, since it can be adapted to target only the infection-causing bacterial species, while leaving the commensal or mutualistic bacteria (i.e.: the 'good' bacteria) of the microbiota untouched (Brives and Pourraz, 2020). Recent research by Delattre et al from INSERM in France focused on using phage therapy to treat AMR pneumonia by *Escherichia coli*, where they created a mouse model to characterize the interactions between phage and bacteria during the course of infection. This was a valuable study because before phage therapy can be administered to humans, the optimal dose, route of administration, and treatment duration must be elucidated, which is notoriously complex in this field. This is because the standardized pharmacology assessments that help to dictate processes of administration, distribution, metabolism, and excretion (i.e.: ADME) of drugs like antibiotics are not designed to be adapted to phage therapy. Their replication in the human body differs between each specific phage-bacteria pairing and their routes of elimination don't follow the standard metabolic pathways through the kidney or liver as pharmaceutical drugs do. The model created by this team incorporated pre-existing *in vitro* and *in vivo* data as well as mathematical models and found that the route of administration determines the success of phage therapy. The mice had better survival when the phages were more quickly administered to their target bacteria, thus the intratracheal route was more favorable than the intravenous route. Interestingly, the model revealed that the dosage given didn't have much bearing on the overall efficacy. The model also incorporated aspects of the animal's immune system, which is imperative in phage therapies since phages work in synergy with the immune system of the host, which aids in the overall process of eradicating the pathogenic bacteria. This study is extremely important in the field of microbiology since it lays out a new approach to create a more well-organized approach to clinical development of phage therapies to treat serious AMR-related infections (Delattre et al., 2022).

Engineering bacteria to follow our orders

Throughout the years, humans have found ways to harness the power of microbes, from the production of fermented foods and alcohol to the production of antibiotics and vaccines, there are many benefits to microbial processes which can be availed of. One

such example is the possible uses of microbes that degrade plastic wastes in the environment (Ru et al., 2020). Probiotic microbes have also become important resources in genetic engineering diagnostic and therapeutic technologies, for example *Escherichia coli* Nissle 1917 (EcN), which has been engineered to successfully diagnose and treat bacterial infections, cancers, gastrointestinal bleeding, obesity, and inflammatory disorders. Thus, EcN strains have garnered attention for further research in medical applications, however, as is the case with the plastic-eating bacteria, these are living organisms, and thus releasing them into the environment or the human body, respectively, will have consequences such as environmental contamination and competitive exclusion of native microbes. Any uncontrolled release of microorganisms poses a biosecurity risk, thus a study conducted by Rottinghaus et al examined the concept of biocontainment circuit designs, focused on preventing uncontrolled proliferation of these microbes in the wild. Such a mechanism could be considered a microbial 'kill switch', preventing the release of such genetically modified organisms into the environment by engineering them to self-destruct under certain circumstances. The researchers inserted multiple kill switches into the EcN genome via CRISPR technology, allowing the EcN to grow under normal gut conditions in the mouse, but to die on the consumption of an inducer, and subsequently get excreted from the body. This research will be important as it offers the opportunity for on-demand, selective removal of engineered microbes from the gut. This mechanism could also be engineered and subsequently applied to many other microbes, with the possibility of altering the kill switch conditions to environmental or chemical conditions. Therefore it offers a novel mechanism for self-regulated biocontainment, with far researching applications in therapeutics and industry (Rottinghaus et al., 2022).

A step in the right direction to develop field tests for Ebola virus

Ebola virus disease (EVD) is a rare but severe disease in humans with one of the highest viral death rates in the world, at an average case fatality rate of 50%.

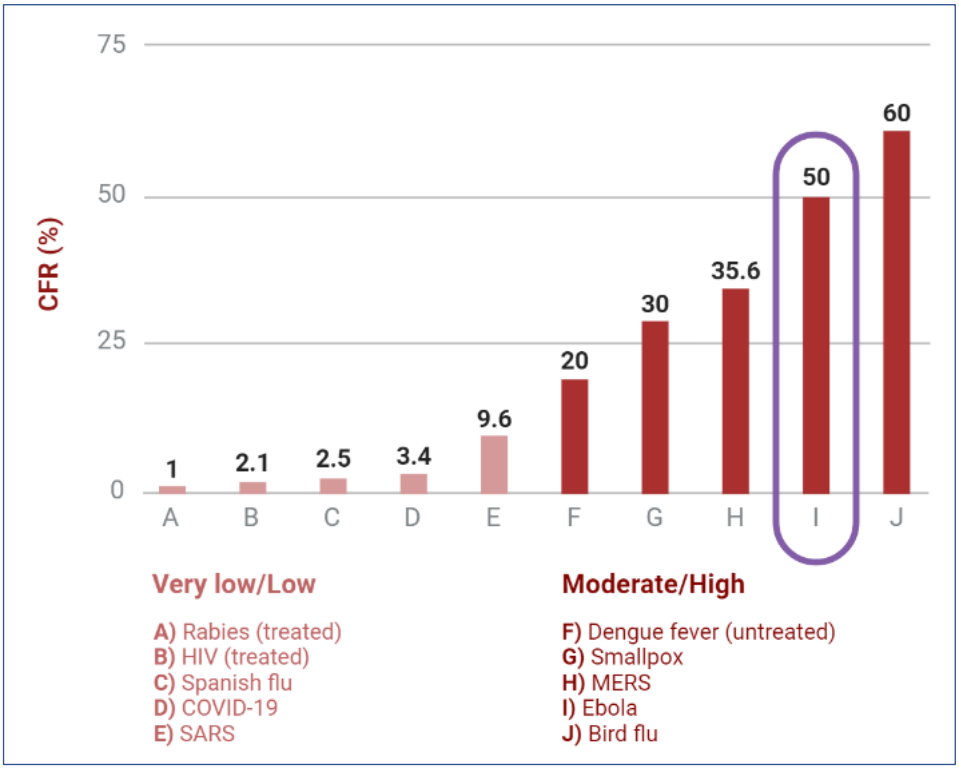

Figure 3: Case fatality rate (CFR) of Ebola virus disease compared to other viral diseases.
(Adapted from "Case Fatality Rate (CFR) of Common Viral Infections", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.)

The incubation period before symptoms appear is 2 to 21 days and symptoms include fever, fatigue, muscle pain and headache followed by vomiting, diarrhoea, bleeding and death (World Health Organisation, 2021). Several severe outbreaks have happened across the African continent in the past few decades resulting in thousands of deaths. Good outbreak control heavily relies on early intervention and treatment; however, this can be difficult in remote settings. This is because the gold standard test for diagnosis is reverse transcriptase polymerase chain reaction (RT-PCR) which requires good laboratory capacity, trained personnel, and transport infrastructure, as well as being expensive and time-consuming with results taking at least 2 days. In an outbreak setting with such a serious viral infection, this time can be too late for some patients. Therefore, much research has been focused on creating rapid diagnostic tests (RDT) that can be used in the field, however this hasn't been an easy feat in terms of reaching the required sensitivity and specificity to compete with RT-PCR (Nouvellet et al., 2015). A recent observational study conducted by Mukadi-Bamuleka et al aimed to address this research gap by evaluating the field performance of three Ebola RDTs during the 2018-2020 outbreak in the Democratic Republic of the Congo. They compared their performance directly with the gold standard RT-PCR and estimated the sensitivity and specificity of each RDT. Unfortunately, the three RDTs did not achieve the desired levels as mapped out by the WHO, however this does not discount their importance in helping to triage people with suspected cases of EVD into high and low risk groups while they await their RT-PCR result, which is extremely valuable in busy and low-resource emergency hospital settings (Mukadi-Bamuleka et al., 2022).

References

Brives, C., Pourraz, J., 2020. Phage therapy as a potential solution in the fight against AMR: obstacles and possible futures. *Palgrave Commun* 6, 100. https://doi.org/10.1057/s41599-020-0478-4

Delattre, R., Seurat, J., Haddad, F., Nguyen, T.-T., Gaborieau, B., Kane, R., Dufour, N., Ricard, J.-D., Guedj, J., Debarbieux, L., 2022. Combination of in vivo phage therapy data with in silico model highlights key parameters for pneumonia treatment efficacy. *Cell Reports* 39, 110825. https://doi.org/10.1016/j.celrep.2022.110825

Ishino, Y., Krupovic, M., Forterre, P., 2018. History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. *J Bacteriol* 200. https://doi.org/10.1128/JB.00580-17

Kwon, J., Namgoong, S., Kim, N.-H., 2015. CRISPR/Cas9 as Tool for Functional Study of Genes Involved in Preimplantation Embryo Development. *PLoS ONE* 10, e0120501. https://doi.org/10.1371/journal.pone.0120501

Mallapaty, S., 2022. China's approval of gene-edited crops energizes researchers. *Nature* 602, 559-560. https://doi.org/10.1038/d41586-022-00395-x

Mukadi-Bamuleka, D., Bulabula-Penge, J., De Weggheleire, A., Jacobs, B.K.M., Edidi-Atani, F., Mambu-Mbika, F., Mbala-Kingebeni, P., Makiala-Mandanda, S., Faye, M., Diagne, C.T., Diagne, M.M., Faye, Oumar, Kajihara, M., Faye, Ousmane, Takada, A., Sall, A.A., Muyembe-Tamfum, J.-J., van Griensven, J., Ari?n, K.K., Ahuka-Mundeke, S., 2022. Field performance of three Ebola rapid diagnostic tests used during the 2018-20 outbreak in the eastern Democratic Republic of the Congo: a retrospective, multi-centre observational study. *The Lancet Infectious Diseases* 22, 891-900. https://doi.org/10.1016/S1473-3099(21)00675-7

Nouvellet, P., Garske, T., Mills, H.L., Nedjati-Gilani, G., Hinsley, W., Blake, I.M., Van Kerkhove, M.D., Cori, A., Dorigatti, I., Jombart, T., Riley, S., Fraser, C., Donnelly, C.A., Ferguson, N.M., 2015. The role of rapid diagnostics in managing Ebola epidemics. *Nature* 528, S109-S116. https://doi.org/10.1038/nature16041

Oliver, A., Xue, Z., Villanueva, Y.T., Durbin-Johnson, B., Alkan, Z., Taft, D.H., Liu, J., Korf, I., Laugero, K.D., Stephensen, C.B., Mills, D.A., Kable, M.E., Lemay, D.G., 2022. Association of Diet and Antimicrobial Resistance in Healthy U.S. Adults. *mBio* e00101-22. https://doi.org/10.1128/mbio.00101-22

Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J., Segata, N., 2017. Shotgun metagenomics, from sampling to analysis. *Nat Biotechnol* 35, 833-844. https://doi.org/10.1038/nbt.3935

Rottinghaus, A.G., Ferreiro, A., Fishbein, S.R.S., Dantas, G., Moon, T.S., 2022. Genetically stable CRISPR-based kill switches for engineered microbes. *Nat Commun* 13, 672. https://doi.org/10.1038/s41467-022-28163-5

Ru, J., Huo, Y., Yang, Y., 2020. Microbial Degradation and Valorization of Plastic Wastes. *Front. Microbiol*. 11, 442. https://doi.org/10.3389/fmicb.2020.00442

Tiruneh G/Medhin, M., Chekol Abebe, E., Sisay, T., Berhane, N., Bekele, T., Asmamaw Dejenie, T., 2021. Current Applications and Future Perspectives of CRISPR-Cas9 for the Treatment of Lung Cancer. *Biologics* 15, 199-204. https://doi.org/10.2147/BTT.S310312

Toinga-Villafuerte, S., Vales, M.I., Awika, J.M., Rathore, K.S., 2022. CRISPR/Cas9-Mediated Mutagenesis of the Granule-Bound Starch Synthase Gene in the Potato Variety Yukon Gold to Obtain Amylose-Free Starch in Tubers. *IJMS* 23, 4640. https://doi.org/10.3390/ijms23094640

World Health Organisation, 2021. Ebola virus disease [WWW Document].
URL https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease

Conflicts of Interest

The authors state no conflict of interest.