University of Nottingham Students Sniffing for Success

A group of undergraduate students from the University of Nottingham have formed a team to design and conduct a novel scientific research project for the iGEM competition (International Genetically Engineered Machine). The project aims to develop a new system that can “sniff out” when food preservation and packaging methods fail, allowing for growth of the dangerous *Clostridium botulinum* bacteria. These bacteria can cause a type of food poisoning that may result in paralysis or even death.

C. botulinum is an anaerobic bacterium, meaning it can grow in conditions that completely lack oxygen. This organism is notorious for producing the deadly botulinum neurotoxin. This toxin, when in the nervous system, prevents the release of messenger chemicals resulting in paralysis. An infection with *C. botulinum* and/or ingestion of the toxin causes the disease botulism.

The History of Botulism

Botulism was first described and identified as a foodborne disease by the German medical writer, poet, and physician, Justinus Kerner, in 1820. His discovery was based on the clinical observations he had made following experiments both on animals and on himself. The name comes from the Latin for sausage, *botulus*, which at the time was commonly associated with botulism. However, it was not until 1896 that the organism was grown by Emile van Ermengem and correctly described as the bacterial source of the toxin.

There are seven different types of the botulinum toxin: A, B, C1, D, E, F and G. However, human botulism is only caused by types A, B, E and occasionally type F. The *C. botulinum* bacterium can also form spores which are highly resistant to harsh environments. Consequently, when they contaminate food that has been improperly prepared, the spores can germinate into toxin producing bacterial cells, which cause botulism when ingested.

There are several different forms of botulism; the form one contracts depends on the route of infection or intoxication: foodborne, infant and wound. Foodborne botulism occurs through preserved or home-canned foods that have been incorrectly processed. Infant botulism occurs when young children ingest tiny amounts of botulinum spores, which would be harmless to older children and adults. They are most at risk due to their small size and underdeveloped immune system and gut microbiota. Natural foods such as honey can be a reservoir for botulinum spores, hence the recommendations to avoid giving honey to children under the age of one. Wound botulism is caused by botulinum spores entering the body through damaged skin and is most common with intravenous drug users.

Foodborne botulism is rare, but when it occurs it is serious. Only 62 cases have ever been formally recognised in the United Kingdom between 1922 and 2005, with the largest outbreak occurring more than 30 years ago. This resulted in the hospitalisation of 27 people, all due to the consumption of contaminated hazelnut yoghurt, which led to the shutting down of a major company. This was an unexpected source of botulism due to the acidic conditions of the yoghurt which usually inhibit *C. botulinum* growth. However, it was later determined that the canned, hazelnut conserve was to blame – not the yoghurt. The preroasted hazelnut mixes had been heat-treated correctly, but were subsequently stored at room temperature for several months. This allowed the bacteria to grow before being added to the yoghurt and assigned a 25-day ‘use-by date’.

Other cases and outbreaks of foodborne botulism have been further afield, such as the mass recall of Alaskan salmon cans in 1982 - the second-largest recall of a product in FDA history (Food and Drug Administration). The incident involved a Belgian couple who, after consuming US canned salmon, contracted botulism from which the husband subsequently died. After a thorough investigation, a “small dented-in black spot and tiny hole” was discovered near the bottom seam of the can – a defect that allowed for contamination by *C. botulinum* spores. The cause of these holes was found to be a specific type of can-manufacturing machine that was used in multiple factories to shape the cans before they were filled with salmon. The filling of these cans resealed the puncture holes, creating an anaerobic environment perfect for the bacteria to grow in.

It’s Not All bad

However, the botulinum toxin does have some advantageous applications. In recent years it has gained prominence in the healthcare and cosmetic industry, e.g. Botox. Purified toxin is extensively diluted, so that it can be given as a treatment for muscle spasms or continuous constriction, such as the eyelid muscles in blepharospasm or bladder neck muscles in bladder outflow obstruction. Another common use of the diluted toxin is reversal of some visible signs of ageing, via cosmetic injections, as they will cause a person’s facial muscles to relax, and smooth out lines and wrinkles.

Challenge Testing

Food companies must subject their products to rigorous trials to determine food expiry dates, in a process called challenge testing. Challenge testing involves injecting test food with bacteria in a lab to determine whether the food preservation method is suitable for preventing bacterial growth. After inoculation, the food is kept for a certain period of time, under specific storage conditions before samples are then tested. The ELISA assay is the golden standard for detecting botulinum toxin protein. The assay determines whether the bacteria have been able to grow and produce the toxin; this can be used to determine the subsequent shelf-life of that product. The cost associated with challenge testing is very high as there are large amounts of food replicates involved and the preparation and extraction of the toxin for the ELISA assay is complicated. Companies take this testing very seriously, as botulism food poisoning outbreaks are estimated to cost the food industry about £25 million per person!

The iGEM Competition

The iGEM competition brings together 375 teams from 45 different countries in an event that resembles the Science Olympics. iGEM offers interdisciplinary teams of students the opportunity to design and test their science products. The students work on biological projects to tackle issues in their local communities and far beyond. Teams solve real-world problems, such as antibiotic resistance and global warming. Projects range from developing bacteria that can degrade plastics, to building a printer that allows you to create your own bacteria! Some teams have even gone on to start successful businesses.

As part of the competition, teams work to develop new Biobricks with novel applications to add to 'The Registry' – iGEM's extensive catalogue of modular DNA parts; this furthers the drive to standardise synthetic biology. Working with Biobricks poses an exciting new challenge for the undergraduates who would otherwise not have the opportunity to work with this technology.

In previous years Nottingham's iGEM teams have worked on projects involving bacterial security with *E. coli* and engineering bacteriophage viruses to be used as an alternative to antibiotics in the treatment of *Clostridium difficile* infections.

This year's iGEM team, from the University of Nottingham, consists of ten science-loving undergraduates studying a range of degrees, from Biotechnology to Computer Science and Mathematics. The students entered iGEM to develop their technical skills, learn more about synthetic biology and experience life in academia. The opportunity allowed them to use their science knowledge to engage in a project that solves a biological problem. Their project focuses on developing a strain of bacteria which could be used to safely monitor the likelihood of botulinum neurotoxin production in food. The reporter strain will be modified to produce the volatile gas, acetone, when induced by the transcription factor BotR.

How It All Works

The *botR* gene encodes the transcription factor BotR (DNA binding protein), which when expressed in the native botulinum bacterium, induces the transcription the neurotoxin. Therefore, they are engineering constructs that contained the *botR* gene under a native promotor of *C. botulinum*, so when the transcription factor is produced it can induce the acetone production genes, mimicking toxin production. Acetone was selected as the volatile gas produced by the reporter strain as it is a simple molecule that *Clostridium acetobutylicum* readily produces but *C. botulinum* naturally does not.

This gas will then be detected using an electronic nose designed and created by the team. The acetone will be detected in the headspaces of inoculated food that have modified atmospheres or are vacuum packed. Once the sensor detects the acetone produced by *C. botulinum*, a voltage is induced and converted to acetone concentration which is displayed on an LED screen. With this alternative method for detecting toxin production in food packaging, the teams aim to provide a cheaper and faster alternative to current ELISA food testing methods and could replace the need for animal testing, which still takes place in many countries.

In October, the team will present their project, 'NoTox - a nose ahead in food safety', at the iGEM conference in Boston with over 6000 attendees. For many of the students, this will be their first time experiencing such an international and prestigious event. They hope to follow in the footsteps of last year's iGEM team and bring home a gold medal!

iGEM website https://igem.org/

Nottingham iGEM team website https://2019.igem.org/Team:Nottingham

References:

- Al-Ghamdi, A.S et al. Botulinum Toxin: Non Cosmetic and Off-label Dermatological Uses. Journal of Dermatology & Dermatologic Surgery. 19(1), 1-8 (2015).
- Hull Hayes Jr, A. The Food and Drug Administration's role in the canned salmon recalls of 1982. Public Health Reports, 98(5). 412–415 (1983).
- Magill, A.J et al. Botulism. Hunter’s Tropical Medicine and Emerging Infectious Disease (Ninth Edition). 56, 511-513 (2013).
- Peck M.W. Bacteria: Clostridium botulinum. Encyclopedia of Food Safety 1, 381-394. (2014).
- Terranova, W. et al. Botulism type B: epidemiologic aspects of an extensive outbreak. American journal of epidemiology. 108(2), 150–156 (1978).