Telephone Conference with Dr Greg Jones (Campden BRI)

Challenge testing is primarily carried out by food testing companies, and we came across one such company known as Campden BRI. We reached out to them about our project and were put into contact with Dr Greg Jones, a microbiologist at Campden who has expertise on challenge testing. He provided us with answers to some questions we had, and he provided constructive feedback on our project as well. A summary of our interaction has been written below.

Aim of the conference:

After visiting Pro-Pak Foods, we decided that it would be better to target food testing companies rather than food manufacturing companies as our device is more likely to be used in this kind of setting. When talking to David Raine, the technical director of Pro-Pak foods, we were introduced to challenge testing and felt that our device could provide a cheaper alternative to it.

Why we chose this approach:

We decided that a phone conference was the best way to approach Campden for Human Practices, as Dr Jones was very helpful and already had some feedback on our project which he wished to relay as soon as possible.

The purpose of our telephone conference with Dr Jones was to receive feedback on our project and how we could alter it to fit the food testing industry better, but also to learn more about challenge testing and how much of a demand there is for it.

Questions we asked:

Dr Jones preferred that we use most of the answers he gave us for our own internal use, rather than publish them in this report. The majority of answers in the report are from the more general questions we asked him regarding *botulinum* and our project, as opposed to Campden BRI.

Project Questions:

1. **Would the industry be interested in a safe strain of botulinum?**

   Our project is useful as it would allow higher volume of experiments to be run since a less complex analysis of results would be required. The end point of our project (a cocktail of different *botulinum* strains) would be useful too.

2. **Is acetone an appropriate reporter?**

   We were advised us to stay away from sulphides - *Clostridia* give off a smell and it's due to sulphides being produced. If we used a sulphide as a reporter, it would get mixed up with the background. We were also advised to run experiments with the non-engineered

organism and see what it produces before we look at reporters. From this data, we will be able to decide on an appropriate one for the purposes of our project.

3. **Should our nose display yes/no for acetone detection, or should it state the concentration of it instead? I.e. would a concentration reading be useful in determining the efficacy of toxin detection.**

A simple yes/no is fine, but our project would be especially useful and industrially viable if we can quantify the amount of toxin produced based on acetone production. For example, X amount of acetone would produce Y amount of toxin. Standard curves of known acetone concentrations against toxin concentrations.

4. **How is food media made for *C. Botulinum?***

Standard *botulinum* media is used. We were recommended to look at sterile foods or make sure food that we do use is sterile if we were to make food media.

5. **Would *sporogenes* be able to grow on the same media as *C. botulinum*?**

Canned foods are better for *sporogenes* as *sporogenes* models group I *botulinum* (which can grow in canned foods). Alternatively, a better model for group II *botulinum* would be *C. Estertheticum* or any other psychrophilic *clostridia.* This is because psychrophilic *clostridia* would have similar growth characteristics to group II *botulinum* as it's also psychrophilic. It's more likely to grow on the same media. (more reasons described under "other").

<u>Challenge testing:</u>

1. **What happens in a challenge test?**

You would inoculate the organism into the product (around 100uL), making sure to disturb the product as little as possible. It would be stored under the proposed temperature and shelf life, monitoring what happens and when growth occurs.

2. **Is there a big demand for challenge testing?**

There is a big demand in the food industry. A safe *botulinum* strain that our project proposes as our endpoint wouldprove to be very useful.

3. **What is challenge testing data used for?**

Setting a safer shelf life/expiry date. It can tell you when *botulinum* grew. You would set the shelf life a few days before growth was recorded.

4. **How reliable is challenge testing?**

Challenge testing is quite reliable. Standardised methods are used for pathogen detection, and challenge testing doesn't produce false positives/negatives.

5. **What is the importance of a challenge test if it's not required by law?**

Food industries must follow the 10-day rule. Food products need to meet some limits e.g. pH, a_w (water activity), thermal process, salt levels for preservation/safety. If these limits aren't met, companies need to prove that a shelf life greater than 10 days for VP/MAP is achievable in some other ways. E.g. a company makes a new product, but it doesn't meet the pH requirement. To prove that the shelf life is greater than 10 days, they would need to challenge test it.

6. **What do companies require the challenge testing for?**

It is used for setting a safe shelf life of a product which claims to be >10 days.

Companies change their products accordingly based on the challenge test. E.g. if shelf life is <10 days then they might add preservatives or more salt etc. They would then challenge test it again to validate.

7. **How much of a priority is *C. botulinum* when it comes to challenge testing?**

Most common organisms that are challenge tested for are *botulinum* and *listeria*. To reach a bigger market we could use the same idea of our project to target *Listeria* in the chilled foods industry.

8. **Is there another way to test new preservation methods?**

Challenge tests, ELISA assays or use lateral flow devices (like pregnancy tests) are used.

9. **Are mice bioassays still used? (injecting mice with food extract and observing any symptoms of botulism)**

The mouse bioassay isn't used in the UK anymore. Most companies use ELISA for toxin detection.

<u>*Botulinum* Questions</u>:

1. **Is there any particular type of food industry which *botulinum* is most prevalent in/more likely to occur?**

People don't tend to look for it – Challenge tests assume the worse-case scenario that *botulinum* is present. The meat industry claims there is little *botulinum* in meat - 1 spore per kilo of fresh meat. This seems like a small figure, but then that means 1000 spores per tonne, and thousands of tonnes are produced daily.

2. **How are different strains of *C. botulinum* tested for, and which ones are more important to test for within the food industry?**

Outside of challenge testing they are distinguished by toxin type using ELISA. It can show that type B toxin is produced for example, but not the specific strain of *botulinum* producing it. Type E is found most commonly in the food industry – it's associated with fish.

3. **The meat industry is under pressure from the government to reduce the salt content of their products. Curing salt contains nitrates/nitrites which inhibit *botulinum*; this salt is used to cure meats. Thereby, won't reducing the salt content of meat allow *botulinum* to proliferate?**

Nitrite is not officially considered to be a controlling factor for *botulinum* growth. It does have an effect, but it is difficult to quantify. There is an anti-botulinum effect in cooked cured meats such as ham, but scientists are unsure why it has an effect though. The effect itself is known as the "Perigo factor".

Other points which were raised:

- *C.sporogenes* has different growth characteristics to group II *botulinum*. It's mesophilic
- *C. sporogenes* is used as a surrogate for group I – our concern is group II if we want to target challenge testing. However, using *C. sporogenes* is fine for proof of concept of our project.
- No surrogates are used for group II, so scientists mainly work on the actual group II *botulinum* itself.
- Group I have other signs of spoilage, so we don't need to look for toxin production in this group.
- It's hard to see other signs of spoilage in group II – our project is useful for this group. Group II (mesophilic) grows at different temperatures to group I (psychrophilic).
- *Clostridium estertheticum* is a better model for toxin production as it's also a psychrophilic species.
- Group I isn't used when looking at toxin production – group I mainly affects the canning industry. This may be due to retorting issues if *botulinum* is found in cans. Challenge testing is done for group II *botulinum.*

Reasons for the questions we chose:

Challenge testing is something which we learned about recently from our visit to Pro-Pak Foods. Campden BRI challenge test extensively and are licenced to use/challenge test for *C. botulinum.* This was why we felt that they were the perfect company to contact about our project, as they would be able to advise us whether it offers a better alternative to challenge testing for wild-type *C. botulinum*.

We asked Dr Jones specific questions regarding our project to determine how we need to alter our project to make it industrially viable/gauge the interest of Campden BRI and other food testing companies. For example, we asked about whether acetone was a good reporter to use – based on this feedback we would change the type of reporter we're using for something more appropriate (or keep the same reporter if acetone is fine).

Since we decided our project suited the food testing industry better than food manufacturing, we felt it would be wise to ask about the details of challenge testing to understand it clearly and see how we could integrate our project into a challenge test, or perhaps even carry out our own.

Similar to what I asked David Raine from Pro-Pak Foods, I asked whether *botulinum* was a priority in challenge testing. As we moved away from food manufacture, we wanted to see if the answer remained the same within the food testing industry as well.

We also learned from Pro-Pak that our project might be of interest to the meat industry due to the reduction of salt and nitrates causing a concern about the proliferation of *botulinum.* We asked Dr Jones to validate their concerns, as we felt it would be a good idea to get a response from an academic with expertise in food microbiology.

What we've learned from this call:

- *C. sporogenes* is not an appropriate surrogate for group II *botulinum* as it isn't psychrophilic.
- We learned about the uses of challenge testing and why food companies want it done.
- We learnt that the quantification of acetone is what will make our electronic nose viable for industry.
- Being able to quantify amount of toxin produced would also give our project an industry advantage.
- Our project will provide a quicker challenge test and require less complex analysis – you would no longer need to plate up the food extract after challenge testing and look for *botulinum* colony growth, which can be time consuming and expensive as an ELISA test is conducted as well.
- If our project can target *Listeria monocytogenes,* it would be incredibly useful as *C. botulinum* and *Listeria* are the most commonly challenge tested organisms – a bigger market for us.

How we can integrate our findings:

- We could investigate other psychrophilic *Clostridial* species and evaluate whether they are a better surrogate to use for group II *botulinum* – Dr Jones recommended *C. estertheticum.*
- If we want to make our sensor industrially viable, we should modify it to display a concentration reading too, rather than just turn on an LED when acetone is detected (however for proof of concept this is fine)
- Using GusA as an alternative reporter would allow us to quantify amount of expression of this gene under the BotR sigma factor and therefore give us an insight into toxin production.
- If we manage to carry out our own challenge tests with our modified organism and sensor, we know to inoculate our sample with around 100 µL of bacteria.

- Canned foods may be good to use as media for *C. sporogenes* when carrying out tests on real food samples.
- Now that we know how to challenge test, we could conduct our own with the food samples provided by Pro Pak.