

Manufacturing phages for livestock farming: An Interview with Proteon Pharmaceuticals as featured in Capsid & Tail

Jessica Sacher from the Phage Directory, and publisher of Capsid and Tail, interviewed Dr. Arkadiusz Wojtasik, a Production Director at Proteon Pharmaceuticals, about how his team uses the Cellexus CellMaker to produce phage for livestock farming.

Jessica: Let's start with your background — how did you come to work at Proteon? What's your role there?

Arkadiusz: I graduated with a degree in microbiology and microbial genetics, and then I did my PhD in biotechnology. After that, I worked for several years as a scientist in the Polish Academy of Sciences, where I developed my skills in microbiology, genetics, and bioinformatics. And in 2009, I got the chance to join an early stage biotech company, Proteon Pharmaceuticals, which was aiming to develop phage therapy products. I recognized that phage-based antibacterials have a real future. My role was to do deep genetic and bioinformatics analysis of phages, to support the selection of the best phages for our products. At that time, work on phages at Proteon was concentrated in the laboratory. It was only a few years later that we had the need to develop phage production technologies. Since the goal of industrial scale production was new, we built our approach to phage production from scratch. Based on skills I had acquired during my PhD studies, I took this responsibility and now I work as Production Director, managing the team, process, supplies, and the further development of Proteon's phage production path.

J: And what does Proteon do with phages? What kinds of phages are you working on, and what kind of applications are you working towards?

A: Proteon uses precision biology for microbiome protection. Our goal is to improve animal and human health, increase environmental sustainability and eliminate the unnecessary use of antibiotics. We use natural and safe solutions developed from our patented phage-platform technology. We aim to cooperate with farmers in the field of animal health, focusing on solutions that improve the economic efficiency of their farms, and also enable ecologically friendly solutions. Since we focus on the livestock industry, our first product, BAFASAL, eliminates Salmonella on the poultry farm. This product is already registered in India and Southeast Asia, and we are at the final steps of the registration process in Europe, North and South America.

J: How was the process for registering your phage products as feed additives?

A: Our products are novel applications everywhere that we register. So it takes a lot of work. For instance, I think we are the first company in Europe that wants to register a phage product as a feed additive. There are questions about how to classify that kind of product, what tests need to be done and things like that. We have had enough success that we are starting to register our other products. We have an aquaculture product, BAFADOR, which prevents and eliminates opportunistic infections in aquaculture, as well as BAFACOL, to combat APEC strains (avian pathogenic E. coli) in poultry. Both are also feed additives and are being registered in selected markets around the world.

J: How much phage are you regularly producing?

A: We can produce a large volume of phages. We are manufacturing phages in lines with the smaller 8-L Cellexus CellMakers and we also have a process line, which is still in the developing stages, where we use 50-L Cellexus machines. I cannot share exact volumes, but when we take into consideration the upstream and downstream processing, we use a significant amount of this capacity.

J: What kind of challenges have been coming up for your phage production?

A: I think the biggest challenge of phage production is to prevent cross-contamination with other phages. Proteon products are all cocktails consisting of several phages. Usually, some phages cover one part of our target production strains and others cover some other part. But sometimes, different phages target the same strains, so more than one phage may be active against a given production strain. This is why cross-contamination can be a serious challenge. The key, in my opinion, is to be extremely careful in all possible fields of operation, and I think one way is to use disposable technology. Cellexus offers that kind of technology, which helps to avoid cross-contamination.



The Proteon Pharmaceuticals team is using Cellexus CellMakers for optimizing phage production technology. Source: Proteon Pharmaceuticals.

J: That makes sense. So you always produce each phage separately even if they're going to be combined in a cocktail?

A: Yeah, each phage should be amplified in separate cultures. And, of course, if we want to have a cocktail, we need to make several separate cultures. But the most important way to avoid cross-contamination is to not produce two similar phages one after the other. Of course this may sound difficult, but it's not. Especially if you are preparing three or four products, as we do, with different phages, you can alternate the phages, and it works.

J: I see. So they all have different host strains that you're propagating them on. Is that right? Or is there some crossover in the strains you end up using?

A: It's different every time. Sometimes every phage has separate host ranges, other times several phages have the same host range. It depends on the product.

J: Got it. So you brought in the Cellexus CellMaker specifically to tackle this cross-contamination problem?

A: Yes, we launched the first CellMaker system seven years ago, when I was searching for a solution for phage amplification using disposable technology. At that time, it was very difficult to find a proper device that would ensure effective multiplication of microbes. The market offered mainly bioreactors for cell culture, and most were rather ineffective in aeration, because cell cultures don't need that. But Cellexus put in an 'airlift' technology, and I think this was very important. This is quite a different approach compared to most known bioreactor manufacturers, but this solution actually gives the possibility of multiplication of bacteria and thus phages. Of course the market is changing, but we started with Cellexus, and the CellMaker is very effective, so we continue to use these machines.

J: Are there other ways that the CellMaker has helped you?

A: Yes, the system is quite simple and it allows for a quick transfer of production from laboratory flask to bioreactor. However, it all depends on the phage strain. If it can be easily optimized in a flask, it will be possible to achieve quick success in the bioreactors, I think. And despite the fact that investments related to the purchase of the bags are necessary, the disposable bag technology lets us avoid many problems related to sterilization and cleaning the machines. In this way, we not only reduce the risk of cross-contamination, but also the risk of bacterial contamination of the operators, which is important when working with microbial pathogens.

J: What other potential pitfalls do you face when scaling up phage production? Do you have issues getting titers high enough, for instance?

A: Amplification is one thing, and you can search for solutions, like changing the media, optimizing the parameters, or culturing for longer, but the bioreactor culture is not the only thing that can be optimized. We have also discovered that the process of purification is also very sensitive. In the case of E. coli, you tend to lose phages using typical methods of purification. So every phage is individual, and although you may rely on your previous experience, it's not the only important thing.

J: So how easy is it to set this up? If you're ordering a new CellMaker in your lab, would you need a lot of background and experience in production, or is it pretty plug-and-play?

A: If someone had no experience in working with bioreactors, a quick startup of any bioreactor will of course not be easy, but it is worth noting that the CellMaker doesn't require additional systems like sources of water, aeration, or temperature. You can connect the bioreactors and start to use them in any laboratory. So I think technically speaking, it is easier to start work with the CellMaker.

J: Do you have any tips for someone considering ordering the CellMaker for their phage work? Would you say it's a good option?

A: Of course, I would recommend trying it. We are also pleased of course that both Cellexus' devices and bags can be purchased quite economically, though if you have experience in bioreactor cultures, remember that working with the airlift technology can generate some changes in processes that you may have already optimized.

Another very important thing to note, in my opinion, is that Cellexus offers the Regular system, a simple version of the bioreactor without the possibility of pH, or DO control, which is really quite inexpensive. But in fact, in the case of some of our phages, we didn't need to monitor these parameters at all. In contrast, for other manufacturers, the removal of pH or DO sensor is treated as a custom product, so it's not cheaper, it's actually much more expensive than the regular one! That is not very customer-friendly. So that might be another tip for laboratories: you may not need all the bells and whistles of advanced manufacturing — the Cellexus bag has a cheaper option that can be suitable. Of course if you want to work with bacteria, and you need precision control of pH, you need to use the more sophisticated option — the CellMaker Plus.

J: With regards to Proteon's upcoming directions, what are you excited about?

A: I'm glad that we are still developing new phage products. We are developing products to combat mastitis in dairy cows, as well as to combat infections caused by bacteria like *Campylobacter* or *Vibrio*. Of course, I'm very excited about how our production will develop, and what we will need to upgrade for those kinds of cultures. But my biggest challenge is that we are expanding our manufacturing footprint because we need to build our capacity further to meet current and future demand.

J: Are farmers excited about your products? And have they found them to be working?

A: Yes, Proteon has had a lot of interest and our clients have had great results. One challenge is that phage products are really new, and not everybody understands how to use them. Some farmers think they can use them like antibiotics, but it's not true. So we give them support, show them how to reduce antibiotic usage and take advantage of our products. As we do this, we also get feedback and it helps us better support farmers. So we see that our products are valuable and helpful and also that our dialogue with clients helps everyone learn.

J: Proteon seems to be taking exciting steps forward with phage bioinformatics and AI. How is that going, and how does that integrate with the rest of your activities?

A: Bioinformatics and AI are valuable. We use and develop both as tools for a number of reasons, for instance to help assess whether a given phage is safe, whether it is lysogenic or not, which can be very difficult if the phage's genes are not similar to known genes. The AI tool we developed, Phage.ai, lets you compare phage genetic sequences with databases and find out if it is likely to be lysogenic or lytic. We see this as an important obstacle for commercial development and have made this platform free for use to the research community because we want other scientists to join us in testing a larger number of phages. With more phages, the machine learns even more and enables the expansion of phage understanding.

J: Awesome. I think it's so cool what you're doing, and so encouraging to hear that you're already at the stage of working with farmers, and that you have this AI angle — I like how it incorporates data from the community too, because that just seems like the smartest way to do it.

A: It's very nice to hear that. Thank you.



Dr. Arkadiusz Wojtasik is a Production Director at Proteon Pharmaceuticals. Proteon uses precision biology for microbiome protection to improve animal and human health, increasing environmental sustainability and eliminating the unnecessary use of antibiotics.

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Contact details

Cellexus International Limited
6 Riverside Court, Mayo Avenue
Dundee, DD2 1XD, UK

Email: enquiries@cellexus.com
Office: +44 (0) 1382 666357
www.cellexus.com

Contact us for more information or
to request a demonstration.