The challenges in commercialisation of Probiotic API manufacturing



Joe Liu[A,B], *Brendan Cook*[A] *and Shaun Roux*[A]

[A]Probiotics Australia

[B]Email: joe@probioticsaustralia.com.au

Abstract. The concept of probiotics is well known and has developed into a high value commodity in recent times. Despite the ever-expanding number of probiotic products on our pharmacy, health food and supermarket shelves, the probiotic culture active ingredient has always been imported until now. In 2019, Probiotics Australia Pty Ltd opened Australia's first and only Therapeutic Goods Administration/current Good Manufacturing Practice (TGA/cGMP) certified facility dedicated to the manufacture of probiotic active ingredients. This article outlines the significant export demand for Australian-made health products and the lengths to which Probiotics Australia have gone to create a facility to meet needs of the probiotics research, commercialisation and consumer market today and into the future.

'Product of Australia' or 'Made in Australia'?

Probiotics is one of the most focused topics in the functional food and complementary medicines markets. An international research company reported that the global probiotics market was valued at an estimated US$49.4 billion in 2018. This market is anticipated to expand at a compound annual growth rate of 7.0%, to reach US $69.3 billion by 2023. One of the primary driving factors for the market to grow rapidly is the increasing awareness of the probiotic health benefits among customers, especially in the Asia-Pacific region including countries like China and Japan[1]. Australian-made complementary medicine and functional food products are renowned for their quality and high desirability, especially in Asia. Much of this can be attributed to rigorous quality of Australian Standard for industry including probiotic manufacturing process. Parallel to this, the regulatory framework in Australia is compulsory in maintaining superior quality of Australian products. Unlike most countries in the world, Australia, through the TGA, categorises health supplements including probiotics as listed medicines instead of food. From the procurement of raw materials, quality control, manufacturing facilities and equipment, production processes, to the packaging and final quality control testing of finished products, all steps are subject to strict regulation and scientific guidelines.

By 2030, CSIRO predicts over $3.2 billion in export revenue to Australia derived from vitamins and supplements, including probiotics[2]. Nevertheless, customers seldom know that the active probiotics ingredient, the highly concentrated pure culture of dried probiotic powder, is imported from overseas, mainly from the US, Europe, China, Japan or India. The imported active probiotics ingredient is further formulated into the end products such as capsules or tablets, or sachets. Some other end products are in the form of functional foods including beverages and dairy products. Therefore, for commercial products containing probiotics, 'Made in Australia' labels do not mean that the probiotics were fermented, purified and tested fully in Australia.

Australia does not lack the technical and scientific knowledge or skills in the field of probiotics. However, most of the expertise is concentrated within the academic world, conducting research focused on the health efficacy and immune functions of different probiotic strains. Some novel probiotic strains have been developed in Australia and commercialised[3]; however, their marketing exposure is very limited on a global scale. This reflects the fact that commercialisation of novel probiotic strains or manufacturing of probiotic active pharmaceutical ingredients (API) has not been the focus in the Australia probiotic industry. On the world stage, major players in the probiotics industry have dominated the market with no significant Australian-made alternative. To compete in a global scale in commercialisation, Australia must focus on helping and bridging academic and industry to work synergistically in boosting the commercialisation of probiotics. Probiotics Australia, as a Queensland-based company is keen to be a key player in probiotic research, commercialisation and industry in Australia.

The challenges of up-scaling: is it just a larger fermenter?

Fermentation is an ancient concept. From wine, sour dough for bread and yoghurt drinks, humans have mastered this technique for thousands of years. In modern fermentation, temperature, pH, agitation and aeration control are just some of the fundamental and critical processing parameters that are closely controlled. In most of the research-focused laboratories, the bio-processing work is usually around optimising the fermentation media and conditions in lab-scale to pilot-scale bioreactors. Although the basic process of probiotic API manufacturing is well studied and familiar to scientists, researchers, processing engineers and technologists in Australia, the manufacturing scale of facilities and utilities are far more complex than the pilot processing of probiotic generation.

Overall, the probiotic manufacturing process can be divided into two main parts, upstream processing and downstream processing.

Upstream processing

Multiple fermentation lines are usually required with the fermenter size ranging from 500 to 100 000 litres in working volume. To achieve the required large commercial volume, fermentation is gradually scaled up from smaller to larger fermenters. In order to economically provide enough heating for sterilisation, industrial pure steam is usually provided from a boiler system that is capable of generating tons of pure steam per hour. As opposed to heating, the temperature maintaining and cooling of fermentation lines is just as critical. The cooling system throughout the process is essential to maintain accurate control over the viability of the cells. It is also crucial to incorporate well designed CIP (clean-in-place) and SIP (sterilisation-in-place) systems to comply with the cGMP cleaning validation requirement to a high standard.

Downstream processing

The downstream process starts from the centrifugation step (Figure 1). Depending on the strains and/or the bioactive components interested in harvest, the concentrating of the biomass is usually carried out by industrial scale filtration or centrifugation. For example, harvesting probiotic cells can be performed in a semi-continuous centrifugation system that is capable of processing hundreds of litres of fermentation solution per hour.

The by-product of the centrifugation is the supernatant. The volume of the supernatant can range from 80% to 99% of the fermentation volume, depending on the strain, fermentation media, equipment and conditions. Therefore, thousands of litres of supernatant can be generated from the process on a daily basis, which will need to be properly treated before disposal under the monitoring of the local city council in Australia. Although there are

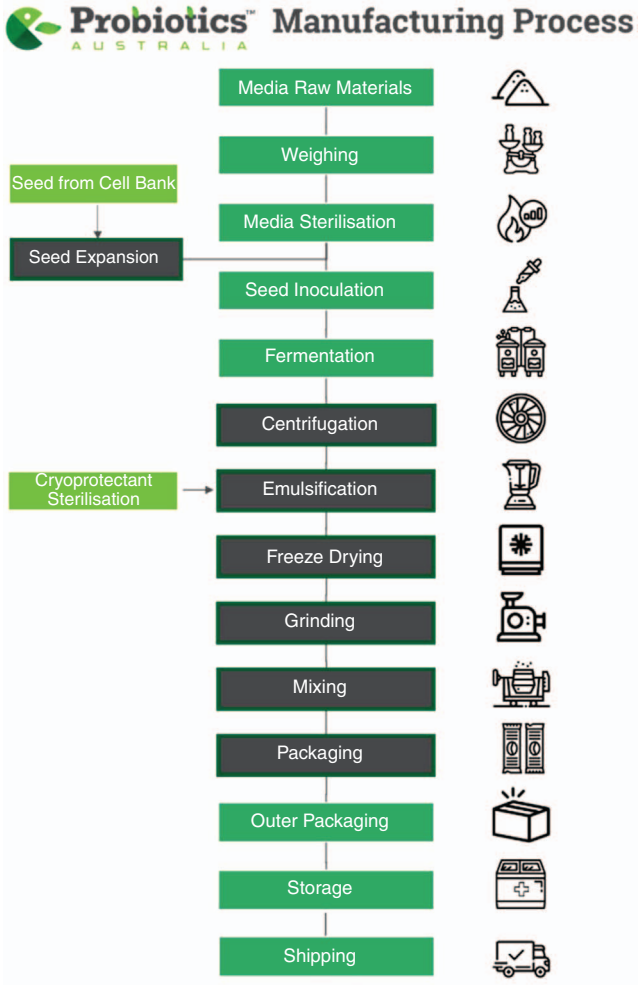

Figure 1. Probiotic manufacturing flow chart.

lots of literature showing benefits or good application of cell-free probiotic supernatants in different fields such as human health, animal health, bio-preservation, agriculture, etc., there does not seem to be many probiotic supernatant-base commercial products in the market.

Probiotics Australia is established and operating to enable all processes as illustrated in Figure 1, including for freeze drying. Freeze-drying cycle usually involves reducing the probiotic temperature down to as low as –190°C, depending on the type of freezing. The entire drying cycle could be more than 70 hours per batch. With multiple freeze dryers operating simultaneously, power consumption could translate into a huge economical problem for the company if the freeze dryer is not well designed, or the cryoprotectant formulation and processing conditions are not optimised.

After freeze-drying, there are multiple steps including harvesting the freeze-dried powder, milling, and mixing with excipients. The exposure of the products in the environment will require the processing facility to be cleanroom equipped with a HVAC system that can accurately control both the temperature, humidity and cleanliness of the air. The RH% (relative humidity) is ideally to be controlled under 30%. As handling probiotic powder can generate large amounts of particles (pure, concentrated and viable microorganism) travelling throughout the facility, high quality HEPA filters and differential pressure design between different processing areas must be an important part of the overall facility design to minimise cross-contamination, to ensure the strain purity of the products.

There are many day-to-day challenges in a modern probiotic API manufacturing plant. Other important utilities include the RO-water generation plant. Using high quality water in fermentation is critical for reducing the batch variation. QC analytical laboratory is also a fundamental component to assist the manufacturing plant for QC monitoring and troubleshooting.

The certification of TGA certified cGMP manufacturer

Probiotics are usually regulated as a food in the countries and regions that dominate this field. The US FDA lists probiotics that are suitable for use in their jurisdiction on a database known as GRAS (Generally Regarded As Safe). There are currently 29 records for 'Lactobacillus' and 17 records for 'Bifidobacterium' with GRAS Notices (as accessed 5 March 2020).

By comparison, regulations outside Australia are usually less strict than those that apply here. Across most of the world, HACCP-base systems from the food industry are usually employed for monitoring probiotics. However, in Australia probiotics could fall in the pharmaceutical category regulated by the TGA. The cGMP certification of a pharmaceutical API manufacturer by TGA is usually governed by the PIC/S (Pharmaceutical Inspection Convention – Pharmaceutical Inspection Co-operation Scheme) guideline Part 2, developed by the Internal Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use[4]. Using the PIC/S Part 2 as the guideline, usually BP (British Pharmacopoeia), USP (US pharmacopoeia), ISO standards are used to implement quality control plans, material testing methods, equipment calibration and validation plans etc. In the PIC/S guide Part 2, the fundamental QA components such as Documentation control systems, Processing parameters validation, monitoring and verification, Product recall systems, etc. are covered. Moreover, there are many additional requirements and system components very specific to the pharmaceutical and bio-processing or biotechnology manufacturers.

For example, cGMP certification for pharmaceutical and biotechnology manufacturers undertake Qualification and Validation of utilities, processing equipment, laboratory instrument, and manufacturing process as a critical part of their GMP certification. For all equipment used on site, from a laboratory thermometer to industrial bioreactors, documentation must be completed to qualify the equipment from its design stage – DQ (Design Qualification), to IQ (Installation Qualification), OQ (Operational Qualification), and PQ (Performance Qualification). The operational range of processing parameters is studied and identified in the OQ stage. This stage could be a very lengthy and expensive exercise for some of the equipment that are not stand-alone, but a set of systems consist of tanks, pumps, pipes with utilities of water, gas, steam connected to it. PQ is usually done through the actual manufacturing stage where real manufacturing data are collected to validate, evaluate and improve the process.

Another component that is quite specific to the biotechnology industry is the Cell Bank System Management. In the PIC/S guide Part 2 Section 18, the specific controls for APIs manufactured by cell culture is given. The starting active material for the fermentation is the 'seed' from the cell bank. The seed in the probiotic industry is the pure culture of intended probiotic strain. The discovery, isolation, purification, characterisation and banking of the probiotic strains involve traditional microbiology culturing methods and modern DNA sequencing identification methods. Other important components in the TGA/cGMP certification process include the quality systems required in the analytical laboratories, and the

quality systems required in the probiotic API manufactured for clinical trials.

The GMP certification process by the TGA is a highly technical, lengthy and extremely costly process. Many technical and regulatory hurdles are required to be overcome prior to the operation of the manufacturing plant.

Untapping the potential of probiotics

The concept for Probiotics Australia was born in 2009 when the opportunity for locally produced probiotic active pharmaceutical ingredients (APIs) was nascent. The vision was to construct a state-of-the-art facility to house research, development and production capabilities that would untap the potential of probiotics. One of the keys to unlocking that potential was to secure TGA certification for cGMP that would demonstrate that Probiotics Australia was delivering the highest quality product and give customers confidence in the probiotics they were consuming. In addition to TGA/cGMP certification granted in July 2019, Probiotics Australia also holds HACCP food license, ACO organic, USA NOP organic and FDA approval.

From their proprietary seed bank, Probiotics Australia can produce probiotic organisms for health, food, agriculture, aquaculture, veterinary and industrial applications. Fermentation capacities range from small scale bench-top experiments through to bulk cell-mass measured in tonnes and freeze dried in one of the largest lyophilisation sites in the southern hemisphere.

The research, development and manufacturing areas of Probiotics Australia are all located in the same building. This provides benefits in terms of rapid implementation of new techniques developed by the research team and offering a contract fermentation or manufacturing service. Through partnerships with universities and research organisations, Probiotics Australia has assisted to bring novel research from the laboratory and *en route* to clinical trials. The goal is for these trials to support the commercialisation of Australian research. Current studies pending publication encompass areas such as gastrointestinal health, Alzheimer's Disease, gut-brain axis, immune response and mother to baby microflora transmission.

An overnight success 10 years in the making, Probiotics Australia has evolved from a great idea to a thriving biotechnology research and manufacturing organisation with over 50 staff. The demand for Australian-made health products, determination of the founders and access to highly skilled scientists has led to the creation of a one-of-kind facility and business.

Conflicts of interest

All authors are employees at Probiotics Australia.

Acknowledgements

This research did not receive any specific funding.

References

1. Research and Markets (2019) Probiotics market by application (functional food & beverages [dairy products, non-dairy beverages, infant formula, cereals], dietary supplements, feed), ingredient (bacteria, yeast), form (dry, liquid), end user, and region-global forecast to 2023. Researchandmarkets.com
2. Wynn, K., and Sebastian, B. (2019) Growth opportunities for Australian food and agribusiness – economic analysis and market sizing. CSIRO Futures.
3. Crittenden, R. *et al.*. (2005) Probiotic Research in Australia, New Zealand and the Asia-Pacific Region. *Curr. Pharm. Des.* **11**, 37–53. doi:10.2174/1381612053382304
4. PIC/S (2009) Guide to good manufacturing practice for medicinal products, part II. https://www.tga.gov.au/sites/default/files/manuf-pics-gmp-medicines-part2.pdf

Biographies

Dr Joe Liu, PhD, is the assistant general manager in Probiotics Australia Pty Ltd. He holds a Bachelor of Biotechnology, Master of Microbiology. Joe started his PhD in CSIRO Food Innovation Centre. His research focus was on novel processing technologies including Ultrasonication and Pulsed Electric Fields on the functional modification of dairy proteins. After graduation, Joe worked as senior microbiologist, technical services consultant and R&D manager in different analytical laboratories to provide technical supports and commercialisation consultancy to different industry sectors. In 2017, Joe joined Probiotics Australia and he was one of the key technical managers to design and construct the state-of-the-art TGA cGMP certified probiotic API manufacturing facilities. He is now leading the technical and R&D teams in Probiotics Australia with the focuses on novel strain discovery, probiotic functionality studies, and optimisation of probiotic API manufacturing technologies.

Brendan Cook, Sales and Marketing Manager, has over 15 years' experience in the Australian biotechnology, healthcare and pharmaceutical industries in manufacturing, technology transfer, sales and marketing roles. His dream to work alongside researchers to bring their innovations to market for the benefit of all is being realised at Probiotics Australia.

Shaun Roux, General Manager, Probiotics Australia, is a highly experienced senior manager with a proven track record leading multi-disciplinary, cross-functional teams. Specialising in technical business culture, complex projects and innovation. His role in international collaborations allowed a transfer of biotechnologies that formed Probiotics Australia, and that is now shaping the probiotic precinct of Australia. He leads the way in product development, innovation and has worked with most of the leading health care brands in Australia producing new and innovative products.