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Review paper

Recent updates on encapsulated probiotics in poultry: a review

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Abstract

The global consumption of poultry is expected to increase by 2032, especially in Asian and European countries. Proper nutrition, including feed additives, plays a role in meeting the global demand for livestock products. In addition, the use of antibiotics as growth promoters resulted in antibiotic resistance issues, leading to the need to investigate alternative methods for replacing the role of antibiotics. The use of probiotic bacteria has proved to improve the growth performance of poultry and suppress pathogenic bacteria growth in the digestive tract. However, micro-environment conditions in the digestive tract affect the survival of probiotic bacteria. Modification of bacteria by encapsulation shows promising potential to protect bacteria from the harsh conditions in the poultry digestive tract. In addition, probiotic encapsulation also showed improvement in performance in poultry. This review will discuss the current encapsulation technology in probiotics applied to poultry and its effects. It also will explore the prospect of encapsulated probiotics, especially in the poultry industry, and its challenge.

Keywords: encapsulation technology, growth performance, nutrition, one health, poultry, probiotic



Introduction

The global consumption of poultry is expected to increase by 15% and account for 41% of meat-based protein sources by 2032. The growth in poultry consumption is primarily driven by rising demand in Asia, particularly in China, India, Indonesia, Pakistan and the Philippines, which has contributed to the overall increase in global poultry consumption over the past decade. In addition, there is a shift in consumption patterns from red meat to poultry in the European Union due to concerns about animal welfare, the environment, health, and carbon footprint issues (OECD-FAO Agricultural Outlook 2023-2032 2023). The anticipated increase in poultry meat consumption presents a challenge for farmers to enhance their production to meet market demand. Proper nutrition and suitable environmental conditions are crucial in maintaining the production performance of poultry (Chen et al. 2021, Barszcz et al. 2024). In addition, feed additives play a crucial role in supporting the poultry's growth and immune system.

Since they were first reported in 1946, antibiotics have been crucial in improving performance as growth promoters in poultry production. However, their use has led to serious issues such as bacterial resistance. As a result, many countries, including those in Europe, have implemented regulations prohibiting antibiotics as growth promoters. (Dibner and Richards 2005).

Probiotics are a useful alternative to antibiotics, and have shown positive results in feed conversion, gut morphology, immune response enhancement, and in inhibiting digestive tract colonisation (Bilal et al. 2021, Fesseha et al. 2021, Gharib-Naseri et al. 2021, Neveling and Dicks 2021). However, probiotic supplementation has limitations in stability that influence microbial survival. This limitation could be affected by temperature, pH, and physical form in storage and the biological mechanisms in the body after administration (Yazhini et al. 2018). In addition, the specific conditions of the digestive tract in poultry, which includes the proventriculus, ventriculus, and small intestine, provide conditions for a very large pH change from 2.0 to 7.0 and can kill bacteria. Therefore, the design of oral probiotics in poultry should be slightly different from that of mammalian oral probiotics in general (Chan and Zhang 2005, Jiang et al. 2017). To overcome these limitations, several probiotic modification reports have been proposed in order to protect and maintain the stability of probiotics, including probiotic encapsulation in the form of micro and nano encapsulation. Therefore, this review aimed to encourage the recent trends in encapsulation technology.

In this review, we searched relevant databases using

PubMed, Web of Science, Google Scholar and Scopus. The research question formulated for this review is: "What is the effect of encapsulated probiotics on growth performance in poultry?". From this question, we defined the search components (SC) into SC1: encapsulation, SC2: probiotics, and SC3: livestock. All articles were managed using Mendeley Reference Manager. During article selection, each article was screened independently. We excluded article types of books and irrelevant articles after examining the titles, abstracts.

Recent encapsulated probiotic development for poultry application

Microencapsulation

Many approaches have been developed to protect probiotic bacteria until they are released at the target site, including incorporating bacterial cells into other materials and eventually into encapsulated probiotics at different scales, from nanometers to micrometres (Asgari et al. 2020). Research in probiotic encapsulation, especially for the poultry industry, is still ongoing, with various studies exploring different methods of encapsulation to improve the viability and effectiveness of probiotics, as shown in Table 1. One promising approach is micro-encapsulation, which involves enclosing the probiotic bacteria in a protective coating to shield them from the harsh conditions in the gastrointestinal tract. This technology has shown potential for enhancing the survival of probiotics during storage and passage through the gut, inhibiting the inflammatory response due to bacterial pathogens, and ultimately leading to improved health benefits for growth performance in poultry (Wu et al. 2024).

As seen in Table 1 and Fig. 1, one of the commonly used methods in probiotic microencapsulation is the pre-encapsulation route. Following this route, the probiotic medium is added after microencapsulation (Dong et al. 2016). In broiler chickens, applying encapsulated *Enterococcus faecalis* with an emulsification system using alginate as an encapsulating matrix resulted in excellent growth performance and decreased feed consumption rate from the starter to the finisher phase in chickens. In addition, pre-encapsulation of probiotics also exhibited antioxidant capacity and immune system improvement (Zhang et al. 2015). A similar emulsification method has also been successfully applied to other bacteria, i.e., *Saccharomyces boulardii* and *Enterococcus faecium* (Qi et al. 2019).

Another simple method of making micro-encapsulated probiotics is the extrusion technique. Stable capsules are formed once the anionic polysaccharide comes into contact with multivalent ions. Before extrusion,

Table 1. List of encapsulation materials and techniques that are applied to several probiotics used for poultry.

Type	Material encapsulation	Technique	Microorganism	Results	Ref.
Microencapsulated probiotic	Alginate	Emulsification	<i>(L. crispatus, L. johnsonii, P. lactis)</i>	<ul style="list-style-type: none"> • Microencapsulate probiotics can mitigate intestinal and liver inflammation and regulate the growth of <i>Salmonella typhimurium</i> followed by growth performance improvement. • Reducing pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) expression, and inducing of anti-inflammatory cytokine (IL-10) expression 	(Wu et al. 2024)
Microencapsulated probiotic	Alginate	Emulsification and spray drying	<i>Enterococcus faecalis</i>	Assisting the efficient functioning of probiotics in broilers	(Zhang et al. 2015)
Microencapsulated probiotic	Alginate	Emulsification	<i>Saccharomyces boulardii, enterococcus faecium</i>	Efficient in the preservation of microbes in in vitro and in vivo environments, with the potential for the mass production of probiotics	(Qi et al. 2019)
Microencapsulated probiotic	Maltodextrin, skim milk powder	Spray drying	<i>Lactobacillus murinus, Streptococcus thermophilus, Pediococcus acidilactici</i>	Supplementation can improve broiler chicken live weight, body weight gain and FCR, followed by improved nutrient absorption by stimulating intestinal epithelial proliferation	(Pradipta et al. 2019)
Microencapsulated probiotic	Alginate, soybean protein isolate	Emulsification	<i>Ligilactobacillus salivarius</i>	The formulation of probiotic supplements targeting bacteria vulnerable to gastrointestinal digestion.	(Babot et al. 2023)
Microencapsulated probiotic	Alginate, sodium carboxymethylcellulose, sucrose, xylooligosaccharide	Extrusion	<i>Enterococcus faecalis</i>	<ul style="list-style-type: none"> • The microcapsules probiotics improve the performance of broiler chicken followed by antioxidant activity and influence the intestinal microbiota. • Boosting their overall health and improving serum biochemical parameters and increasing cecal microflora diversity in laying hens. 	(Han et al. 2013, Dong et al. 2016)
Microencapsulated probiotic	Agar-alginate	Emulsification	<i>Lactobacillus plantarum</i>	Enhancing the performance of broiler chicken in a tropical climate, optimizes the composition of bacteria in the cecum, maintains the health of the digestive system, and enhances the absorption of nutrients.	(Vimon et al. 2023a b)
Microencapsulated probiotic	Alginate	Extrusion	<i>Lactobacillus lactis, Bifidobacterium bifidum</i>	Improve the total serum protein, albumin, and globulin values, followed by lowering mean total cholesterol, LDL, and triglycerides.	(Yazhini et al. 2018)
Microencapsulated probiotic	Alginate	Extrusion	<i>Lactobacillus plantarum TN8</i>	Encapsulated probiotics able to improve the broiler performances and reduce the biochemical parameters in the blood serum such as serum cholesterol, LDL and triglycerides.	(Trabelsi et al. 2016)
Nano encapsulated probiotic	Chitosan Alginate	Ionic Gelation	<i>Bacillus amyloliquefaciens</i>	Enhancing bioavailability and gut barrier function and reducing the number of pathogenic microbes like <i>Campylobacter jejuni</i> .	(Alkushi et al. 2022, Ismail et al. 2023)
Nano co-encapsulated prebiotic and probiotic	Alginate, CaCl ₂ , tween 80	Ionic Gelation	<i>Lactobacillus plantarum, Lactobacillus paracasei, Saccaromyces cerevisiae</i>	Stronger antimicrobial and antifungal activity compared to non-encapsulated probiotics	(Hashem et al. 2023)

bacteria are suspended in a hydrocolloid solution and then dropped into the hardening solution through nozzles to form microbeads. The most important step in this technique is the application of nozzles with specific diameters to determine the size of the product to be encapsulated (Krasaekoopt et al. 2003, Rajendran et al. 2022). In broiler chickens, micro-encapsulated *Enterococcus faecalis* in sodium alginate, sodium carboxymeth-

ylcellulose, sucrose, and xylooligosaccharide mixtures exhibited enhanced growth performance and health (Han et al. 2013). Using the same technique, micro-encapsulated *Enterococcus faecalis* gave significantly improved serum biochemical parameters and increased cecal microflora diversity in laying hens (Dong et al. 2016).

In addition to the pre-encapsulation route, the

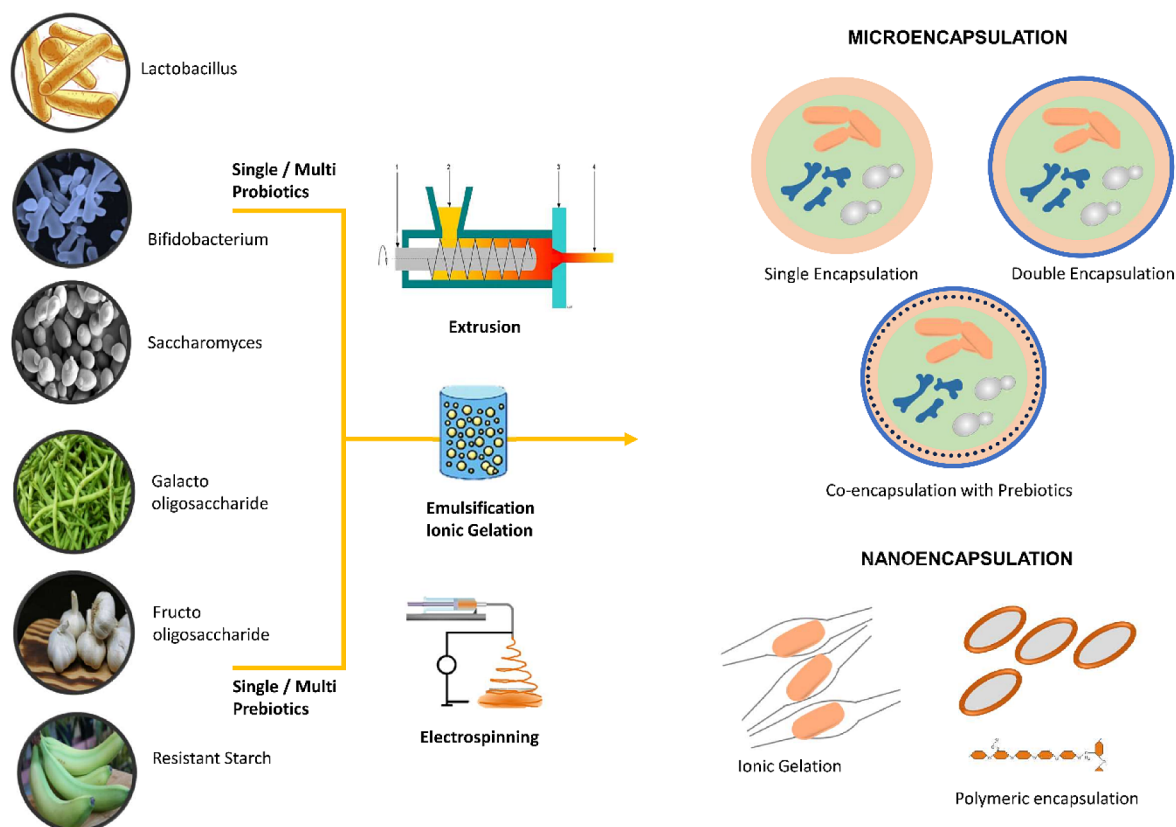


Fig. 1. Encapsulation routes of probiotics for poultry application. Single and multi-probiotics could be encapsulated using several methods creating micro and nano encapsulation. Double encapsulation and co-encapsulation with prebiotics offer enhanced properties including survivability in gastrointestinal fluid and long-term storage stability. Several images were taken from openclipart.org under Creative Commons Attribution CC0 1.0 (Universal Public Domain Dedication), Images were created using Inkscape version 1.3.2.

pro-encapsulation route using the spray drying method in which full-grown probiotics are dried with aqueous solutions of various polymers to form a micro-encapsulated product can also be used as a potential technique. Pradipta *et al.* used maltodextrin and skim milk powder that contains lactose and protein to protect cellular membrane damage during drying. This study showed encapsulation of *Lactobacillus murinus*, *Streptococcus thermophilus* and *Pediococcus acidilactici* using a spray drying technique improved the body weight gain and feed conversion rate with villus improvement to increase nutrient absorption in broiler chickens. However, the pro-encapsulation route using the spray-drying technique results in challenges during the encapsulation process which requires high temperature with high pressure. These conditions will stress the bacteria. Therefore, the selection of bacterial species and encapsulation materials play an important role in this technique (Zhang *et al.* 2015, Pradipta *et al.* 2019).

Nanoencapsulation

Studies on the development and application of nano-encapsulated probiotics are still challenging,

but they have considerable potential for poultry use. Most of the research has focused on developing probiotics using microencapsulation techniques. So far, Ismail *et al.* (2023), have mentioned the use of nano-encapsulation for probiotics with chitosan and alginate as encapsulating material to replace the use of antibiotics in poultry (Alkushi *et al.* 2022, Ismail *et al.* 2023). Hashem *et al.* (2023) found that nano-encapsulated prebiotics and probiotics could enhance antimicrobial and antifungal activity compared to non-encapsulated probiotics and levelled the conventional antibiotics' performance (gentamicin) (Hashem *et al.* 2023). Apart from the ionic gelation method that has been used in the nanoencapsulation of probiotics for poultry applications, there is a potential method that could also be applied in the fabrication of nano probiotics for poultry applications, namely the electrospinning method used commonly for polymeric encapsulation of bioactive compounds, including probiotics. This method offers stability, high encapsulation efficiency and release control, thus increasing oral bioavailability (Wen *et al.* 2017).

Encapsulating materials

The consideration of material selection for probiotic encapsulation is crucial to protect the bacteria from the harsh conditions in the gastrointestinal tract, such as the effects of digestive enzymes and pH changes from different areas of the gut. One of the most common encapsulating materials is sodium alginate. Alginate is a water-soluble polyanionic polymer consisting of 1, 4-linked- α -L-guluronic acid and β -D-mannuronic acid residues. It is biocompatible, biodegradable and mucoadhesive. Encapsulated probiotics will be released from the alginate capsule depending on the environment's acidity. Release study of chitosan-alginate microcapsule exhibited a low-release rate in the simulated gastric medium with acidic pH. In addition, higher release occurred in the alkaline pH of the simulated intestine fluid. Protection in acidic pH is provided by the formation of alginic acid due to the conversion of $-\text{COO}^-$ into $-\text{COOH}$, which prevents the release of a core substance (Yousefi et al. 2020).

However, a disadvantage of alginate use is its low stability in the presence of chelating agents such as phosphate, lactate, and citrate, which affect cell release from the beads. Therefore, several studies added cross-linking with cationic polymers, coating with other polymers, mixing with starch, and incorporating additives to maintain the stability of the beads (Krasaekoopt et al. 2003). A study of combination protein with polysaccharide, soy protein isolate (SPI)-alginate, effectively protected the *Ligilactobacillus salivarius* in the gastric environment. This combination also protected the encapsulated probiotic from proteolytic activity (Babot et al. 2023).

A combination of Agar-alginate that combines agar matrices and alginate charges under ionic crosslink through an o/w emulsion system for probiotic incorporation resulted in prolonged bacterial viability under heat treatment. This combination is suitable for use in tropical climates. The performance of broiler chickens in tropical climates showed improvement in average daily gain and feed conversion rate, although there was no effect on daily feed intake. It also improved the cecal microbiota, gut integrity and nutrient utilisation (Vimon et al. 2023a, b).

Chitosan is not suitable for use as a single material for probiotics since protonated amino groups under acidic pH will release the core substance from the capsule. Therefore, it is commonly used as an outer layer of the encapsulated probiotics. For instance, a combination of chitosan-alginate can increase the adhesion of encapsulated probiotics to the intestinal mucosa through a layer-by-layer method (Anselmo et al. 2016, Cao et al. 2019).

Effect of encapsulated probiotics

The final purpose of encapsulated probiotics is to improve the growth performance of the poultry by improving gut health. Therefore, the selected bacteria and the encapsulated material will also determine the final performance of the poultry. As shown in Table 2, the encapsulated and microencapsulated probiotics for poultry are mostly specified to improve the immune system, reduce the number of pathogenic intestinal microbes, provide antioxidant effects, improve blood serum, and enhance growth. By targeting pathogenic microbes inside the gut and increasing production, post-production aspects such as meat quality are also improved before consumption. The probiotic also maintains the homeostasis of the intestinal tract by increasing the immune system (Hernández-Granados et al. 2022, Gyawali et al. 2022).

The use of probiotics is also expected to reduce the use of antibiotics during poultry production, since the overuse of antibiotics can lead to drug resistance. For instance, the application of *Bacillus amyloliquefaciens* nano-probiotics in poultry, which resist gastric and bile acid, exhibited the ability to suppress pathogen bacteria such as *Enterobacteriaceae* and *Clostridium* species inside the intestine. Therefore, the abundance of *Bifidobacterium* and *Lactobacillus* species significantly increased, and gut health improved (Ismail et al. 2023). Another study by Zhang et al. also showed that the microencapsulation of *Enterococcus faecium*, which is currently becoming an alternative to antibiotics, can make the microbe grow better inside the GI tract and provide slow release compared with unencapsulated probiotics. This slow release will reduce the negative impact of the microbe when growth is too fast inside the GI tract (Zhang et al. 2024). Figure 2 emphasizes the effect of encapsulated probiotics in poultry in three main functions. The function starts with the reduction of pathogenic microbes, which could be achieved with more probiotics surviving in the gastrointestinal tract by nanoencapsulation. Probiotics also improve the immune system of poultry, thus replacing the use of antibiotics. Finally, feed efficiency and high meat quality are the main effects of the encapsulation of probiotics for poultry application.

As a consequence of the ability to suppress pathogenic bacteria and maintain intestinal microflora, encapsulated probiotics could repair the injured intestine by restoring the height of the villus in the ileum and the depth of the crypt affected by pathogens. Therefore, nutrient absorption can be enhanced (Wu et al. 2024). In addition, when the probiotics are fully released and active inside the gut, they will benefit by increasing protein synthesis, which affects meat quality (Poorbaghi et al. 2016).

Table 2. Current research on encapsulated probiotics and their applications for poultry.

Type	Microorganism	Encapsulating material	Function	Result	Ref
Encapsulated probiotics	<i>Lactobacillus paracaesi</i>	Polyacrylate resin	Growth promotor, gut health and microflora improvement	Encapsulated probiotics improve the health benefits of broiler chicken alongside the enhancement of the immune system and the potential antioxidant effects.	(Gyawali et al. 2022)
Microencapsulated probiotics	Bacteriophage (BP) FGS011	Polymer Eudragit L100	Pathogenic bacterial control	Enable the control of the pathogenic intestinal microbial <i>Salmonella</i> during the rearing period.	(Lorenzo-Rebenaque et al. 2022)
Encapsulated probiotics	<i>Lactiplantibacillus</i> (LAB) strains	Sodium alginate solution	Growth promoters, and infection prevention and treatment	The encapsulated probiotics can demonstrate potential probiotic characteristics.	(Zhang et al. 2024)
Microencapsulated probiotics	<i>Enterococcus faecalis</i>	Sodium alginate solution	Growth performance improvement and antioxidant ability	Enhanced the growth performance and serum immune responses. It also showed antioxidative functions and benefited the cecal microflora.	(Dong et al. 2019)
Microencapsulated probiotics	<i>Pediococcus acidilactici</i>	Gum Arabic Skim Milk	Improving the health and performance of broiler chicken	The microencapsulated product showed antibacterial activity against the <i>Salmonella</i> strain and improved GI conditions of broiler chicken.	(Kamwa et al. 2024)
Microencapsulated probiotics	<i>Lactobacillus reuteri</i> KUB-AC5	Sodium alginate solution Chitosan solution	Feed additives for growth promoters	Enhance the potential intestinal targeted probiotic delivery system for broiler chicken.	(Rodklongtan et al. 2014)
Encapsulated probiotics	<i>Bifidobacterium animalis</i>	Agave fructans CaCl ₂ 2% solution	Growth performance promoter, health improvement and immune responses	Enhance the feed conversion index and improve GI tract and immunomodulatory properties.	(Hernández-Granados et al. 2022)
Microencapsulated probiotics	<i>Lactobacillus acidophilus</i>	Calcium alginate	Meat quality improvement	Improve the physicochemical properties of chicken breast muscle.	(Poorbaghi et al. 2016)

Besides probiotics, a recent study also used bacteriophages to control the pathogenic microbe inside the GI tract. Using microencapsulation slightly improves the delivery, especially when application via drinking water is known to face more challenges (Lorenzo-Rebenaque et al. 2022).

Microencapsulated probiotics protect the bacteria from poor environments, especially the pH change during the digestive process. Probiotics also improve nitrogen metabolism, and they are known to form ammonia as a side product.

Future prospects and challenges of probiotics encapsulations

The International Scientific Association for Probiotics and Prebiotics (ISAPP) introduced a wide range of products, such as baby food, drugs, and nutraceuticals containing probiotics for health benefits and nutrition (Hill et al. 2014). The health benefits of probiotics are mainly attributed to enhancing the epithelial barrier, modulation of insulin-sensitive tissues, synthesis of antimicrobial substances, multi-pathogen competition, and induction of mucin secretion (Plaza-Diaz et al. 2019). Further, probiotics can attach to the surface of the epithelium, eliminating pathogenic microbes. Additionally, immune modulation by probiotics inclu-

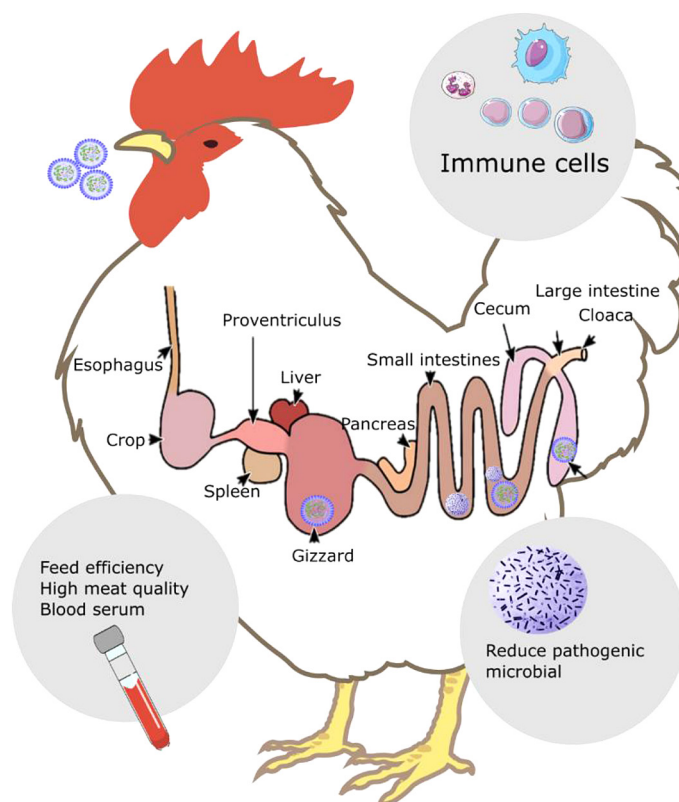


Fig. 2. Mechanism of encapsulated probiotics inside the chicken gastrointestinal tract. The encapsulated material of probiotics may vary depending on the purpose. After encapsulated probiotics are administered to chickens, they will pass through different parts of the GI tract and be involved in different parts of the intestine, especially the small and large intestines. The encapsulated probiotic then reduces the pathogenic microbes and improves the immune system. Several images were taken from openclipart.org under Creative Commons Attribution CC0 1.0 (Universal Public Domain Dedication). Images were created using Inkscape version 1.3.2.

des upregulation of anti-inflammatory cytokines and growth factors, differentiation of T-regulatory cells (Tregs), and facilitating interaction with the gut-brain axis (GBA) by endocrine regulation and neurological functions (Plaza-Diaz et al. 2019). It is now well established that probiotics possess anti-inflammatory, anti-allergic, anticancer, antidiabetic, immunomodulatory, anti-obesity, and anti-pathogenic activities. Administration of *Bifidobacteria* can even improve neuronal plasticity and cognitive behaviour (Rocha-Ramírez et al. 2020, Talani et al. 2020). However, there is a lacuna in exploring the probiotic microflora for their nanobiotechnological potential.

Probiotics are crucial in addressing diverse health issues, such as enhancing gastrointestinal health, strengthening the immune system, supporting metabolic regulation, and influencing mood. Studies suggest that probiotics can also help prevent viral infections through the gut-lung connection (Shahbazi et al. 2020, Baindara et al. 2021). This suggests the potential use of probiotics in managing respiratory illnesses caused by viruses, including those experienced during the COVID-19 pandemic in 2020, as well as neurological conditions such as influenza virus infection (de Oliveira

et al. 2021, Baindara et al. 2021). The encapsulation of probiotics has demonstrated potential in safeguarding these microorganisms and improving their precise delivery (Yao et al. 2017). As our knowledge of probiotics deepens, their crucial role in human health and potential for addressing various diseases becomes increasingly evident. Encapsulation technology holds significant value for probiotics, providing researchers and industry professionals with the latest tools to advance this technology from the laboratory to industrial applications. It is important to note that, although numerous research studies have been published in this area, only a few commercially available products incorporate encapsulated probiotics. Manufacturers will need to consider factors such as cell viability and probiotic functionality to make legitimate health claims. In addition, encapsulated probiotics issues such as size stability and production cost are two major factors challenging large-scale production. Therefore, laboratories face the challenge of developing technologies that are feasible for industrial production while maintaining appropriate scale and cost control.

Conclusion

In conclusion, encapsulated probiotics, such as micro-encapsulated and nanoencapsulated bacteria, have shown promising results as feed additives to improve poultry health. This area has become an interesting subject for research, especially for nanoencapsulated bacteria, which have become interesting aspects of research. The prospects for developing the probiotics market are also considerable, and laboratory research holds the potential to transition towards industrialisation.

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