

# Microbial Biopreparations in Broiler Nutrition: Production, Safety, Quality, and Regulation

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## Abstract

The increasing global demand for sustainable and high-quality protein sources in poultry production has intensified the exploration of microbial alternatives to conventional feed ingredients. This review provides a comprehensive overview of microbial biopreparations used in broiler nutrition, focusing on their production, composition, functionality, and regulatory considerations. The manuscript begins by addressing the current methods for obtaining biopreparations, with emphasis on fermentation technologies and biomass recovery processes. Special attention is given to microbial protein sources, including single-cell proteins derived from bacteria, algae, and yeasts. Among microbial biotechnology products, yeasts and mixed microbial cultures are discussed as versatile bioresources with high protein content, and potential probiotic benefits. The integration of brewery by-products and brewers' spent yeast as substrates or feed ingredients is highlighted as a sustainable approach to waste valorisation and circular economy principles. The review further explores the qualitative aspects of microbial biopreparations, such as protein digestibility, bioavailability, and amino acid composition, which are critical for optimizing broiler performance. Safety concerns, including microbial contamination, mycotoxins, and the presence of anti-nutritional factors, are discussed alongside quality assurance practices. The manuscript also outlines the current legal frameworks and international standards governing the use of microbial proteins in animal nutrition, identifying challenges and opportunities for market integration. Lastly, the impact of microbial protein inclusion on broiler health, growth performance, gut microbiota, and immune response is critically evaluated based on recent experimental studies. This review aims to consolidate current knowledge and identify knowledge gaps in the field of microbial biopreparations, supporting their advancement as sustainable and functional alternatives in broiler nutrition.

**Keywords:** bacteria, broiler nutrition, protein, single cell protein supplements, yeasts

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## 1. Introduction

Over the past sixty years, the global human population has witnessed an unprecedented rise of approximately 250%, growing from 2.2 billion to around 6.9 billion people. Projections by the United States Census Bureau suggest that if this upward trend persists, the world population could reach 9 billion by the year 2050 [1]. This dramatic forecast accompanies significant challenges in meeting the global demand for food. Relying

solely on traditional sources such as agriculture, livestock, and fisheries may not be sufficient to support the expanding population. While agricultural practices have seen substantial advancements in many industrialized nations, numerous regions continue to grapple with persistent issues like hunger, malnutrition, food scarcity, and diet-related health problems [2, 3]. Feed represents approximately 70% of the total expenses associated with broiler farming systems [4]. Among the primary components of these diets, corn serves as the main energy source, while soybean meal (SBM) is widely used as a protein-rich supplement. Despite its widespread use, soybean meal presents several drawbacks. Among

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its anti-nutritional factors are the oligosaccharides raffinose and stachyose, which poultry cannot digest. These sugars have been linked to decreased energy utilization, impaired fibre breakdown, and altered gastrointestinal transit [5]. Furthermore, its reliance on global trade and elevated market price poses financial limitations, particularly in regions without access to domestic alternatives. Although broiler diets generally limit soybean meal inclusion to around 30%, it still accounts for 40–50% of the total expense in formulated feeds [6]. Beyond economic aspects, soybean farming is associated with environmental degradation, especially due to deforestation activities in Brazil's Amazon basin, prompting broader socio-economic and ecological concerns [7]. Moreover, animal nutritionists are encouraged to explore cost-effective and sustainable alternatives that do not compromise animal productivity. When selecting alternative feed options for poultry, several factors must be carefully evaluated, including nutritional profile, accessibility, taste acceptability, and formulation consistency [8]. Promising substitutes including microbial sources, all of which offer potential as economical and effective replacements for conventional animal-based feedstuffs.

Microorganisms are emerging as promising agents for developing alternative food products, with single-cell proteins gaining recognition for their nutritional value. In addition, microorganisms such as yeast microalgae, bacteria and fungi have been identified as an especially potent source of protein. It can be efficiently cultivated both in controlled laboratory conditions and in open-water systems using minimal nutrient inputs. Bacteria are protein-rich microorganisms [9], with cell walls composed largely of peptidoglycan and classified on structural differences, including the presence of a lipopolysaccharide layer in the latter. Their high protein content has led to their use as alternative protein sources in feeds, although the digestibility of their thick cell walls varies, potentially influencing nutrient absorption and gut health in monogastric animals [10]. The yeast cell walls are rich in bioactive proteins such as mannoproteins [11] and  $\beta$ -glucans, which interact with host immune receptors (TLRs, dectin-1, dectin-2) to modulate immune responses, enhance gut integrity, and promote beneficial microbiota, contributing to improved nutrient utilization and growth performance in pigs.

Mannoproteins can block pathogen adhesion through mannose-specific binding, while  $\beta$ -glucans stimulate anti-inflammatory cytokines and gut cell activity, making yeasts effective protein-based feed additives with antibiotic-like benefits [12]. Microalgae exhibit elevated protein concentrations (12–65%, depending on species) [13], making defatted biomass a valuable protein supplement. Supplementation with microalgal proteins improved average daily gain [14], and feed efficiency [15], while slightly decreasing average daily feed intake in broilers [16]. Despite their promising nutritional and functional potential, microbial protein sources remain underexplored in animal nutrition. Current knowledge is limited regarding their digestibility, bioavailability, and long-term effects on animal health and performance. This review aims to consolidate current knowledge and identify knowledge gaps in the field of microbial biopreparations, supporting their advancement as sustainable and functional alternatives in broiler nutrition.

## 2. Materials and methods

This review presents a focused and analytical overview of recent advancements in obtaining microbial biopreparations, safety and quality criteria and regulatory framework regarding novel microbial biopreparations, emphasizing technologies that facilitate novel protein resources, in line with circular economy strategies. The research was guided by a systematic search protocol applied to leading academic databases, including Web of Science, Scopus, PubMed, MDPI and ScienceDirect. Search queries combined standardized descriptors and targeted keywords such as “microbial protein”, “single cell protein”, “nutrient recovery processes,” and “bioconversion of food waste”.

The selection criteria were confined to peer-reviewed articles in English, published between 2021 and 2024, with an emphasis on experimental data, critical reviews, and case analyses relevant to food production and agricultural sustainability. Supplementary materials, such as regulatory guidelines and industry reports, were also examined to enrich contextual understanding. Findings were categorized by primary treatment goals-microbial protein, yeast protein recovery, nutrient extraction, and isolation of valuable

bioactive molecules-and evaluated based on practical implementation, efficiency, and potential for scaling up.

### 3. Microbial protein sources

#### *Protein derived from bacteria biomass*

Bacteria are increasingly recognized as a promising source of single-cell protein (SCP) due to their rapid growth rates (20-30 minutes) and their ability to utilize a diverse range of substrates for energy [17]. Their biomass yield and protein content, which can reach up to 80%, are notably higher than those of other microorganisms used for SCP production [18]. However, due to the potential presence of pathogenic strains, extensive screening is required before bacterial biomass can be used as a food supplement, and the small size of bacterial cells complicates their separation. Moreover, commercial production ceased primarily due to economic factors, including rising oil prices and the competitive cost of traditional protein sources [19]. Recent research has focused on methane-oxidizing bacteria, such as *Methylococcus capsulatus*, which exhibit high efficiency in converting methane into protein-rich biomass [20]. Methane, a highly energy-efficient carbon source, supports significant microbial growth, making it a viable substrate for producing microbial protein for poultry [21]. Despite their high nucleic acid content, which may limit their direct use in animal feed unless processed, bacterial proteins are a promising alternative for animal feed applications [22].

#### *Protein derived from yeasts biomass*

*Candida utilis*, *Yarrowia lipolytica*, and *Saccharomyces cerevisiae* are rich in protein, containing up to 55% protein content in their total biomass, making them potential protein sources for animal feed [23-25]. Although yeasts are particularly high in lysine, they are low in sulfur-containing amino acids, requiring the addition of methionine when included in feed formulations [26]. Yeasts are also utilized by the intestinal microbiota, which convert them into short-chain fatty acids (SCFAs) that support the energy needs of intestinal epithelial cells and contribute to overall gut health [27]. The inclusion of yeast protein in animal diets has been associated with a minor decrease in average daily gain and feed intake, but it can enhance feed efficiency on

monogastric [28–30]. Despite this, other studies have demonstrated that substituting yeast for conventional protein sources such as soybean meal has beneficial effects on growth performance, nutrient digestibility, and gut structure in broiler fed [31–35]. These positive effects are mainly attributed to the presence of  $\beta$ -glucans and mannoproteins in the yeast cell wall, which promote animal health [36]. However, certain studies suggest that replacing soybean meal with yeast does not significantly influence intestinal health, immune function, or liver markers in poultry [37, 38].

#### *Protein derived from algae biomass*

Microalgae are notable for their high content of essential fatty acids, vitamins, and minerals [39,40]. The primary structure of their cell walls consists of cellulose, with smaller amounts of pectin, fucan, xylan, and mannan [41]. *Spirulina*, *Arthospira platensis* is traditionally harvested from alkaline lakes, it has long been consumed by both human and animals. Of the various *Spirulina* species, *Arthospira platensis* stands out for its exceptional nutritional profile, containing up to 70% protein, along with essential minerals, vitamins, amino acids, and fatty acids, making it a potential protein source [42] for poultry nutrition. In addition, *Pyrrophyta*, which possess two flagella, contain chlorophyll and bioactive compounds like carotenoids and xanthophylls, and can store starch through photosynthesis [43]. In contrast, *Chrysophyta microalgae* have a cell wall made of cellulose, silica, and calcium carbonate, and are proficient in accumulating lipids, including omega-3 fatty acids [44]. Moreover, microalgae are a rich source of polyunsaturated fatty acids, particularly n-3: eicosapentaenoic acid and docosahexaenoic acid, making them valuable in the starter and grower broiler diets [45]. However, microalgae-based feeds are not economically competitive with other protein sources due to high production costs and limited animal acceptance [46].

#### *Protein derived from mixed cultures*

Microbial protein can be obtained through mixed cultures for both solid state [25] and submerged fermentation [47], which offer several advantages over monocultures. The combination of different microorganisms allows for the optimization of metabolic pathways, enhancing protein yields and

diversifying the nutrient profile of the product. For example, bacterial and yeast cultures can complement each other by synergistically improving growth rates and nutrient uptake, leading to more efficient protein production. Additionally, mixed cultures have the potential to contribute to sustainable protein production, as algae can utilize sunlight for growth while bacteria and yeast can metabolize organic substrates [48]. However, challenges exist, such as the need for careful management of environmental conditions,

competition for nutrients, and the risk of contamination. A recent study [49] regarding mixed culture of *Candida utilis* and *Brevibacterium lactofermentum* indicate higher amounts of crude protein and amino acids, by submerged fermentation.

Despite these challenges, mixed cultures hold promise for producing microbial protein for applications in animal feed [50], and aquaculture [51], offering a sustainable and cost-effective alternative to traditional protein sources.



Figure 1. Advantages and disadvantages in obtaining microbial protein for broiler feed (after Sharif et al. (2021) [18])

#### 4. Current methods for obtaining microbial protein

The efficiency of productivity is largely influenced by the correlation of the specific metabolic pathways, the type of culture medium (whether organic or inorganic), strain/phyla characteristics, and the environmental conditions of growth. Achieving optimal protein biomass requires careful balancing of microorganism's nutritional needs and cultivation conditions, which can enhance carbon utilization and growth rates while maximizing metabolite production [52]. Carbon and nitrogen are essential sources of energy for microorganisms, with their availability

important in supporting growth, thus protein synthesis. Adjusting the substrate composition can influence these outcomes, though some studies suggest that nutrient limitations, particularly nitrogen, can induce stress that boosts protein production in certain strains, such as *Saccharomyces cerevisiae*. Furthermore, the type of carbon source used as substrate can alter the protein profile. The choice between organic and inorganic nitrogen sources also influences production, with organic sources like yeast extract boosting protein yields [26]. Additionally, residual agro-industrial waste, such as beer wastewater or sludge, provides an underutilized yet valuable source of nutrients for yeast, particularly in

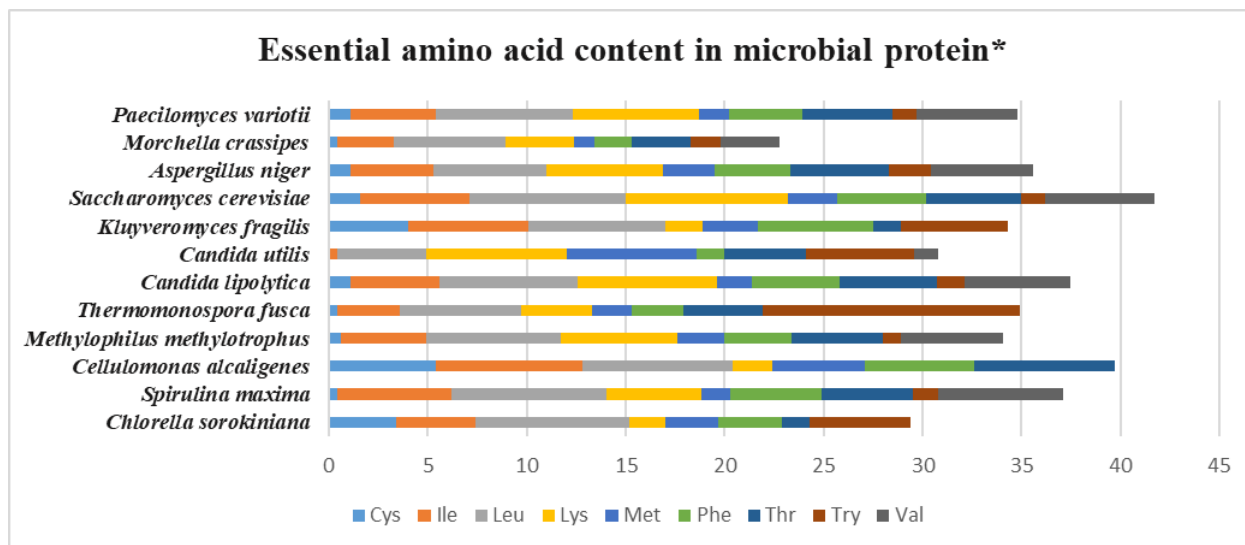
developing countries and can contribute to more sustainable yeast-based metabolite production [53].

In obtaining microbial protein encompass a multitude of biotechnological approaches, involving both cultivation and harvesting of microorganisms in controlled and uncontrolled environments. The most common technique is fermentation, in bioreactors under controlled physic-chemical, and biological parameters: strain purity, temperature, pH, and nutrient supply for maximize growth and protein yield recovery [54]. Microorganisms such as *Corynebacterium glutamicum* known for amino acid production [55] and *Methylococcus capsulatus* as for bacterial protein [56], are grown on a variety of substrates, including agricultural waste, methane, or hydrocarbons. Microorganisms such as *Saccharomyces cerevisiae*, *Escherichia coli*, and *Arthrospira platensis* are commonly employed for obtaining high protein biomass [57]. Following microbial growth, the biomass is harvested using methods like centrifugation or filtration, with the resulting biomass being processed into protein-rich forms such as powders or pellets suitable for animal feed or human consumption. The harvested protein biomass can be used in various applications, including animal nutrition and aquaculture, and its production often involves optimizing growth conditions to maximize protein synthesis. Furthermore, bioreactor technology has been integral to large-scale microbial protein

production, enabling the precise control of environmental factors to maintain optimal growth conditions, allowing for continuous microbial culture, by enhancing the efficiency of protein production [58]. In addition, waste utilization or bioconversion has become an increasingly important approach, where agricultural by-products or industrial effluents are converted into valuable microbial protein. This not only reduces waste but also provides an environmentally sustainable source of protein. The development of genetic engineering techniques has further advanced microbial protein production, with genetically modified strains of microorganisms being designed to enhance protein yield, growth rates, or to produce proteins with specific amino acid profiles [59]. Lastly, the biorefinery approach integrates the production of multiple products, such as proteins, lipids, and biofuels, from the same microbial biomass, increasing the overall efficiency and economic viability of microbial protein production.

### 5. Microbial protein digestibility, bioavailability, and amino acid content

Microbial protein has garnered attention as a potential nutrient source for both humans and animals, due to high levels of crude protein and amino acids (Figure 2); however, several challenges hinder its widespread adoption.



\*Expressed as g/16g N

Figure 2. Amino acid content in microbial protein (after Upadhyaya et al. (2016) [62])

One major issue is the high nucleic acid content found in microbial proteins, which is characteristic of rapidly growing organisms. This high nucleic acid concentration, ranging from 17 up to 25 g per 100 g of dry weight, can lead to increased uric acid levels in the blood, potentially causing health problems such as gout and kidney stones [50]. Additionally, bacterial protein sources are associated with complications such as high ribonucleic acid content, contamination risks during production, and difficulties in cell recovery. The nitrogen composition of microbial protein, with 70-80% of total nitrogen present as amino acids and the remainder as nucleic acids, can also affect its nutritional value [60]. Another concern is the indigestibility of microbial cell walls, which can reduce the bioavailability of proteins. Furthermore, microbial proteins, particularly from algae and yeast, may present undesirable sensory characteristics, such as unpleasant colors and flavors, and may cause adverse reactions such as skin irritation and gastrointestinal distress. Algal proteins, with the exception of *Arthrospira platensis*, are often rich in chlorophyll and unsuitable for human consumption. The low density of algae biomass (1-1.9 g dry weight per liter of substrate) and the high contamination risk during cultivation add further production challenges [61].

## **6. Current legal frameworks and international standards governing the use of microbial proteins in animal nutrition**

The regulation concerning protein products derived through specific technical processes that serve as direct or indirect protein sources and are introduced into the European Union (EU) market as feed or as components of feeding stuffs. The feed protein sources are often developed by the animal feed industry, are typically substitution products generated using innovative manufacturing methods. As such, their marketing within the EU requires regulation to ensure safety and efficacy. It is mandatory that before a new product is permitted for inclusion in the relevant groups, it must be verified that it possesses the required nutritional value. Furthermore, it must be demonstrated that these products, when used appropriately, do not pose any risks to human or animal health or the environment, nor do they compromise the quality of animal-derived

products consumed by humans. In some cases, labeling may be required to safeguard against fraud and to enable users to maximize the benefits of the products. Furthermore, the EU and national framework regulation: (EC) No. 767/2009, (EC) No. 183/2005, (EC) No. 178/2002, and Directive 2002/32/EC supports the feed and food industry with recognition for food and feed additives, safety standards concern, and labeling requirements.

The European Union legislation, specifically Regulation (EU) No. 2017/1017, supports the use of proteins derived from microbes. Since 2007, the European Food Safety Authority (EFSA) has kept a Qualified Presumption of Safety (QPS) list that includes microorganisms claimed safe for food and animal feed applications. Furthermore, the EU has established a strategic framework through the "Food 2030" policy initiative with the goal of tackling food security, health, and sustainability by the year 2030. Microbial proteins are an important pathway, aiding the transition towards alternative protein sources that encourage dietary modifications while reducing environmental consequences

## **7. Assessment of the impact of microbial protein alternatives on poultry growth performance, and health**

The use of microbial protein into poultry diets has shown promising effects on growth performance and overall health (Table 1). Microbial proteins, such as those derived from bacteria, yeasts, and algae, offer high-quality amino acid profiles, enhanced digestibility, and valuable bioactive compounds that contribute to improved feed efficiency and weight gain in broilers. Several studies have demonstrated that replacing conventional protein sources with microbial proteins can maintain or even enhance growth rates without compromising carcass quality. Additionally, these alternatives support gut health by promoting beneficial microbiota, strengthening intestinal barriers, and modulating immune responses, thereby reducing the incidence of disease and the need for antibiotic interventions. Among the evaluated microbes, *Spirulina platensis* stands out for its high protein content and rich profile of vitamins and essential fatty acids. It has consistently demonstrated positive effects on both growth performance and immune

function in poultry, making it one of the most promising candidates for inclusion in feed [63]. *Methylococcus capsulatus*, a methane-oxidizing bacteria, has shown strong potential as a fishmeal replacement, particularly in aquaculture, but its benefits have also been observed in poultry. It not only improves growth rates and meat quality but also supports gut health and reduces mortality, reflecting its nutritional and functional benefits [64]. Similarly, *Schizochytrium sp.*, a microalga rich in omega-3 fatty acids, enhances both growth and intestinal health, highlighting its dual nutritional and therapeutic value [65]. *Corynebacterium glutamicum* has proven effective as a soybean meal supplement, promoting growth and immune responses without the endotoxin concerns often associated with Gram-negative bacteria. In contrast, *Candida utilis* and *Saccharomyces cerevisiae* show more nuanced

outcomes. While they support gut health, nutrient absorption, and provide important micronutrients: B-vitamins and lysine, their impact on growth performance appears less consistent, with some reports of slightly reduced weight gain, possibly due to imbalanced amino acid profiles or variable digestibility [34, 35, 66]. *Methylophilus methylotrophus*, though beneficial for gut microbial balance, demonstrated a negative impact on broiler growth performance, suggesting limitations in its amino acid composition or digestibility [70].

Finally, *Desmodesmus sp.*, a green marine microalga, has been shown to improve both growth and feed efficiency, likely due to its valuable protein content and omega-3 profile, though more research is needed to fully evaluate its health effects in poultry [71].

**Table 1.** The use of microbial proteins in poultry nutrition

Microbial protein source	Impact on growth performance	Impact on health	Observations	References
<i>Spirulina platensis</i>	Positive effects on growth and feed efficiency	Improved immunity and disease resistance	High in protein, vitamins, and essential fatty acids.	[63]
<i>Methylococcus capsulatus</i>	Improved growth performance, meat quality	Enhanced intestinal health and reduced mortality	Used as a substitute for fishmeal in aquaculture diets.	[67]
<i>Schizochytrium sp.</i>	Improved growth and weight gain	Enhanced intestinal health	Rich in omega-3 fatty acids	[65]
<i>Corynebacterium glutamicum</i>	Positive growth effects when replacing SBM	Increased immune responses, higher Ig levels	Safe for consumption; endotoxin-free	[68]
<i>Candida utilis (torula yeast)</i>	Slightly reduced growth performance in poultry	Improved gut health, energy production	High in lysine but lacks sulphur amino acids	[69]
<i>Saccharomyces cerevisiae</i>	Slight reduction in growth rate	Improved nutrient digestibility and intestinal morphology	Provides essential B-vitamins and bioactive compounds	[34]
<i>Methylophilus methylotrophus</i>	Negative impact on growth performance in broilers	Improved intestinal health and microbial community	-	[70]
<i>Desmodesmus sp.</i>	Improved growth and feed efficiency		Source of protein and omega-3 fatty acids	[71]

## 8. Conclusions

This review highlights the growing need to explore sustainable alternatives to conventional protein sources in broiler nutrition, such as soybean meal, which, despite their nutritional adequacy, face limitations related to cost, availability, and environmental impact. Microbial

proteins have emerged as a promising solution, offering favourable amino acid.

Current biotechnological methods for microbial protein production, ranging from fermentation to biomass cultivation, have advanced considerably, enabling scalable and cost-effective outputs. Moreover, the inclusion of microbial proteins in broiler diets has shown positive effects on growth

performance, nutrient utilization, and health markers, although the degree of benefit varies depending on the microbial strain and processing method. Legal frameworks and international standards governing the use of microbial proteins in animal feed are evolving, aiming to ensure safety, efficacy, and environmental sustainability. Overall, microbial protein sources represent a viable and innovative approach to enhancing poultry nutrition while addressing the global demand for more resilient and eco-friendly feed solutions.

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