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REVIEW ARTICLE

Bacillus-based Probiotics: An Antibiotic Alternative for the Treatment of Salmonellosis in Poultry

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A B S T R A C T

Poultry is the second largest industry among the world`s various industries. This industry is highly affected by “salmonellosis” causing clinical signs like diarrhea, inappetence, vomiting, and sometimes death. This disease is becoming deadly due to antibiotic resistance which has been curtained as a worldwide security risk. Probiotics are microbes that can help chickens in the fight against pathogens in their gastrointestinal tracts. The most commonly and effectively used probiotics are those derived from *Bacillus* species. Probiotics establish cross-feeding among various bacterial strains in the gut environment, lowering blood cholesterol levels. In current years, there has been an increase in the occurrence of infections due to some strains of *Salmonella* which are resistant to multiple antibiotics. The use of antibiotics in poultry causes an enormous impact on the selection of antibiotics. A significant fraction of the world's consumption of antibiotics is being used in poultry. There is a knowledge vacuum regarding the establishment of bacterial resistance from poultry in resource-constrained contexts. The probiotics market share is rapidly growing. Asia is going to make the largest part of it as the growth is maximum in India, Pakistan, China and Japan. The aim of this review is to emphasize and overview the use of *Bacillus* probiotics instead of antibiotics to treat resistant genes of *Salmonella*.

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INTRODUCTION

One of the most significant food manufacturing sectors in the world is poultry farming (Sharopatova and Pyzhikova, 2020). As a result, the requirements for food safety are stringent, and larger production facilities tend to maintain them better than smaller ones (Qaim, 2020). Salmonellosis is the most prevalent disease of poultry worldwide (Zhou *et al.*, 2022). It causes diarrhea, inappetence, vomiting, and sometimes death (Johnson *et al.*, 2014). Each year, there is a salmonellosis outbreak in a number of nations (Popa and Papa, 2021). Humans develop salmonellosis through eating raw chicken meat, contaminated vegetables, shellfish, and beef (Harker *et al.*, 2014). The eating of chicken meat and eggs, thus most frequently results in salmonellosis. The prevalence of egg-borne salmonellosis has been widely recognized in various nations (Chousalkar and Gole, 2016). Human salmonellosis outbreaks have reportedly been seen in England and Wales. Consumption of tainted eggs was the main cause of these epidemics (Dominguez *et al.*, 2007). According to reports, between 1990 and 2003, eggs in Spain were the source of 48% of human salmonellosis cases. In Japan, instances of food-borne salmonellosis peaked between 1998 and 2004 (European Food Safety Authority, 2021). Consumption of eggs and infected raw meat was also linked to these occurrences (Toyofuku, 2008). *Salmonella* contamination of eggs can happen either horizontally or vertically (Gul and Alsayeqh, 2022). In horizontal transmission, it spreads through the environment of the poultry farm or by the excrement of the infected chicken (Chousalkar and Gole, 2016). It spreads vertically by passing through the contaminated eggs' yolks, albumin, and shells (Howard *et al.*, 2012). It is thought to affect 1.4 million people each year. Surprisingly, consuming raw meat has been linked to a high number of human outbreaks, especially when it comes to infected or undercooked poultry like chicken and turkey (Bryan and Doyle, 1995).

Antibiotic resistance has been identified as a security risk in the USA (George, 2018). A combined introvert plan is needed to overcome antimicrobial resistance (AMR) and to protect the health of the population (Smith and Coast, 2002). In this prospect, many programs for monitoring have been launched for the purpose of protecting humans as well as animals (Alexandra *et al.,* 2013). Finding an alternative to antibiotics is an important program to overcome this haphazard use of antibiotics (Lazzaro *et al.,* 2020). Probiotics, specifically those are derived from *Bacillus* species, are used as an alternative for eradicating salmonellosis (Tazehabadi *et al.,* 2021). The word "probiotic" came from a phenomenon discovered amongst co-cultured organisms in which some microbes produced growth-promoting chemicals which consequently enhanced the growth of the host (Jeni *et al.,* 2021). Probiotics are microbes that can help chickens fight against pathogens in their gastrointestinal tracts as well as increase their overall health and disease prevention (Abd El-Hack *et al.,* 2020) as shown in Fig. 1. Probiotics, for example, are useful because of their inexpensive production costs and wide range of applications in a variety of host species (Gaggìa *et al.,* 2010) as elaborated in Table 1. Through bile salt hydrolase activity, probiotics establish cross-feeding among various bacterial strains in the gut environment, lowering blood cholesterol levels (Arsène *et al.,* 2021). This review paper emphasizes the use of probiotics as a viable alternative to antibiotics in poultry. Probiotics derived from *Bacillus* species are the most widely and effectively used.

Selection of ideal *Bacillus* probiotics: *Bacillus* probiotics, which are used in animals and cross-species, can be isolated from the animal's own gastrointestinal tracts (GITs) and feces of many species of animals such as chickens, pigs, ruminants, and other terrestrial and aquatic animals (Mingmongkolchai and Panbangred, 2018). Although isolated *Bacillus* strains are frequently used across species, they are also frequently obtained from the GIT of the animal (Jayaraman *et al.,* 2013; Fan *et al.,* 2015). Soil, dust, water, and air are also the sources of saprophytes like *Bacillus* species. These bacteria are typically allochthonous to the GITs and are acquired through the consumption of microbes from contaminated food and soil (Duc *et al.,* 2004; Xu *et al.,* 2012; Wu *et al.,* 2014).

What are *Bacillus* probiotics: Live bacteria known as probiotics can help the host's health when given in sufficient doses. Since 1958, when the Italian product *Enterogermina*® was approved as an over-the-counter supplement in Italy, *Bacillus* species have been utilized as probiotics. In the past years, scientists have become interested in using *Bacillus* species as probiotics, and various major reviews have been published on the topic (Mazza, 1994; Sanders *et al.,* 2003; Hong *et al.,* 2005). *Bacillus clausii, Bacillus cereus, Bacillus licheniformis, Bacillus coagulans,* and *Bacillus subtilis* are the species that have undergone the most thorough examination (Celandroni *et al.,* 2019). When compared to other species which do not form spores like *Lactobacillus spp.,* spores have a variety of advantages, including the capacity to be dried and stored at room temperature without negatively affecting viability. Furthermore, these species can bear the low pH of the GIT barrier (Spinosa *et al* 2000; Barbosa *et al.,* 2005), although this is not liable for all *Lactobacillus spp.* (Tuohy *et al.,* 2007). Therefore, a certain dose of spores can be kept without refrigeration for an endless period while the complete number of eaten germs will reach the small intestine unharmed (Lysenko *et al.,* 2021).

Bacillus coagulans: The inaccurate name *Lactobacillus sporogenes*, which is not a recognized species name, is frequently used to describe this species. *B. coagulans* is produced in India as a food component and is being exported in different countries like Europe and USA after rebranding. This species is the source of probiotics. A bacteriocin called Coagulin, which is secreted by *B. coagulans*, is active against a variety of intestinal bacteria. Recently, FDA in the US awarded one strain, known as *GanedenBC30*, self-affirmed GRAS certification. It is sold by Ganeden under the brand name *GanedenBC30*®, and it is utilized in many goods like *Sustenex* as well as foods where it sterilizes the meals by the application of gentle heat treatment (AlFadil and Raga, 2017). *B. coagulans* has been demonstrated to have significant efficacy as a therapy for treating the symptoms of rheumatoid arthritis (Mandel *et al.,* 2010).

***Bacillus licheniformis* and *Bacillus subtilis*:** The genetics and physiology of *B. subtilis* have been thoroughly investigated. *B. subtilis* is frequently listed as an ingredient in probiotic goods, and historically, this is likely due to a mistaken belief that *B. subtilis* makes up the majority of aerobic spore formers (Calvert, 2022). Similarly, the products that carry *B. subtilis* also contain other species. This bacterium can also be used for soybean fermentation that creates natto, a traditional Japanese food. Consuming Natto has long been linked to health advantages, including the stimulation of the immune system. Natto contains up to 108 live spores per gramme of product (Hosoi *et al.,* 2004). Natto vegetative cells release a serine protease known as Nattokinase, which has been demonstrated to decrease blood clotting by fibrinolysis (Cutting, 2011). Almost all strains of *B. subtilis* produce Nattokinase, but the Natto strain produces most of it. This is the first of several crucial considerations. Secondly, it is possible that Natto's purported health benefits call for the eating of both bacteria and soybeans rather than only the latter. The USA manufactures Nattokinase, which is a pure enzyme, from a bacterium and is being sold as a health supplement all over the world. It has GRAS accreditation (Mohamed *et al.,* 2021). Various pathogenic species of chickens such as *Escherichia coli, Clostridium perfringens* and *Salmonella enterica* are minimized by the oral administration of *B. subtilis* spores (La Ragione *et al.,* 2001).

Antimicrobial Resistance against Salmonellosis: Although, the lavish use of various antibiotics in the food chain system has many advantages, including increased productivity, better animal health, and occasionally a decrease in food-borne infections but *Salmonella* infections that are resistant to several different antibiotics have dramatically increased in frequency in recent years (Lagadinou *et al.,* 2021).

Among the various species of *Salmonella*, *S. pullorum*, which causes a disease known as bacillary white diarrhea, is a prominent pathogen that has been eradicated from most

Table 1: Strains of *Bacillus* species used as probiotics and their benefits

Strains	Benefits	Characteristics	Brand	Mode of action	Reference
Bacillus amyloliquefaciens	Enhances growth performance and gut health	Root-colonizing bacteria used to fight plant pathogens in hydroponics aquaculture and agriculture	Enviva®	Cecal metabolites involved in glyceride and amino acid metabolism are changed	(Cao *et al.*, 2018)
Bacillus licheniformis	Growth enhancer and prevents necrotic enteritis	Common bacteria in soil	GALLIPRO® MS	Improves the antioxidant enzyme activity in liver, ilium and serum	(Zhao *et al.*, 2019)
Bacillus coagulans	Growth enhancer and improves gut histomorphology	Characteristics of both Bacillus and Lactobacillus genera	ATCC-7050®	Intestinal flora balance is improved because of which feed conversion ratio is improved	(Hung *et al.*, 2012)
Bacillus subtilis	Enhances laying and helps to improve gut health and immune system	Bacteria of GIT of humans and ruminants	SPORULIN®	Increase in butyrvibrio, butyrate producing bacteria in stomach	(Jiang *et al.*, 2021)

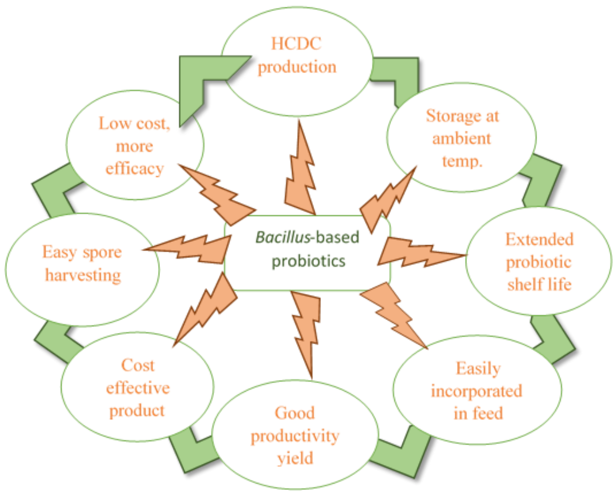

Fig. 1: *Bacillus* probiotics and their benefits.

of the world (Shah and Korejo, 2012). Similarly, *S. gallinarum* causes fowl typhoid in chickens and turkeys which may be acute or chronic; nevertheless, similar infections have also been documented in game or wild birds. Despite being vaccinated, against fowl typhoid, is still a threat to the global poultry industry (Penha *et al.*, 2016). *S. enteritidis* causes food poisoning and layer-by-layer ovarian infection (Majowicz *et al.*, 2010).

The ability of microorganisms to withstand the effects of major therapies practiced against them has been noted and is increasingly recognized (Dewachter *et al.*, 2019). Resistance rates are flourishing rapidly but the situation varies with the type of isolates. For instance, the number of figures for *S. aureus* resistant to *methicillin* escalates from close to zero in 10-15 years; it occurs nearly 66.9% in Korea and the Republic of Japan, 13.2% in Belgium, and 53.3% in the USA by 2019. Resistant rates of *Streptococcus pneumonia* were smaller than 13.2% in Belgium, Italy, and Inland, but 7% in Germany, 6.2% in Asia Pacific region, 6.9% in Latin America, in Iran 47.8%, and 48.3% in Europe (Carvalhaes *et al.*, 2021; Farajzadeh *et al.*, 2021). Hence, AMR requires a master plan for effective control and management.

Resistant genes of *Salmonella:* *Salmonella* strains are recognized as potent pathogens in poultry, causing salmonellosis, which also infects humans, causing vomiting, diarrhea, and abdominal pain. To treat bacteria, prudent therapies are used; however, the choice of antimicrobials is dependent on the susceptibility of microorganisms to achieve the desired response (Mor-Mur and Yuste, 2010). As a result of human concerns, clinicians have very few antimicrobial options. Hence, the repetition of the same salts evokes antimicrobial resistance (Mooljuntee *et al.*, 2010). Imprudent use of antimicrobials produces resistance in both beneficial as well as harmful organisms, and more importantly, it has been observed that antibiotic selection pressure is high in poultry and it sheds in waste material (Guardabassi *et al.*, 2018). AMR is due to resistance in chromosomal genes, which are present in plasmids that help transmission (Von Baum and Marre, 2005). To evaluate the resistance of microorganisms, a disc diffusion assay is routinely used, which gives a pragmatic idea about prescribing authentic antibiotics. Nevertheless, this technique fails to find resistant genes that might be carried by a microbe or may be transferred from other bacteria (Chijioke *et al.*, 2013). Because of the large volume of resistant genes, molecular testing methods like PCR, microarrays, and hybridization are used (Perreten *et al.*, 2005). Salmonella has a wide variety of resistant genes against different antibiotics, e.g., *tetracyclines (tetA, tetB, tetC, tetD, tetE,* and *tetG*), *sulfonamides (sul*1, *sul*2, and *sul*3), *chloramphenicol* (*cat1, cat2,* and *cat3*), *cmlA, cmlB,* and *floR*) and *aminoglycosides* (*aph (3)11a, aac (3)11a,* and *aac6)* as shown in Table 2.

List of resistant antibiotics: For decades, antimicrobials have been used in humans as well as in animals for treatment and control. In modern farming, these antimicrobial agents are included in the feed, which not only serve as growth promoters but also act as prophylaxis. Therefore, antibiotic selection pressure is very high in poultry, and as a result, fecal flora content contains a high number of resistant bacteria. However, misuse of antibiotics for these purposes, predominantly for growth improvement, has come under inspection, as it has been shown that it results in the prevalence of antibiotic-resistant bacteria, mainly of human importance (Van den Bogaard, 1997). Salmonellosis is an infectious disease that is a potential hazard to poultry, causing considerable loss to the poultry industry. Salmonella is normally present in the intestine and in favorable circumstances it can cause disease. The European Parliament, WHO, FDA, and many other scientific organizations acknowledge the elimination of drug resistance as a priority action (Rodriguez *et al.*, 2006). In the poultry industry, the control of bacterial diseases is very difficult because of the development of antimicrobial resistance. Different drugs develop different resistances, such as ciprofloxacin 81.7%, tetracycline 58.1%, gentamicin 76.1%, ampicillin 65.8%, neomycin 43.9%, chloramphenicol 6.2%, oxytetracycline 33.4%, norfloxacin 30.1%, nalidixic acid 42.2% and streptomycin 61% respectively (Salihu *et al.*, 2014). The multidrug resistance index of *Salmonella* strains ranged between 0.08 and 0.083 (Gyang *et al.*, 2019) as shown in Table 3.

Table 2: Genes of *Salmonella* resistant against several antibiotics

Sr. No.	Tetracycline resistant genes	Sulfonamide resistant genes	Chloramphenicol resistant genes	Aminoglycosides resistant genes	Reference
1.	*TetA* (94.5%)	*Sul1*(82.6%)	*Cat1*	*Aph(3)11a*	(Randall *et al.,* 2004)
2.	*TetB* (86.4%)	*Sul2*(56.5%)	*Cat2*	*Aac(3)11a*	(Hai *et al.,* 2020)
3.	*TetC* (50.0%)	*Sul3*(30.4%)	*Cat3*	*Aac6*	(Hai *et al.,* 2020)
4.	*TetD* (45.5%)	*Sul4* (4.3%)	*CmlA*		(Hai *et al.,* 2020)
5.	*TetE* (13.6%)		*CmlB*		(Hai *et al.,* 2020)
6.	*TetG* (9.5%)		*FloR*		(Hai *et al.,* 2020)

Table 3: Antibiotics and their percentage resistance in last 10 years

Sr. No.	Antibiotic	Resistance developed (%)	References
1.	*Ampicillin*	65.8	(Salihu *et al.,* 2014)
2.	*Oxytetracycline*	33.4	(Salihu *et al.,* 2014)
3.	*Chloramphenicol*	6.2	(Salihu *et al.,* 2014)
4.	*Tetracyclines*	58.1	(Zhu *et al.,* 2017)
5.	*Ciprofloxacin*	81.7	(Zhu *et al.,* 2017)
6.	*Gentamycin*	76.1	(Zhu *et al.,* 2017)
7.	*Neomycin*	43.9	(Zhu *et al.,* 2017)
8.	*Norfloxacin*	30.1	(Salihu *et al.,* 2014)
9.	*Nalidixic acid*	42.2	(Salihu *et al.,* 2014)
10.	*Amoxicillin*	36.9	(Zwe *et al.,* 2018)
11.	*Cefotaxime*	7.9	(Zwe *et al.,* 2018)
12.	*Ceftiofur*	9.8	(Cortés *et al.,* 2017)
13.	*Cefepime*	0	(Cortés *et al.,* 2017)
14.	*Ertapenem*	No resistance	(Maciel *et al.,* 2019)
15.	*Imipenem*	No resistance	(Maciel *et al.,* 2019)
16.	*Meropenem*	No resistance	(Maciel *et al.,* 2019)
17.	*Levofloxacin*	No resistance	(Maciel *et al.,* 2019)

***Bacillus*-based probiotics mode of action against salmonellosis:** Unlike other *Bacillus*-based probiotics, they are not present in the gut. But they are highly beneficial because they reach the intestine efficiently, and prevent the carcinogenesis and formation of neoplastic lesions in the small intestine as shown in Fig. 2. Moreover, the *Bacillus* spores release antimicrobial substances against gram-positive bacteria by inducing the production of gamma IFN, T-cell proliferation, and CD4+ cells (Higgins *et al.,* 2010). In the intestine, *Bacillus* probiotics provide the environment for metabolic activity and colonization of bacteria that are not able to tolerate stomach pH as the *Bacillus* spores are very resistant to different types of environments; they can survive harsh and dry physical and chemical conditions, the concentration of bile, even more than 1%, so they are bile tolerant. They can be stored in dry form and can tolerate the process and temperature of baking. Moreover, *Bacillus* is immunogenic, hence it can disseminate the mesenteric lymph nodes and Payers patches. The apoptosis and NF-kB complexes are regulated and *Salmonella,* being an intracellular pathogen, is reduced by an apoptosis mechanism.

Working of the *Bacillus*; spore formers: Since their spores are easily recovered from soil, *Bacillus* species are frequently regarded as soil organisms. These spores are also genetically engineered through the gastrointestinal tracts of animals, who regularly consume them, as the isolation of vegetative bacteria from the soil is a difficult process (Rashid *et al.,* 2023). According to research, when a small portion (> 10%) of *B. subtilis* spores is inoculated, it results in the germination and sporulation in the different body organs of the animal like in the small intestine, growth, and multiplication occurs, and the consequence in the sporulation (Hoa *et al.,* 2001; Tam *et al.,* 2006) as shown in Fig. 3. As a result of this, spores are shed in feces and accumulate in the soil because of peristalsis. Why spores can be discovered in the guts of insects, animals and humans is explained by the intestinal habitat of spore formers (Barbosa *et al.,* 2005; Fakhry *et al.,* 2008). According to previous studies (Fakhry *et al.,* 2008; Hong *et al.,* 2009), bacteria may be easily extracted from the human GI tract using biopsies and feces analysis. *Bacillus* spore concentrations in the latter are around 104 spores/g of feces, which is several logs higher than can be reliably predicted from dietary intake alone. In the process of germination, spores can stimulate strong immune responses in the GI tract of mouse models as demonstrated by various studies. Hence, spore-forming probiotics play a pivotal role in this immune stimulation. One of the most insightful yet little-known pieces of research looked at how bacteria are taken orally affected the growth of the gut-associated lymphoid tissue (GALT) in young rabbits (Rhee *et al.,* 2004). In this research, *B. subtilis* was demonstrated to be more significant for the development of GALT than other commensal bacteria. Of course, other characteristics, including the release of antimicrobials like Coagulin, Amicoumacin, and Subtilisin, may also further provide a probiotic benefit by inhibiting the growth of competing microorganisms and enteric pathogens. Although it can be difficult to distill studies that demonstrate efficacy, the following examples are strong ones. In a poultry model, *B. subtilis* spores were demonstrated to inhibit pathogenic *S. enterica* infection.

Future threats: A critical worldwide health issue is the emergence, transmission, and persistence of AMR. A significant fraction of the world's consumption of antibiotics is due to animal husbandry, particularly poultry. There is a knowledge vacuum regarding the establishment of bacterial resistance coming from poultry in resource-constrained contexts despite the rising corpus of studies examining AMR inside industrial farming systems (Hedman *et al.,* 2020). Demand for high-quality sources of animal protein will rise as nations continue to move from low to middle-income status. AMR exposure concerns to poultry, wildlife, domestic animals, and human populations could increase with further promotion of intensive poultry production, which could help with issues of food security (Silbergeld *et al.,* 2010). Monitoring is required to assess the effects on people, other animals, and the environment because intensively farmed chickens can serve as AMR animal reservoirs. The demand for poultry meat along with the demand for antimicrobial-free meat is also in practice where antibiotics are used for growth as well as treatment of many poultry diseases (Schar *et al.,* 2018). Due to economic and production stress, the quality of meat is being compromised and antimicrobials are used for betterment, which pose their own side effects (Hedman *et al.,* 2020).

Future Prospects: The probiotics market share is expected to increase by 7% in the coming years (Elshaghabee *et al.,* 2017). Asia will account for the majority of it, as growth is

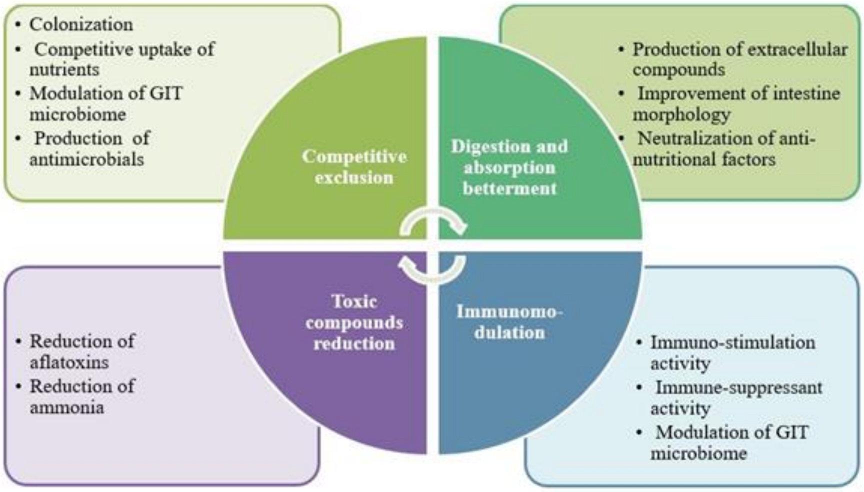

Fig. 2: Prophylactic mode of action of *Bacillus* probiotics.

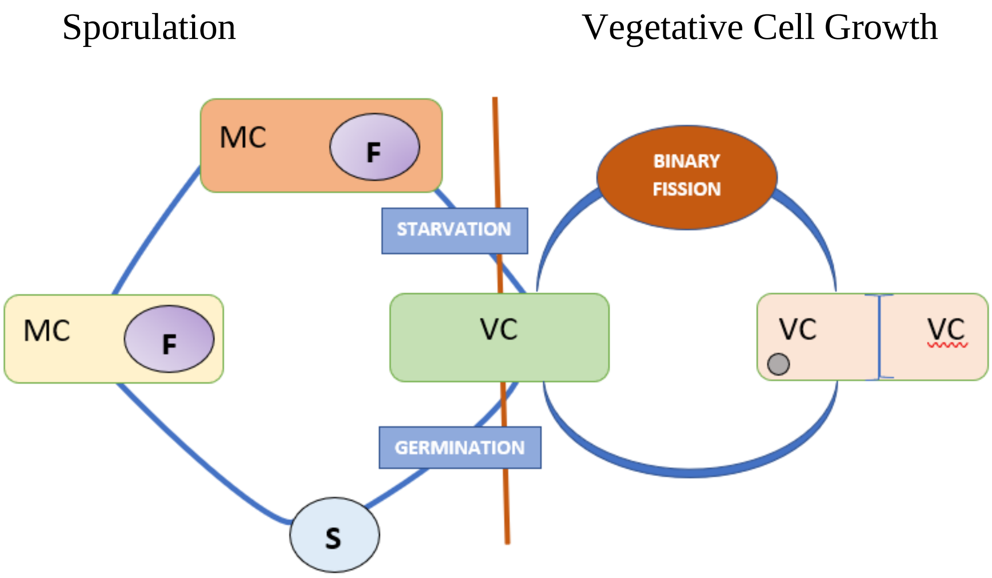

Fig. 3: Spore cell *Bacillus* formation: MC: Mother Cell Compartment, F: Fore Spore Compartment, B: Sporulation, VC: Vegetative Cell.

strongest in India, Pakistan, China, and Japan. These countries contribute 60% of this share and according to experts, will show maximum growth in upcoming years. Probiotics are widely used in poultry without any expected side effects. The probiotic market share has increased because of a number of crucial factors, including rising health and wellness awareness as well as concerns with metabolic and digestive illnesses (Salehi and Bonab, 2006). Additionally, the existence of foreign businesses is drawing more consumers to these health-promoting products. If we examine the probiotic market according to the types of organisms, we may divide it into five groups: spore formers, *Lactobacillus*, yeast, *Bifidobacterium*, and others. As a result, spore-forming probiotics play a significant role in the worldwide nutraceutical and medicinal markets.

Conclusions: The development, spread, and persistence of antibiotic resistance is a serious global health problem. To evaluate the effects on people, other animals, and the environment, monitoring is necessary. Antibiotic resistance in the case of *Salmonella* is predominantly increasing. *Salmonella* causes approximately 1.4 million human illnesses annually. Food-borne salmonellosis and egg salmonellosis are regarded as serious losses to the industry. Probiotics are part of normal gut flora but when these microorganisms are given in high amounts, they improve antibiotic susceptibility as well as promise better production. Out of commercially available probiotics, *Bacillus* probiotics are regarded as the best.

Probiotic products typically list *B. subtilis* as an ingredient. The fermentation of soybeans using this bacterium results in natto, a classic Japanese meal. Natto consumption has traditionally been associated with positive health effects, including immune system stimulation. The selection of microorganisms with antibiotic resistance is impacted by the prolonged use of antibiotics in animal husbandry.

Authors contribution: All authors contributed almost equally in the preparation of this review article.

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