

## Application of probiotic bacteria for controlling *Salmonella* contamination in poultry and enhancing food safety

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### ABSTRACT

**Consumption of food contaminated with pathogenic bacteria results in diarrheal diseases inflicting over 90 million people worldwide and causing over 150,000 deaths, annually. Human pathogens transmitted to consumers through food are termed foodborne pathogens.**

***Salmonella* is one of the major foodborne pathogens of concern for the agro-food sector as it is highly widespread in agricultural settings and may persist through food processing. Poultry are a known reservoir of *Salmonella* and despite intensive intervention efforts, *Salmonella* outbreaks related to poultry (meat and eggs) occur in both developing and developed countries. This article describes the isolation of probiotic lactic-acid bacteria from *Salmonella*-free hens, which possess antagonistic activity against *Salmonella*. Utilization of such probiotics as additional measure against *Salmonella* can be incorporated as part of a multi-hurdle approach to control *Salmonella* in poultry. Effective mitigation of *Salmonella* through all stages of the production and supply chain should reduce both the occurrence and the level of subsequent human exposure and will reduce illness and the associated public health burden.**

### FOOD SAFETY AND FOODBORNE PATHOGENS

Food safety is related to the presence of food safety hazards at the time of consumption, which include physical, chemical and biological hazards that can occur at any stage of the food chain from farm-to-fork. Quality and safety of food are the most important factors influencing consumer choice and are critical for food manufacturers, including farmers. Microbial food safety refers to the presence of hazardous microorganisms that may cause a disease following consumption. Disease-causing microorganisms that are transmitted by food are called 'foodborne pathogens'.

Various foodborne pathogens are implicated in food contamination. These include bacteria, such as *Escherichia coli*, *Salmonella enterica*, *Campylobacter*, *Listeria monocytogenes*; viruses, such as norovirus, hepatitis A; and protozoa, such as *Cyclospora* and *Giardia*. Foodborne illness is a major cause of morbidity worldwide with a yearly estimate of over 90 million infected people and over 150,000 deaths. Besides human suffering, foodborne illness results in substantial economic losses to patients, food producers and national economies. Intervention efforts by agricultural- and food industry sectors have dramatically improved food safety in recent years. However, despite some great human achievements in many areas, we are still facing a considerable burden of foodborne illness in both developing and developed countries.

Among bacterial foodborne pathogens, non-typhoidal *Salmonella*, hereafter-designated *Salmonella*, is one of the most important causes of foodborne diseases, globally. For example, in the USA, *Salmonella* is considered the most common bacterial foodborne pathogen, causing over 1 million cases of illnesses annually, including approximately 20,000 hospitalizations and 400 deaths.

*Salmonella* is an enteric pathogen that usually infects the human gut and causes Salmonellosis, a disease that is typically characterized by diarrhea, fever and abdominal pain, and is generally self-limited. Young children under 5 years-old and elderly are among the most susceptible populations. *Salmonella* may be acquired by consumption of contaminated food, such as meat, eggs, milk, and water, as well as by consumption of contaminated fruits and vegetables, or by contact with infected food animals and reptiles.

The main reservoir of *Salmonella* in industrialized countries is the intestinal track of food-producing animals. Poultry is one of the most important reservoirs of this pathogen and may serve as a vehicle to transmit *Salmonella* to humans through the food chain. Both broilers and layer hens may carry *Salmonella* in their intestinal track without detectable symptoms, hence exacerbating the problem. Spread of the pathogen in the flock is facilitated by modern intensive rearing of mass production, and contamination of carcasses is facilitated by the industrial mass-processing resulting in cross-contamination. The wide spread

utilization of antibiotics in poultry production, as prophylactic, therapeutic or growth-enhancing treatments, contributed to the development of antibiotic-resistance in *Salmonella*, which poses an additional threat to consumer health.

## THE PROBLEM

*Salmonella* control in poultry includes surveillance, biosecurity and vaccination implemented in broilers' rearing and egg production farms. In Israel, all birds that produce eggs for human consumption are obligated by the Veterinary Services to be vaccinated for *Salmonella* (serovars Enteritidis, Typhimurium and Infantis). However, despite moderate success of these measures, poultry still serves as a major source of *Salmonella*. In the United State, for example, ninety-two people were infected, in the last several months, with a multi-drug resistant strain of *Salmonella* serovar Infantis, in a multistate outbreak linked to consumption of tainted chicken products. Additionally, more than 200 million eggs were recently recalled because they may be contaminated with a *Salmonella* strain involved in a multi-state salmonellosis outbreak. Accordingly, additional intervention strategies are needed to mitigate *Salmonella* contamination in poultry.

## BIOCONTROL OF SALMONELLA BY PROBIOTIC BACTERIA

Probiotics are defined as live microbial preparation consisting of high number of bacteria that is given as feed additive and results in a beneficial effect on the host intestinal microbial population. Although probiotics preparations are sometimes used in poultry production, different interactions may occur between the probiotic bacteria and the bird's host depending on the genetic-lineage of the flock, the genetics of the probiotic bacteria, as well as the nature of the feed consumed. Thus, it may be favorable to define a specific probiotic formulation that will be most suitable based on the specific bird's genetics and the production setting. Broilers are grown in large production areas where *Salmonella* can easily be transmitted from

one bird to another through common feed and water systems, contact with contaminated litter as well as through direct contact with each other. Consequently, even if only a single bird becomes a carrier of *Salmonella*, it is conceivable that during the production period the entire flock will become contaminated with the pathogen. Nevertheless, it is possible that even in highly contaminated flocks individual birds will remain free of *Salmonella*. It was hypothesized that such *Salmonella*-free birds are colonized by unique intestinal microflora that exerts antagonistic properties that inhibit *Salmonella* colonization of the chicken gut. Based on this hypothesis, we have screened more than eight hundreds fresh fecal dropping from several flocks of broilers and layers in Israel for *Salmonella*-free fecal samples, which conceivably harbor microbial populations with *Salmonella*-antagonistic properties. Those *Salmonella*-free fecal samples were used as a starting material for screening and isolating of anaerobic lactic acid bacteria (LAB) with potential anti-*Salmonella* activity. LAB are commonly used as probiotic bacteria and hence are considered safe for animal and human consumption. The screening procedure used in this study is depicted in **Figure 1**. Briefly, fresh birds' droppings were collected from the production farm in sterile plastic tubes and transferred under refrigeration to the laboratory, where a portion of the sample was tested for the presence of *Salmonella* by standard



Figure 1. Screening and isolation of antagonistic bacteria from *Salmonella*-free fecal samples. Fresh litter samples were collected from chicken house (upper left panel) and transferred to the laboratory. Bacteria were extracted by mixing with buffer and spreading on *Salmonella*-selective agar plate. *Salmonella* colonies are shown as black dots on red agar plate (upper right). *Salmonella*-free litter samples were enriched for the growth of anaerobic bacteria using anaerobic jar (bottom right). Individual colonies were tested for their ability to inhibit the growth of *Salmonella* on agar plates. An example of a transparent inhibition zone around two antagonistic bacteria grown on a lawn of *Salmonella* is shown in the left bottom panel.

→ procedure. *Salmonella*-negative fecal samples were then streaked on LAB-selective agar plates and incubated under anaerobic conditions. Putative LAB colonies grown on these agar plates were selected and tested for their ability to inhibit growth of *Salmonella* (see, agar-plate image at the bottom of **Figure 1**). Briefly, LAB were put on the center of a lawn of *Salmonella* cells on agar plate, and incubated for 24 hours. The presence of an

inhibition zone surrounding the LAB (see, bottom of **Figure 1**) is indicative of *Salmonella*-growth inhibition. The identity of selected number of LAB was determined following sequencing of the 16S-ribosomal DNA gene. The three most potent LAB strains were identified as *Enterococcus faecium*, *Lactobacillus salivarius*, and *Lactobacillus reuteri*.

Antimicrobial activity of the three isolates was tested against

the five most common *Salmonella* strains derived from poultry (**Table 1**). The tested isolates had variable antagonistic activity against the different *Salmonella* strains as can be seen by their variable Inhibition zones. In order to proliferate within the chicken gut, probiotic bacteria should be capable of growing in the presence of bile salts present in the bird's intestinal track. Consequently, the growth of the three selected probiotic isolates was also tested in the presence of increasing concentrations of bile salts (**Figure 2**). In most cases, except for *E. faecium*, addition of bile salts up to 2% has augmented the growth of the probiotic bacteria. These findings support the potential application of these three LAB isolates to serve as probiotics feed-additive in order to control *Salmonella* carriage in poultry. Further *in-vivo* studies, using chicks, are required to assess the effect of the probiotics on carriage of *Salmonella* in the chicken's gut. While strains of the species *L. salivarius* and *L. reuteri* are well-established probiotic treatments, some strains of *E. faecium* were reported to cause disease in humans and may also carry antibiotic-resistance genes. Therefore, the safety of the *E. faecium* isolate should be determined before commencing further studies.

Salmonella serovar	E. faecium	L. salivarius	L. reuteri
Typhimurium	++	+	++
Infantis	+	++	+
Hadar	+	++	++
Virchow	+	+	++
Enteritidis	+	++	++

Table 1. Table 1. Probiotic bacteria inhibit the growth of main *Salmonella* serovars in poultry.

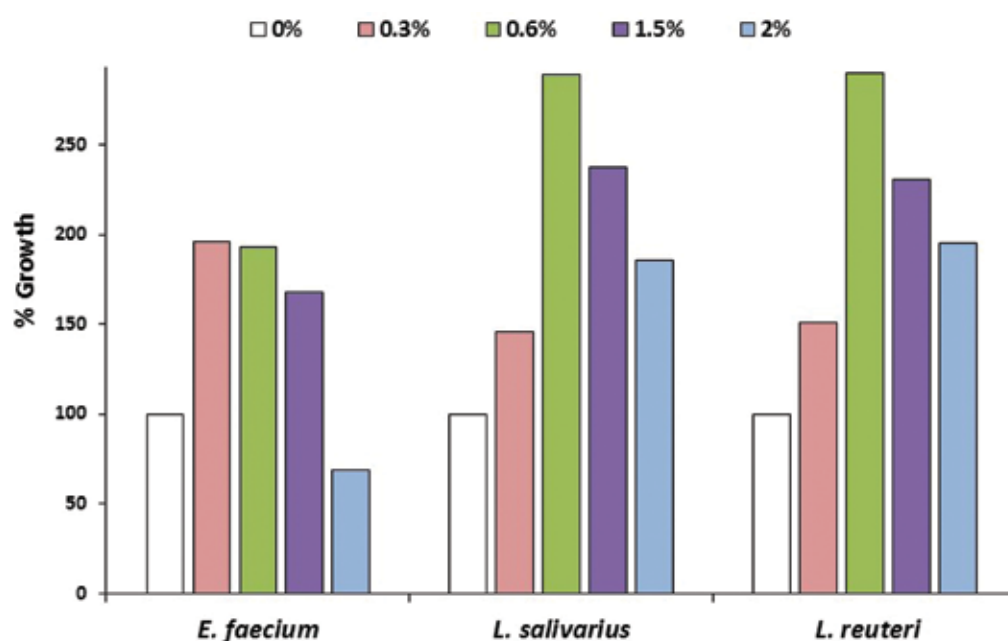


Figure 2. Growth of probiotic bacteria in the presence of increasing concentrations of bile salts.

table antagonistic activity against the different *Salmonella* strains as can be seen by their variable Inhibition zones. In order to proliferate within the chicken gut, probiotic bacteria should be capable of growing in the presence of bile salts present in the bird's intestinal track. Consequently, the growth of the three selected probiotic isolates was also tested in the presence of increasing concentrations of bile salts (**Figure 2**). In most cases, except for *E. faecium*, addition of bile salts up to 2% has augmented the growth of the probiotic bacteria. These findings support the potential application of these three LAB isolates to serve as probiotics feed-additive in order to control *Salmonella* carriage in poultry. Further *in-vivo* studies, using chicks, are required to assess the effect of the probiotics on carriage of *Salmonella* in the chicken's gut. While strains of the species *L. salivarius* and *L. reuteri* are well-established probiotic treatments, some strains of *E. faecium* were reported to cause disease in humans and may also carry antibiotic-resistance genes. Therefore, the safety of the *E. faecium* isolate should be determined before commencing further studies.

## CONCLUSIONS

It should be noted that utilization of probiotics in poultry is not expected to completely eradicate *Salmonella*, but rather reduce *Salmonella* carriage in the intestinal track of the chicken. It is expected that a successful treatment should reduce both the number of *Salmonella* cells in the chicken intestinal track as well as the number of excreted *Salmonella* cells during the production stage, which in turn should reduce cross-contamination during the production stages. It is foreseen that application of antagonistic probiotic bacteria should serve as another component of a multi-hurdle approach, in addition to surveillance, vaccination, and strict hygienic practices throughout the production chain in order to reduce salmonellosis and protect consumer's health. □

## Prof. Shlomo Sela (Saldinger)

Prof. Shlomo Sela (Saldinger) is a senior researcher and currently the Head of the Department of Food Science of the Institute of Postharvest and Food Sciences. His research activity is centered on microbial food-safety with focus on understanding how food-borne pathogens persist in the agro-food environment in order to develop novel control approaches to limit food contamination. Prof. Sela is currently member of the COST action 16110, a pan-European network of excellence focusing on 'Control of human pathogenic Microorganisms in Plant Production Systems'. He is currently leading a multidisciplinary project funded by the chief scientist of the Ministry of Agriculture of Israel (Project No. 20-14-0032) aiming at mitigation of *Salmonella* and *Campylobacter* contamination in poultry using 'One Health' system approach. As part of this project, his research group, led by Dr. Yulia Kroupitski, is developing probiotics-based biocontrol treatments against *Salmonella*.

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