Inhibition of pathogenic E. coli and Salmonella spp in Europe

Keeping poultry healthy is key in modern farm management. Prevention is much more effective and cost-efficient than having to treat disease. Probiotics are increasingly becoming one of the strategies used to ensure health. Proven, effective probiotics are a sustainable, natural solution and play an integral part of prevention programmes on the farm.

by Jean-Christophe Bodin, MSc., Agr., Sr. Product Manager Poultry, Chr. Hansen. www.chr-hansen.com

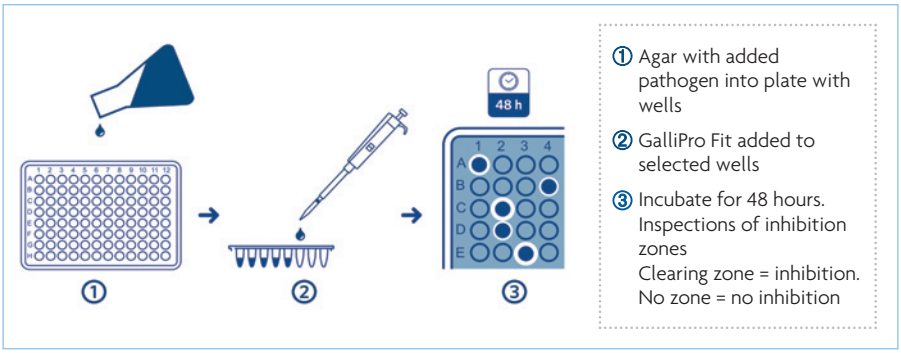


Fig. 1. Agar plate diffusion methodology.

Bacillus-based probiotics are particularly well-suited for use in broiler feeds. Their spores are metabolically dormant and resilient to environmental stresses, including pelleting.

With Bacillus-based probiotics, the primary mechanisms of action contributing to bird-health balance are biofilm creation at the top of the villi at enterocytes level, competitive exclusion, bacteriocin production and immune system modulation.

Bacillus strains can inhibit pathogens

Some Bacilli are specifically strong in production of bacteriocins against unfavourable bacteria. Bacillus strains from Chr. Hansen have the ability to produce peptide-based bacteriocin substances like bacillilactin, fengycin, iturin, mycosubtilin and surfactin that inhibit both Gram-negative or Gram-positive pathogens.

Table 1. Results of inhibition zones per pathogen isolate and country of origin.

Species	Source	Country of origin	Inhibition by GalliPro Fit
E. coli	Broiler chicken	Poland	+++
E. coli	Laying hen	Poland	++
E. coli	Turkey	Poland	+
Salmonella enteritidis	Laying hen	Poland	++
E. coli	Broilers	France	+
Salmonella typhimurium	Layer	France	+
Salmonella enteritidis	Layer	France	+
Salmonella derby	Broilers	France	++
Salmonella mbandaka	Breeders	France	+
Salmonella enteritidis	Broiler chicken	France	++
Salmonella typhimurium	Broiler chicken	France	+
Salmonella enteritidis	Broiler chicken	France	+
E. coli	Broiler chicken	Spain	+
Salmonella sp.	Laying hen	Spain	+
Salmonella sp.	Broiler chicken	Spain	++

Bacteriocins are cationic (positively charged) peptides that display hydrophobic or amphiphilic properties and, in most cases, the bacterial membrane is the target of their activity. Several models have been proposed demonstrating the mechanism of action of these cationic peptides. The thrust of this action involves the formation of channels through which ions can pass and (or) the disruption of bacterial cytoplasmic membranes; this leads to a lethal effect on bacteria via the formation of pores in the bacterial membrane.

Three principal steps are required for these effects:

- Binding of peptides to the bacterial membrane.
- Peptide aggregation within the membrane.
- Formation of channels.

For these Gram-negative bacteria, bacteriocins must cross the negatively-charged outer wall which contains lipopolysaccharides (LPS), as for Gram-positive bacteria they have to pass through the outer cell wall which contains acidic polysaccharides. Production of this peptide was proven and measured for Chr. Hansen Bacillus based probiotics.

GalliPro Fit, a combination of three Bacillus strains, has been selected for its capacity to inhibit Salmonella spp., E. coli as well as Clostridium spp.

On-farm validation

In order to validate the inhibitory potential of GalliPro Fit towards European Union

Continued on page 33

Continued from page 31
customers' pathogens E. coli and Salmonella spp., 15 pathogen samples were directly isolated from poultry farms in Poland, Spain and France. Both type of pathogens were isolated from organs of five or seven dead or sick birds from the same flock having related issues (lesions, airsacculitis, enteritis, etc). Selective media were used (for example MacConkey, Salmonella Chromogen Agar, etc) to ensure pure isolates.

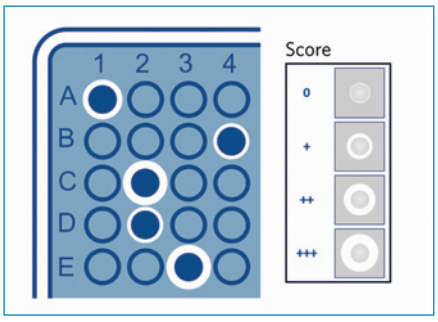
The pathogen isolates were transported to the Chr. Hansen Animal Health Innovation Laboratory, where they were suspended in agar pour plates. Lids were applied to the plates to make wells in the agar. Diluted GalliPro Fit product (1 x 10^8 CFU/g) was applied in the wells (5 x 10^5 CFU/well) (n = 5 replicates plate). The plates were incubated aerobically at 30°C for 48 hours after which inhibition zones were read.

Fig. 1 describes the Agar plate diffusion assay methodology and Fig. 2 presents a plate view of an E. coli strain tested against GalliPro Fit. An associated scoring system is used to assess the results.

Inhibition zone results

Fig. 3 summarises the results obtained from the three countries where E.coli and Salmonella spp. isolates were collected. Inhibition zones were recorded around all inoculated wells with GalliPro Fit. Thus, all 15 tested pathogens were inhibited by GalliPro Fit. A score of + or +++ was used to describe the extent of the inhibition zone.

Fig. 2. Agar well diffusion assay, E. coli. strain 1, GalliPro Fit in n = five wells (dark spots) and scoring system.



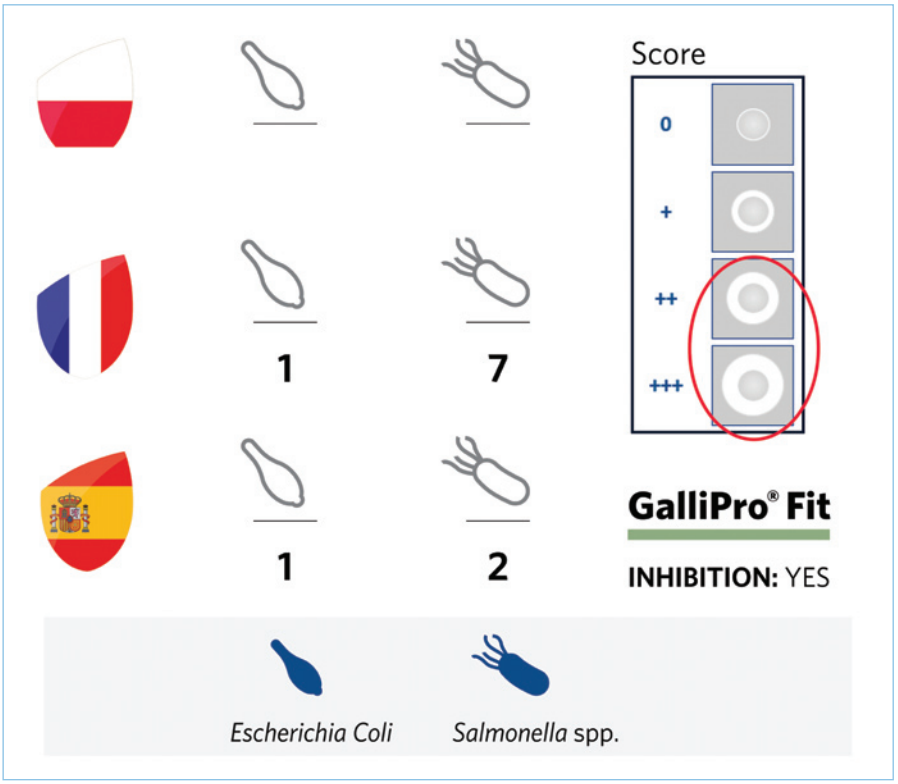


Fig. 3. Summary of the inhibition test from different EU countries (broilers of different ages, layers and turkeys).

Growth inhibition of Gram negative pathogens

These in vitro results demonstrate the potential of GalliPro Fit to get a wide spectrum of inhibition on multiple strains of E. coli and Salmonella spp. collected in field conditions throughout Europe.

A Chr. Hansen pathogen inhibition assay (agar well diffusion) represents an excellent tool to illustrate the potential of GalliPro Fit's vital contribution to pathogen mitigation programmes in commercial conditions.

In addition, it is well known that Chr. Hansen Bacillus strains and especially those included in GalliPro Fit, have the ability to colonise the top of the intestinal villi.

The following illustrates why this is an important benefit for pathogen control.

- Protection of a very important location of nutritional absorption.
- Reduction of undigested nutrients potentially available for pathogens.
- Bacteriocins are produced at the most vital anatomic location.
- Creation of an unfavourable micro-environment for pathogens such as E. coli, salmonella and Clostridium spp. ■

References are available from the author on request