

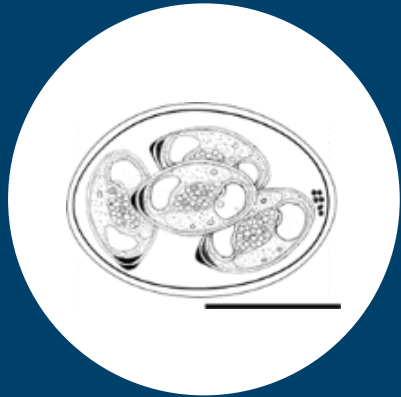
In vitro influence of probiotic *Bacillus* strains on the growth of avian *Enterococcus cecorum*



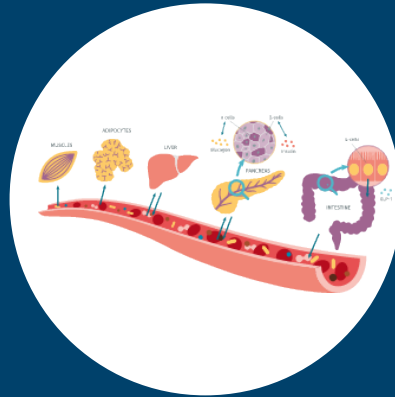
Marion Bernardeau*^{1,2}, Sara Medina Fernandez², Marina Cretenet², Kirsty Gibbs¹

CHICKEN CLINICAL AND SUB-CLINICAL DISEASES

KEEPING PRODUCTION SYSTEM ON ITS TOES



Parasitic diseases



Metabolic/nutritional diseases



Behavioral diseases



Infection diseases
Virus – Fungi - Bacteria

Impair overall animal health status
and performances

When commensal bacteria become opportunistic pathogens...
Case of *Enterococcus* spp.

ENTEROCOCCUS GENUS: A DIVERSE & UBIQUITOUS GROUP

With many faces...

Digestive systems



Commensal
Human
Animals
Insects

Natural biomes



Water
Sewage
Soil
Arable lands



- Immune homeostasis
- Immunomodulatory effect
- Produce bacteriocins against pathogens
- Role in digestion (metabolism of carbohydrates & proteins)
- Lowering cholesterol level



- Potential pathogens, translocation in the circulatory system
- Transferable virulence and resistance factors between species & genera
- Foodborne pathogens



- Biotherapeutic / Probiotic
- Microbial feed additive to improve growth performances



AAFCO (2018) approved list
E. cremoris; *E. diacetyllactis*
E. faecium; *E. intermedius*
E. lactis; *E. thermophilus*

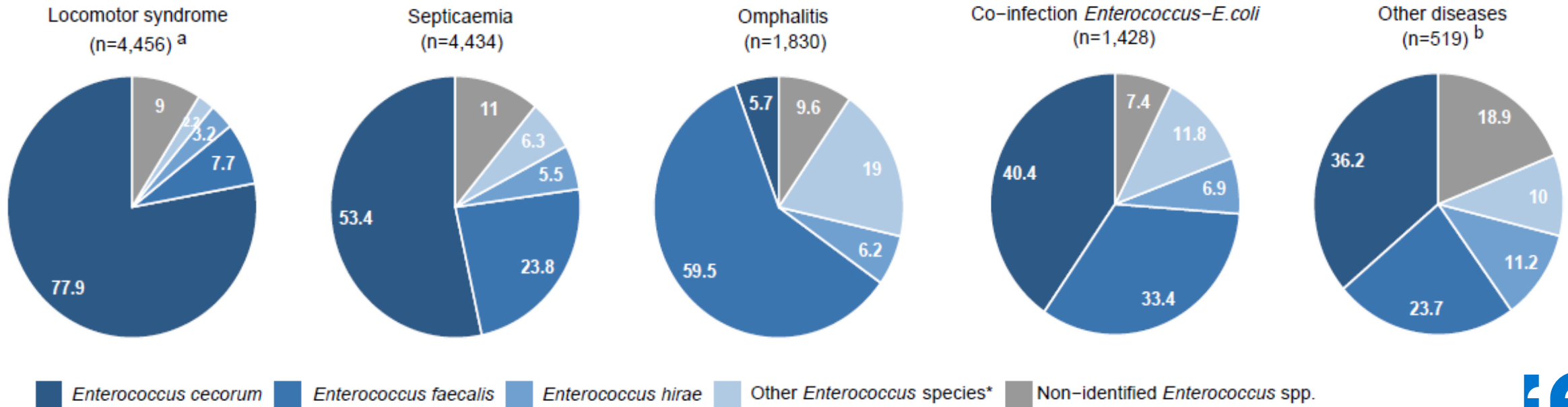


EU_QPS list
Ø *Enterococcus* spp.

ENTEROCOCCUS GENUS ENCOMPASSES 38 SPECIES

<i>E. faecium</i>	<i>E. avium</i>	<i>E. faecalis</i>	<i>E. cecorum</i>	<i>E. gallinarum</i>	Non-classified species
<i>E. faecium</i> , <i>E. canis</i> , <i>E. durans</i> , <i>E. hirae</i> , <i>E. mundtii</i> , <i>E. ratti</i> , <i>E. villorum</i> , <i>E. asini</i> , <i>E. phoeniculicola</i> , <i>E. canintestini</i> , <i>E. thailandicus</i>	<i>E. avium</i> , <i>E. devriesei</i> , <i>E. gilvus</i> , <i>E. malodoratus</i> , <i>E. pseudoavium</i> , <i>E. raffinosus</i> , <i>E. pallens</i> , <i>E. hermanniensis</i> , <i>E. vikkiensis</i>	<i>E. faecalis</i> , <i>E. caccae</i> , <i>E. haemoperoxidus</i> , <i>E. moraviensis</i> , <i>E. silesiacus</i> , <i>E. termitis</i>	<i>E. cecorum</i> , <i>E. columbae</i>	<i>E. gallinarum</i> , <i>E. casseliflavus</i>	<i>E. aquimarinus</i> , <i>E. dispar</i> , <i>E. saccharolyticus</i> , <i>E. sulfureus</i> , <i>E. italicus</i> , <i>E. camelliae</i>

Enterococcus spp. isolated from birds with clinical disease



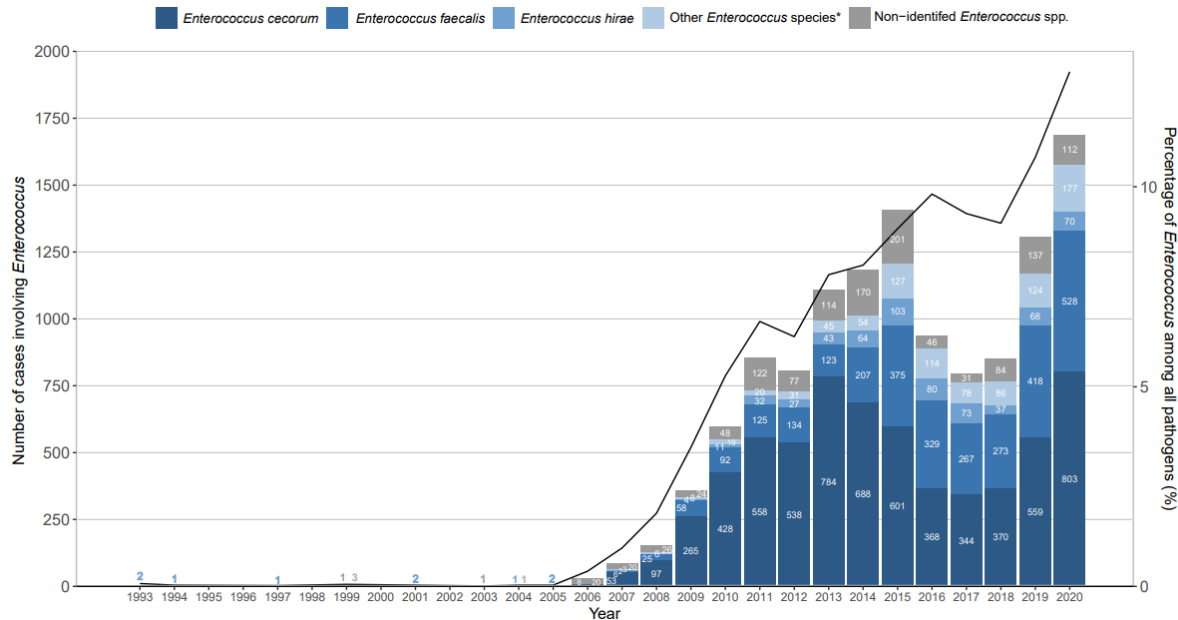
* Other *Enterococcus* species covers *E. gallinarum*, *E. faecium*, *E. casseliflavus*, *E. durans*, *E. avium* and *E. columbae*.

EMERGING OR RE-EMERGING PATHOGEN?

Definitively increasing worldwide

Ex: French epidemiological data from 1993 to 2020

Evolution of the relative frequency of *Enterococcus* and of the number of *Enterococcus* species for all poultry production sectors (n= 12,177 *Enterococcus* cases)

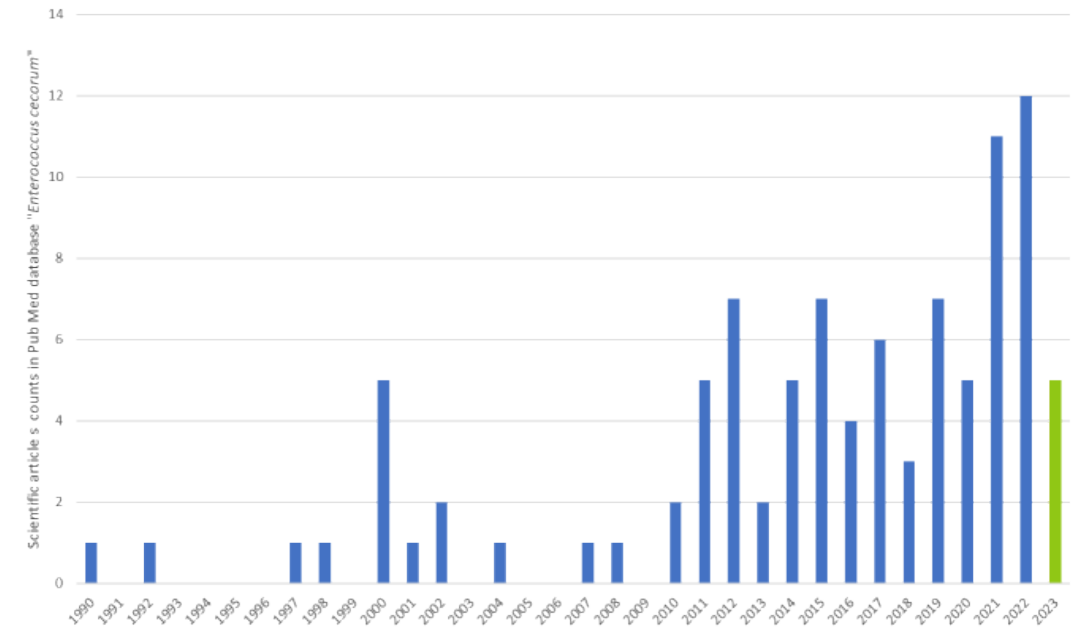


* Other *Enterococcus* species covers *E. gallinarum*, *E. faecium*, *E. casseliflavus*, *E. durans*, *E. avium* and *E. columbae*.

Souillard et al., 2022 Vet Microbiol.

Triggering scientific interest

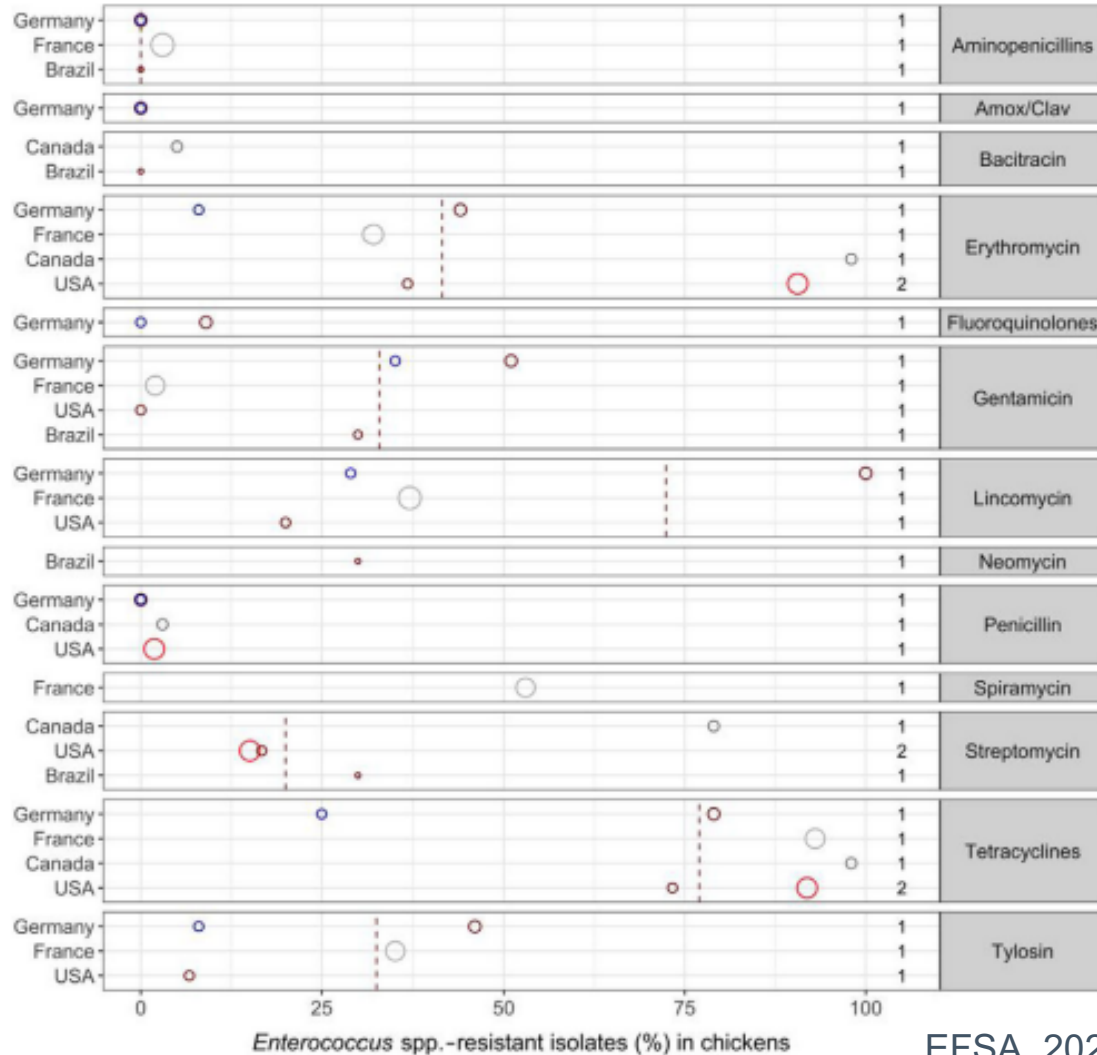
PubMed database hits for *Enterococcus cecorum*



ANTIBIOTICS ARE NOT THE MOST SUSTAINABLE & LONG-TERM SOLUTION

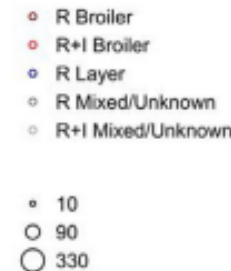
Due to the high prevalence of ABR and ability to acquire and transfer ABR genes

At the genus level across continents



At the species level

Prevalence of the resistance of *E. cecorum* poultry isolates (n=118)

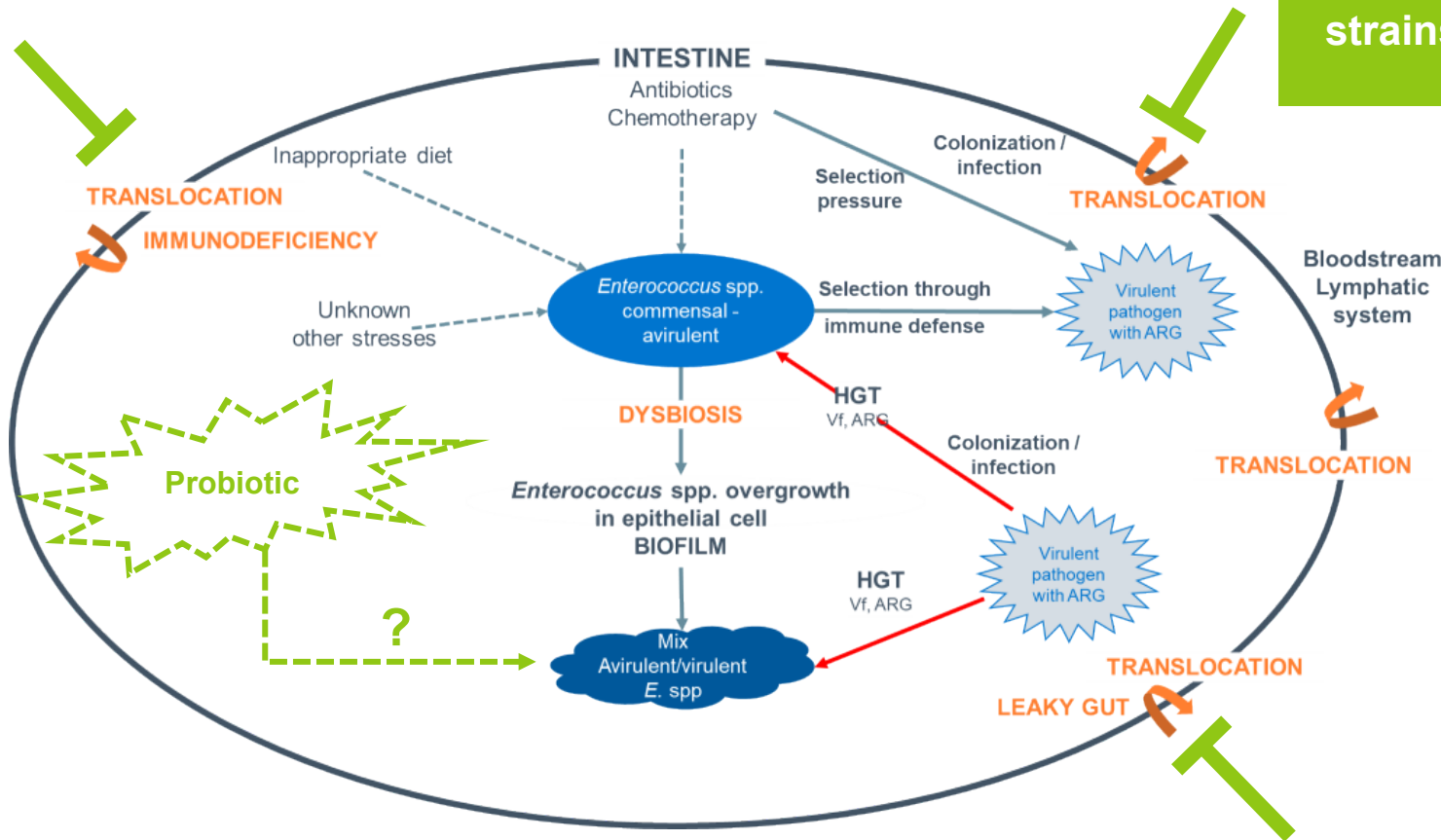


95%
TETRACYCLINE

75%
MLS
Macrolide/Lincosamide/Streptogramin

ENTEROCOCCUS SPP. IN THE TRANSITION TO PATHOGENICITY

Can microbial feed additives influence this transition?



Documented probiotic properties of 3 *Bacillus velezensis* strains BS8, 15AP4, 2084 with the potential to contain transition from commensal to virulent

- Reduce *Campylobacter* translocation - Gibbs et al., 2021 JAAN
- Augment innate immune response on broiler chicken Lee et al., 2015 Animal Feed Science & technology
- Strengthen tight junction and barrier integrity Murugesan 2013
- Inhibit causative agents of avian colibacillosis and Necrotic enteritis

Can these 3 probiotic strains exhibit on top a direct antimicrobial effect against *E. cecorum* isolates?

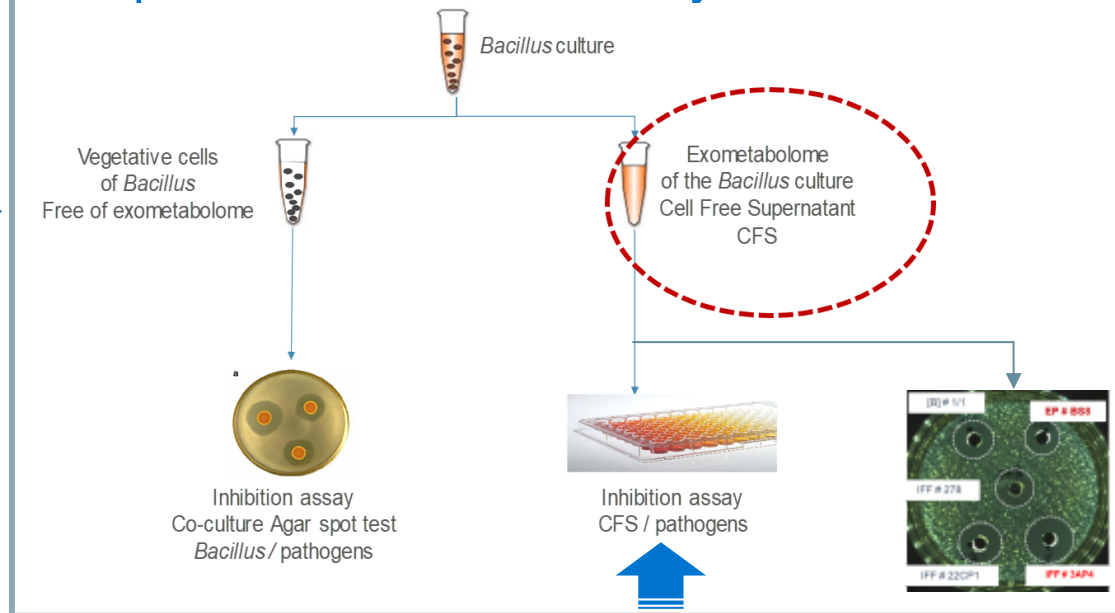
Adapted from Krawczyk, et al., 2021. Microorganisms

OVERVIEW OF THE EXPERIMENTAL DESIGN

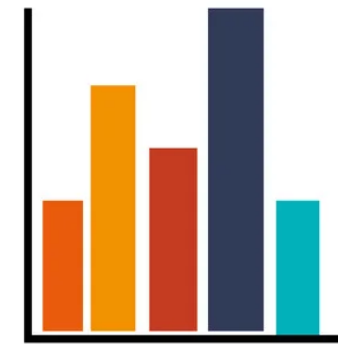
1. Capture “sort of” pathogen diversity



2. Optimize the *in vitro* assay



3. Determine and compare the inhibitory activity of *Bacillus* probiotic strains against *E. cecorum* farm isolates



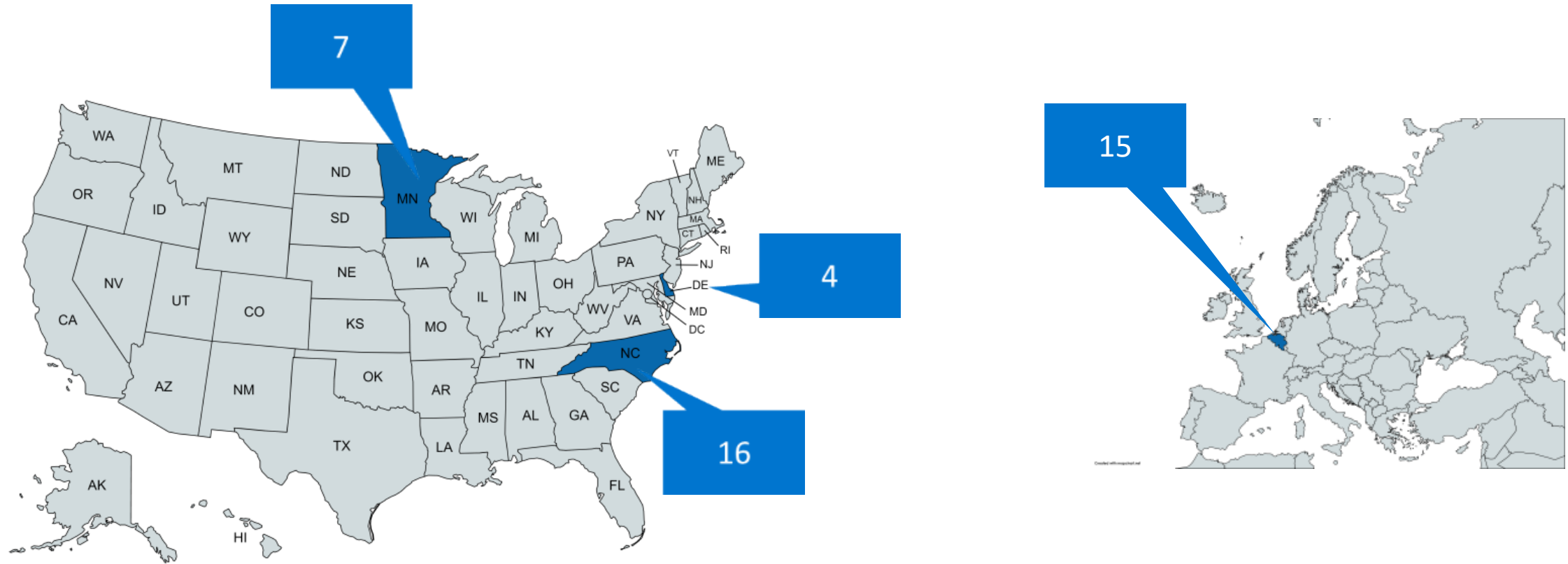
IFF *Bacillus* probiotics strains

- *B. velezensis* BS8
- *B. velezensis* 2084
- *B. velezensis* 15AP4

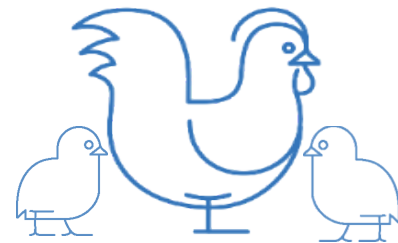
Other *Bacillus* probiotics strains available on the market

- *B. velezensis*
 - DSM 15544
 - PTA-6736
 - #10B/1
 - #10B/4
- *B. subtilis*
 - DSM 17299
- *B. licheniformis*
 - DSM17236
 - #10/4

E. CECORUM ISOLATES, SAMPLING ORIGIN



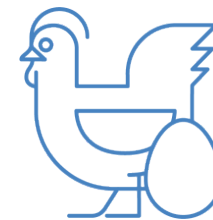
Created with mapchart.net



Breeders
29

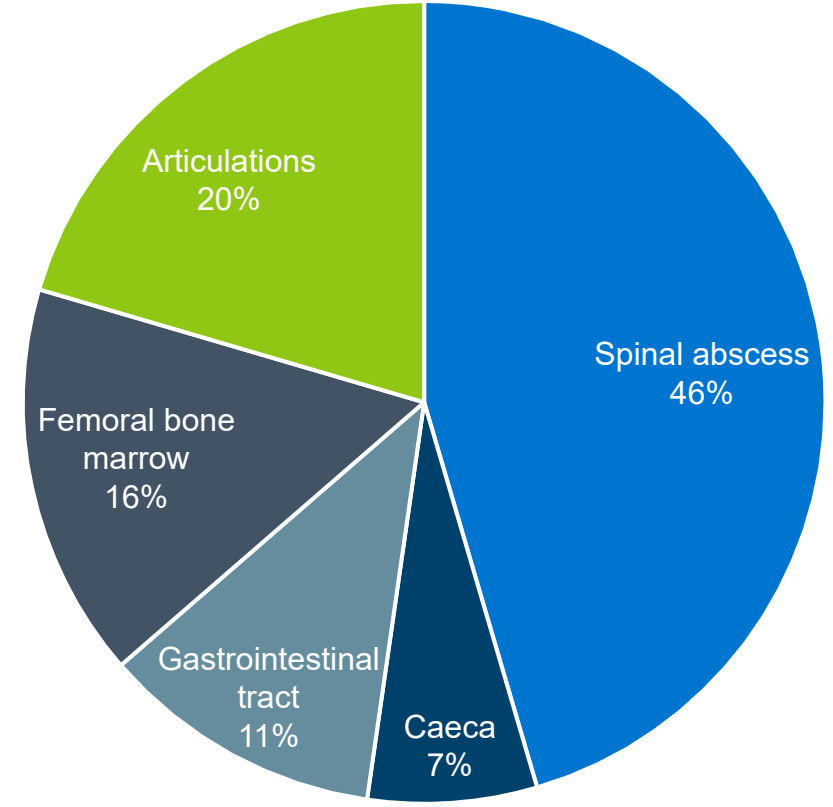
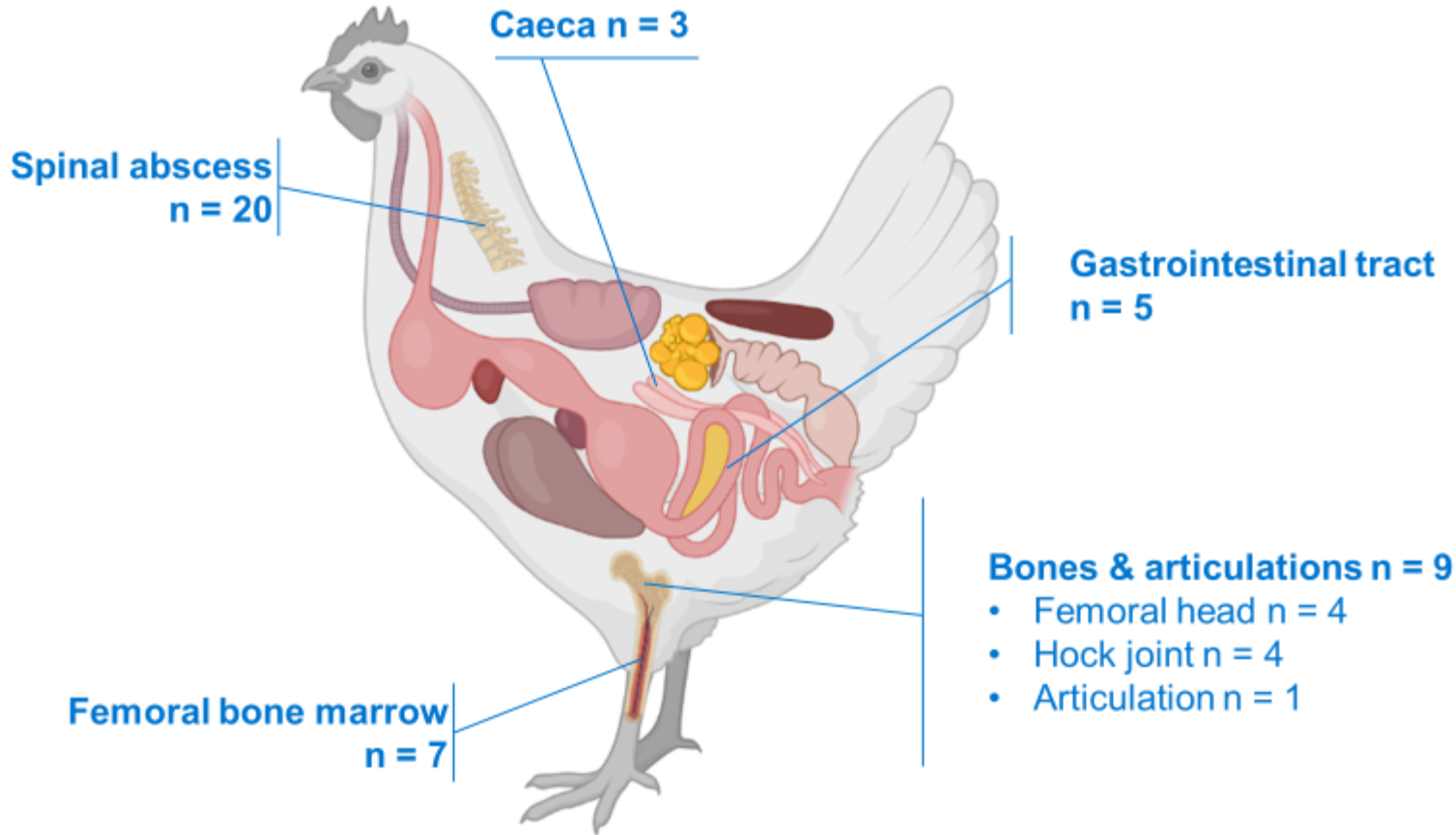


Broilers
12



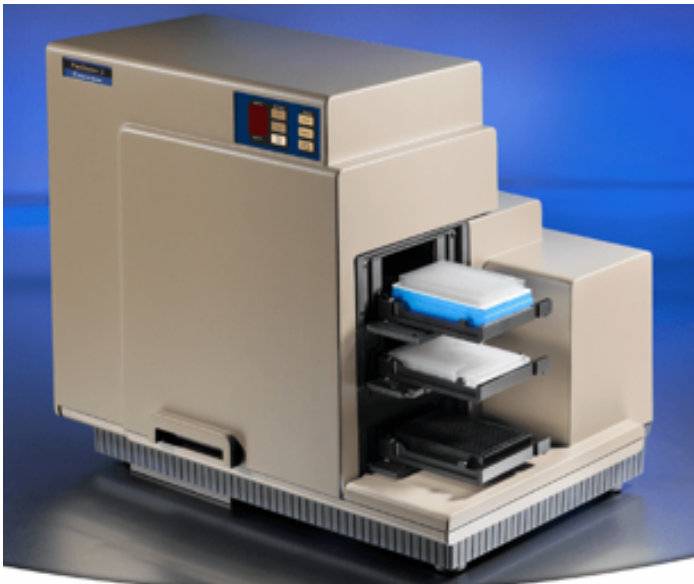
Layers
1

BIOLOGICAL ORIGIN OF THE 42 ISOLATES

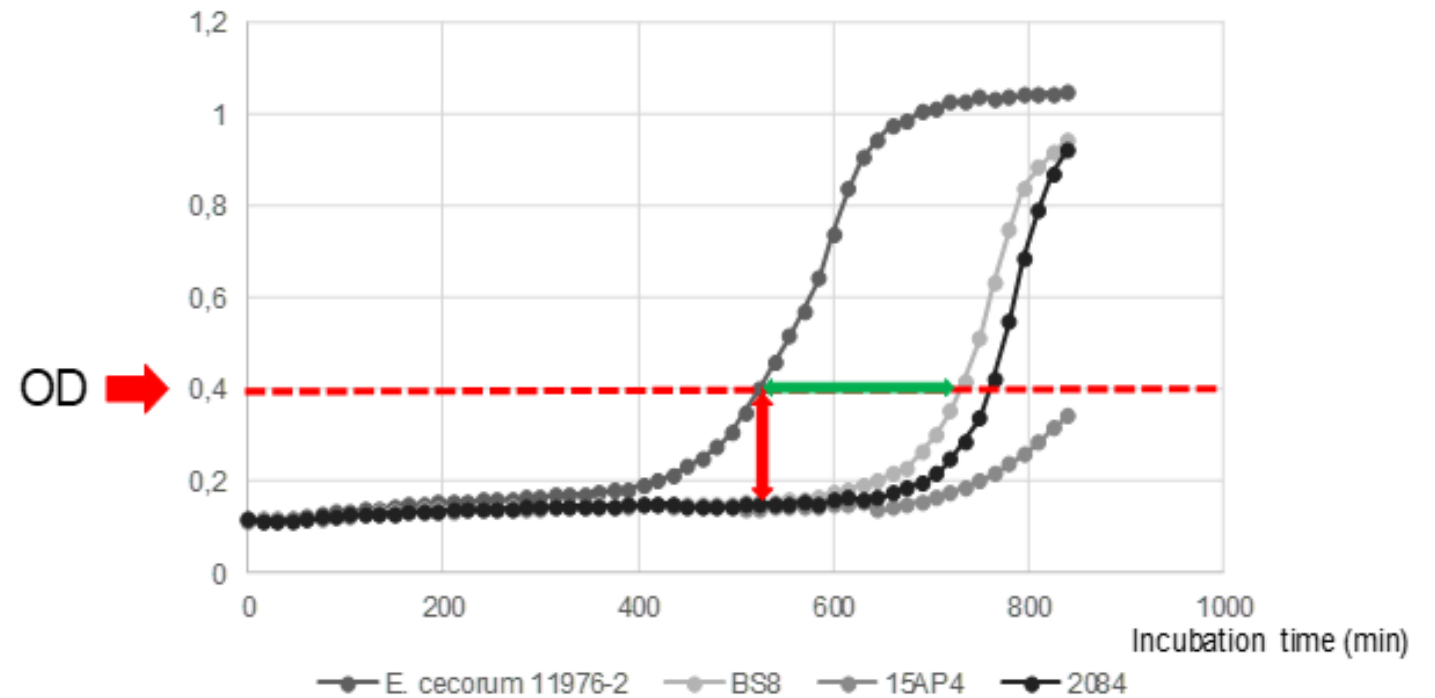


THE IN VITRO MODEL

FlexStation® multi-mode microplate reader coupled with Soft Max pro software
(Molecular Devices LLC, US)



Antimicrobial activity of *Bacillus* CFS against *Enterococcus cecorum* 11976-2



Delay of growth

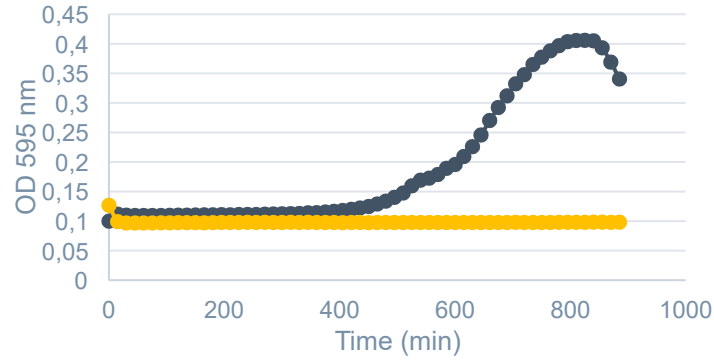


% of inhibition

PATHOGEN GROWTH KINETICS WITH W/O *BACILLUS* CFS

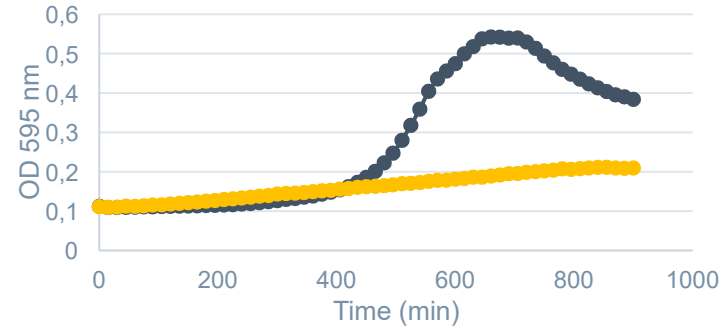
Interpretation of different growth profiles

Fully inhibited



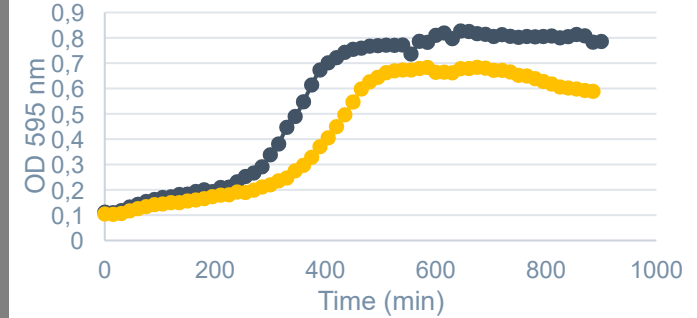
● E. cecorum 11957-3 ● E. cecorum 11957-3 + CFS #BS8

Growth prevented



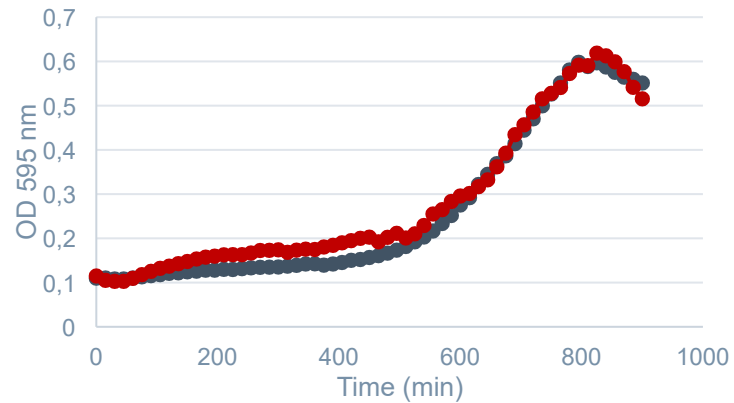
● E. cecorum 12696M-1
● E. cecorum 12696M-1 + CFS #2084

Growth delayed



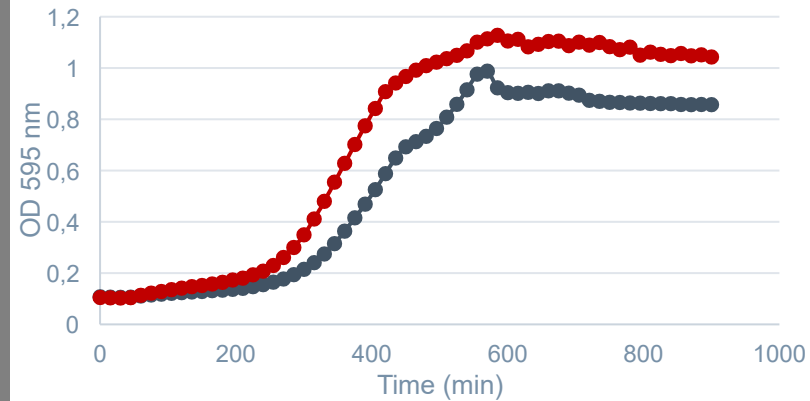
● E. cecorum D4211
● E. cecorum D4211 + CFS 15AP4

No effect



● E. cecorum 11951-1 ● E. cecorum 11951-1 + CFS #10/4

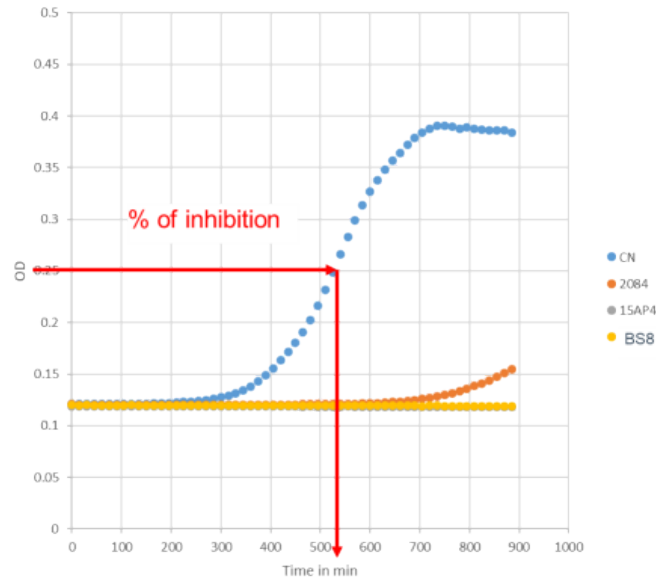
Growth promoted



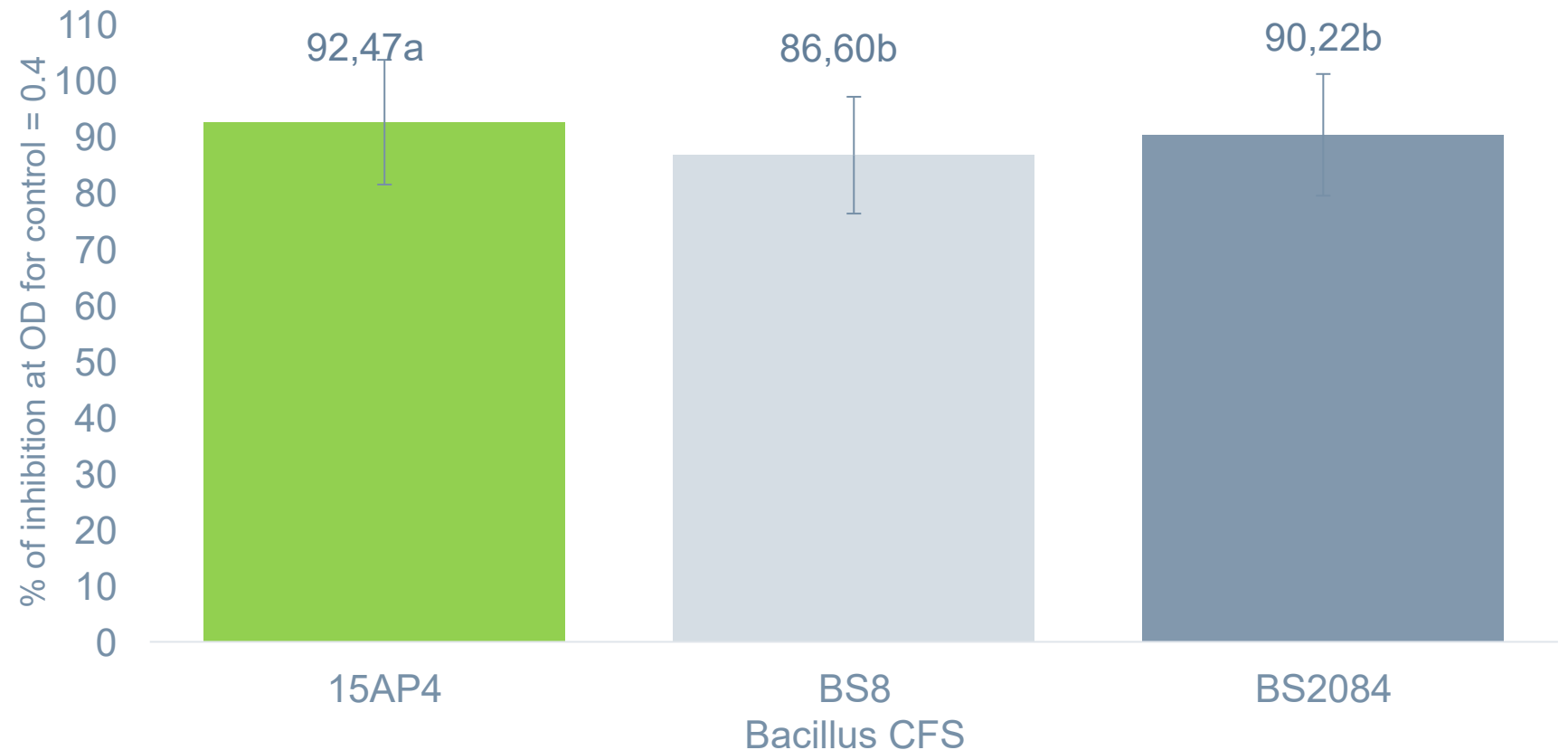
● E. cecorum G7517 ● E. cecorum G7517 + CFS #12/1

RESULTS

Probiotic *Bacillus* strains -BS8, 15AP4 and 2084- prevent growth of avian *E. cecorum* isolates in a robust and consistent manner

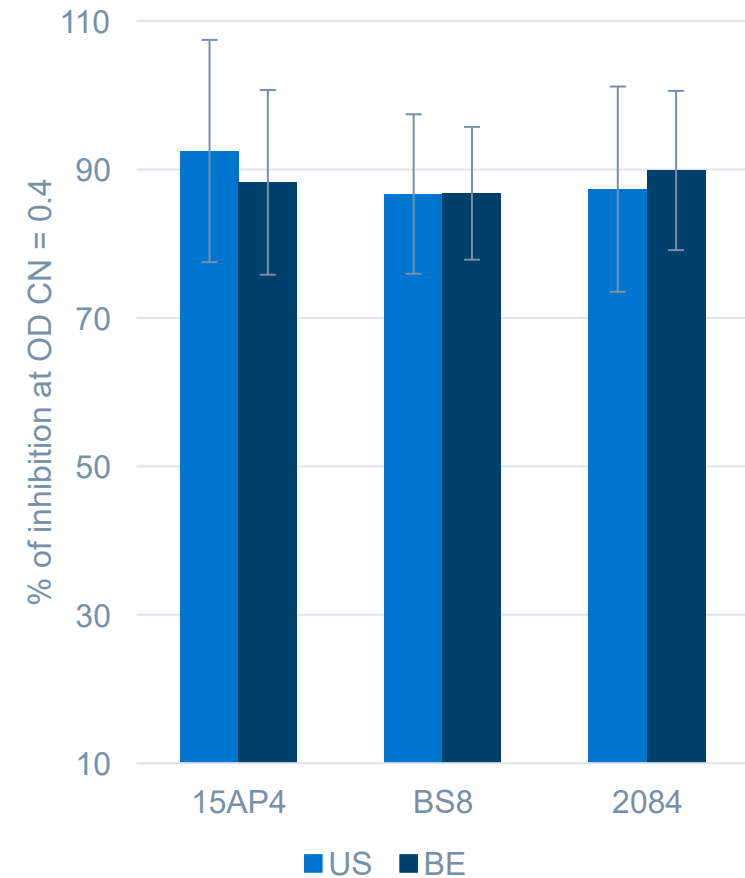
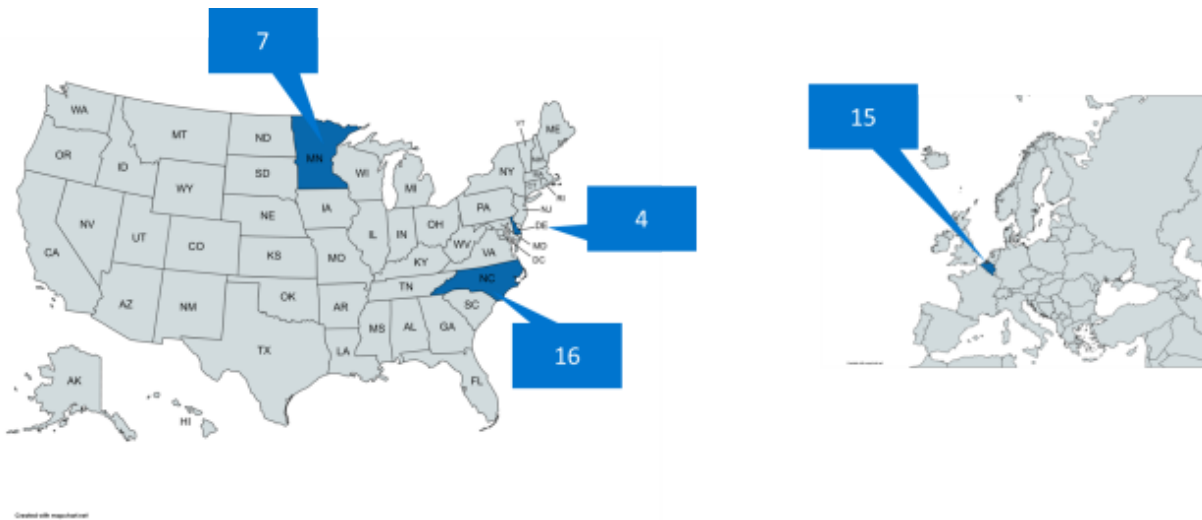


Inhibitory effect of *Bacillus* strains against *E. cecorum* (n=42)



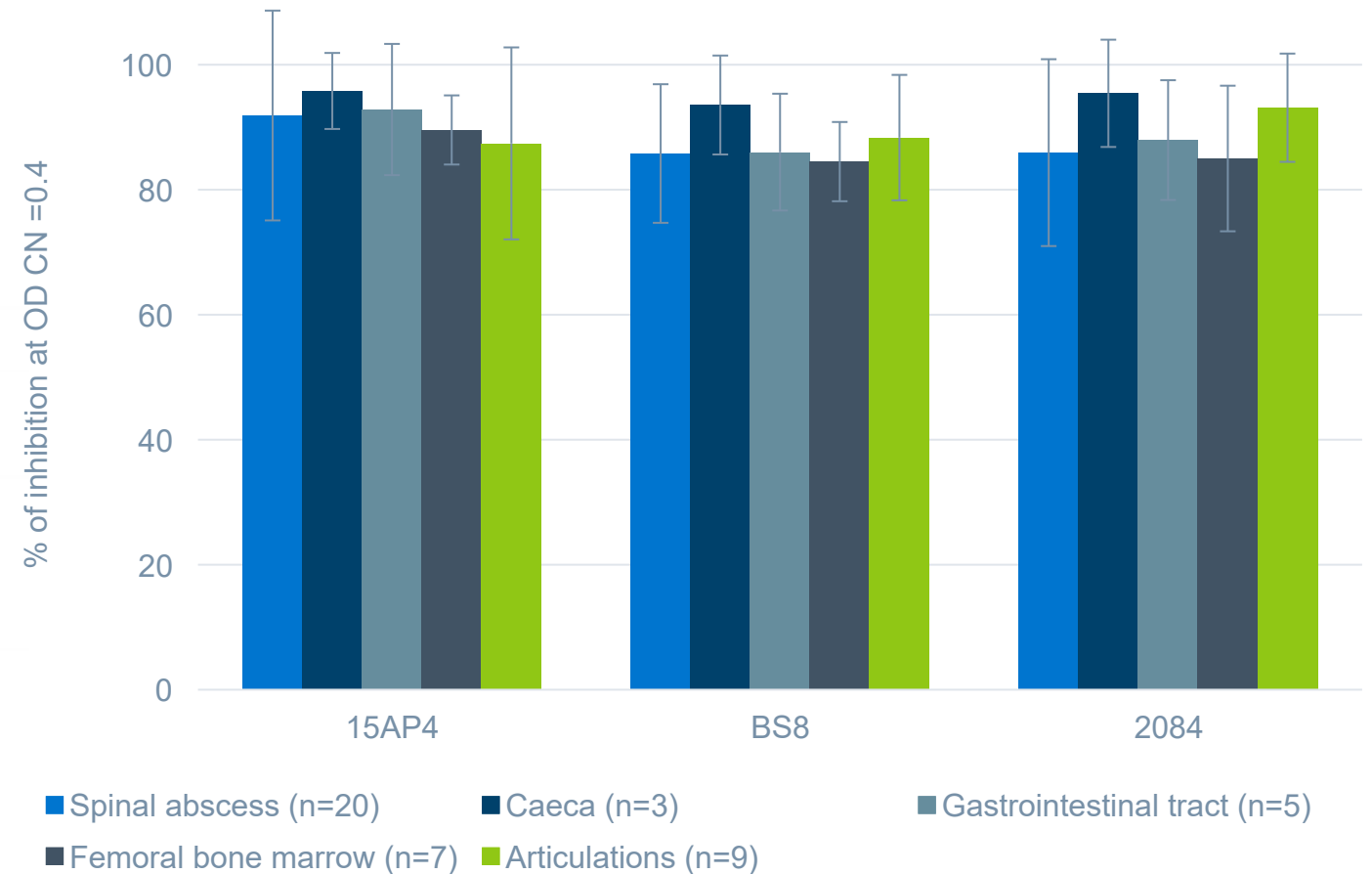
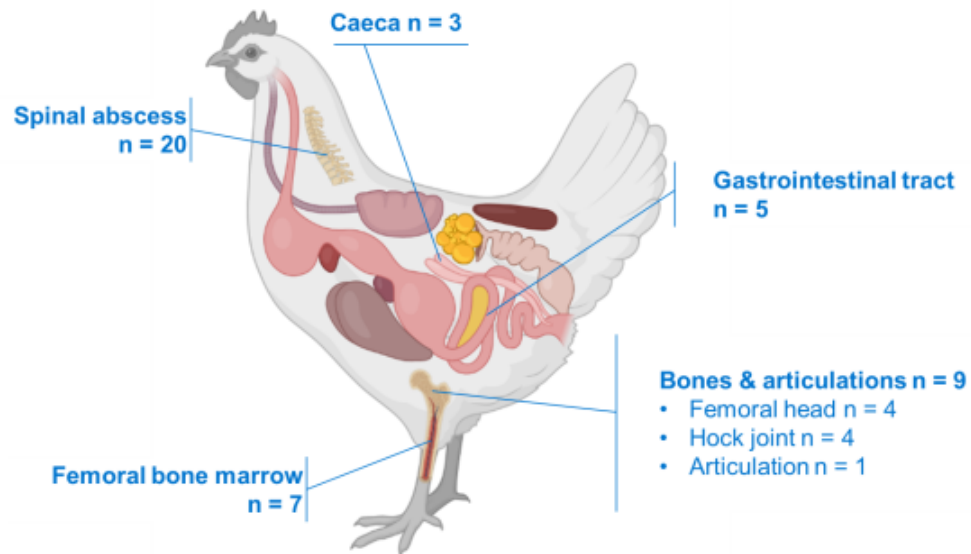
POTENTIAL OF THE 3 *BACILLUS* STRAINS IS CONSISTENT...

Whatever the geographic location of the *E. cecorum* isolates



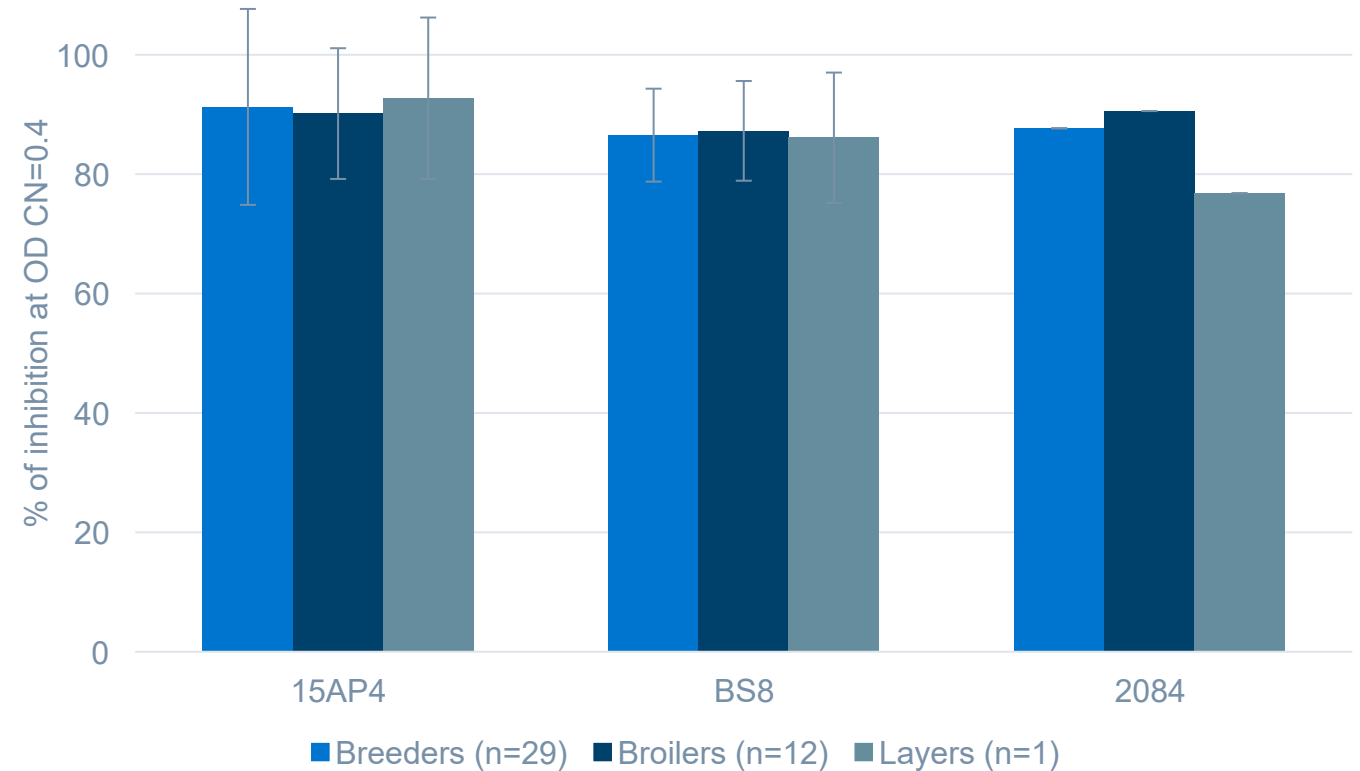
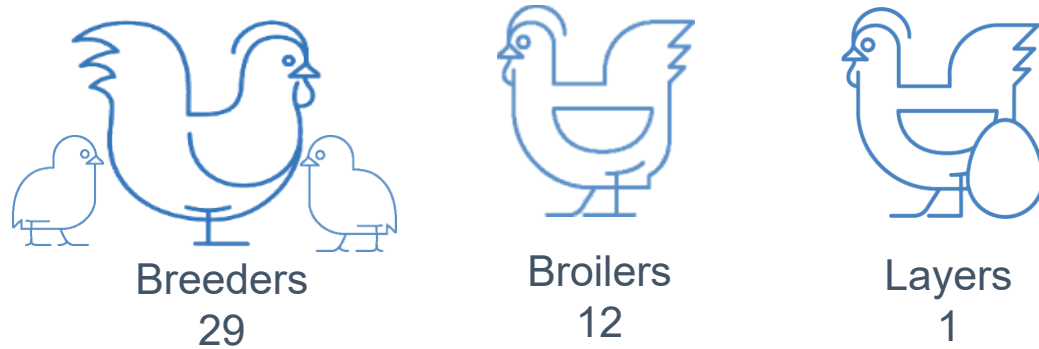
POTENTIAL OF THE 3 *BACILLUS* STRAINS IS CONSISTENT...

Whatever the site of isolation of the *E. cecorum* isolates



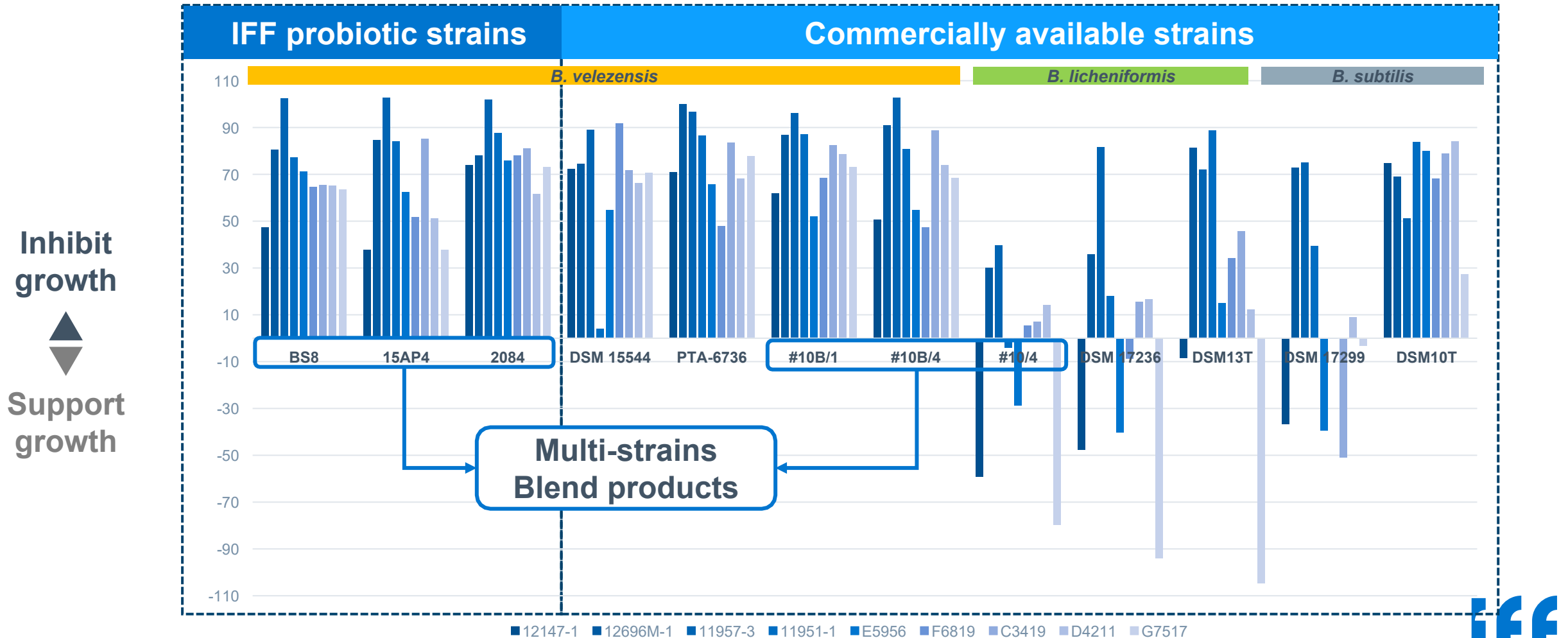
POTENTIAL OF THE 3 *BACILLUS* STRAINS IS CONSISTENT...

Whatever the host origin of the *E. cecorum* isolates



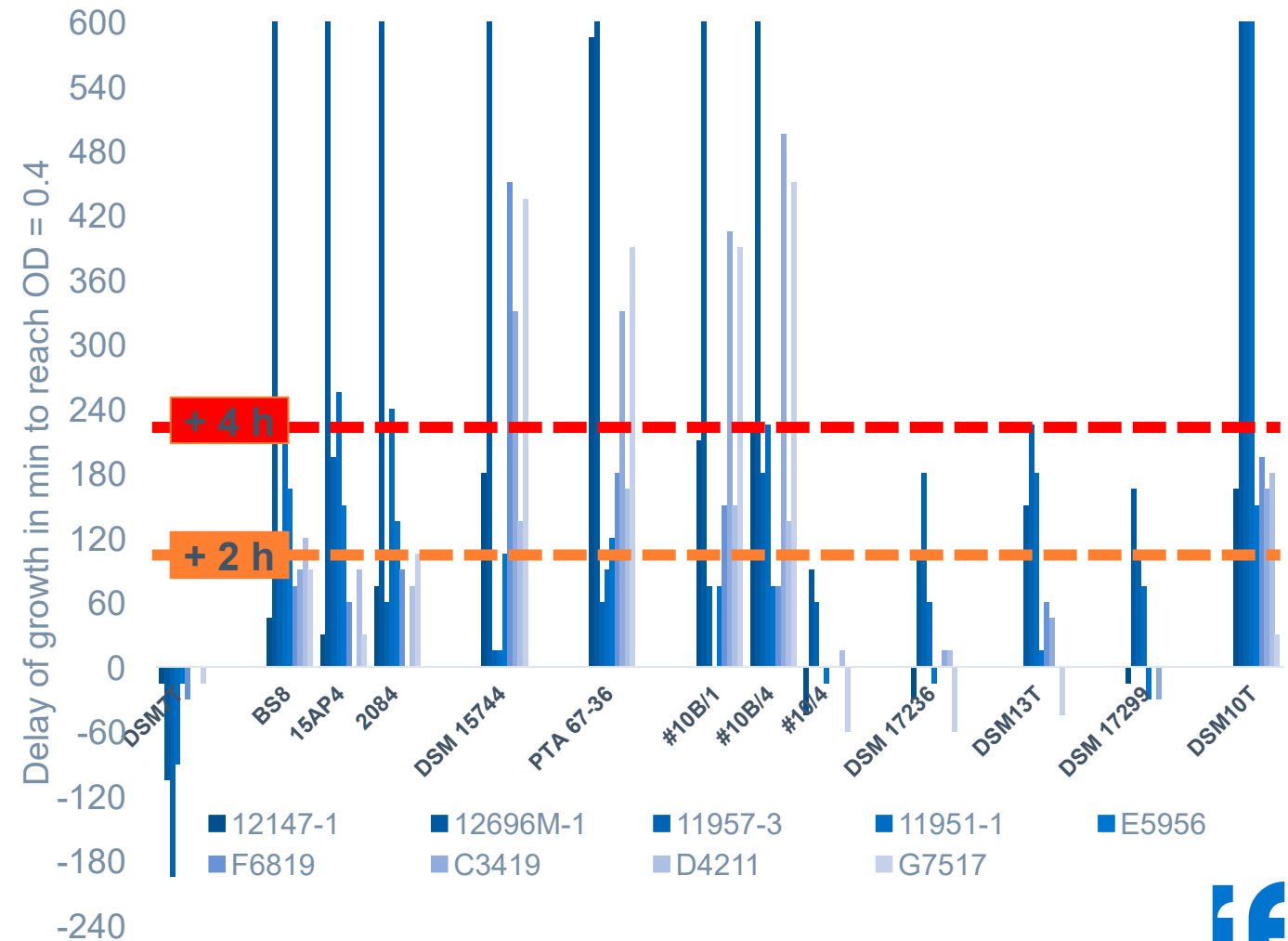
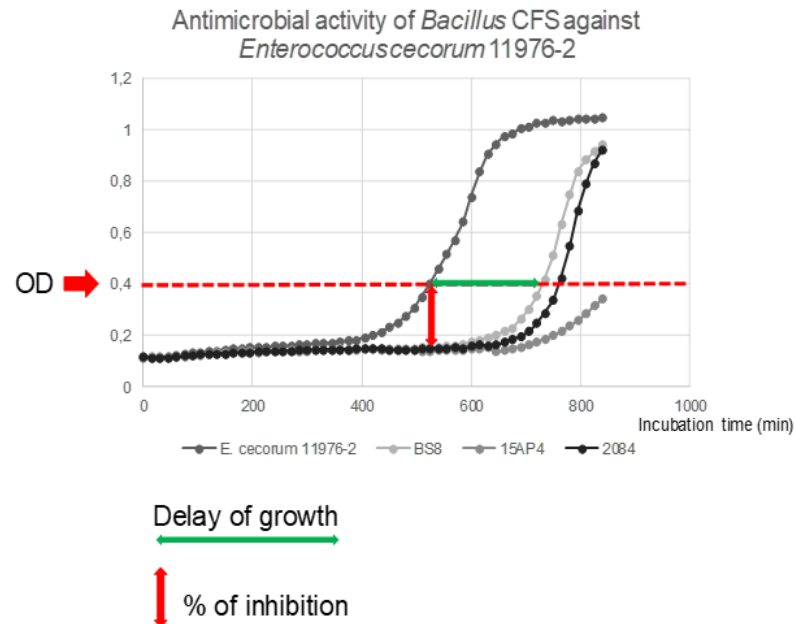
NOT ALL *BACILLUS* STRAINS ARE THE SAME

Comparison of *E. cecorum* inhibitory activity amongst *Bacillus* strains collected from commercially available feed additives (% of inhibition; 9 strains investigated *in vitro*)



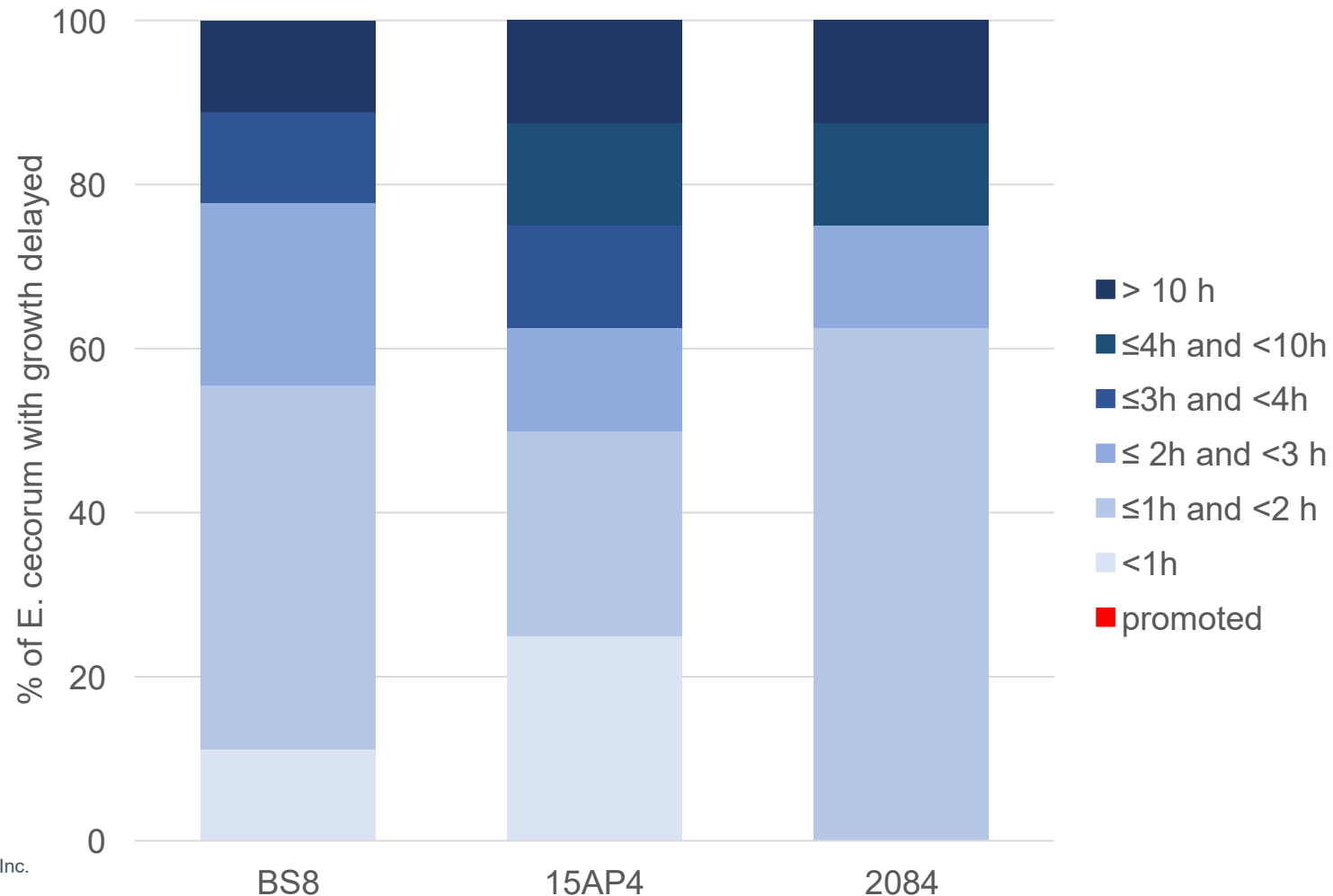
INDIRECT POTENTIAL PREVENTIVE EFFECT ...

... delaying the growth of *E. cecorum* isolates

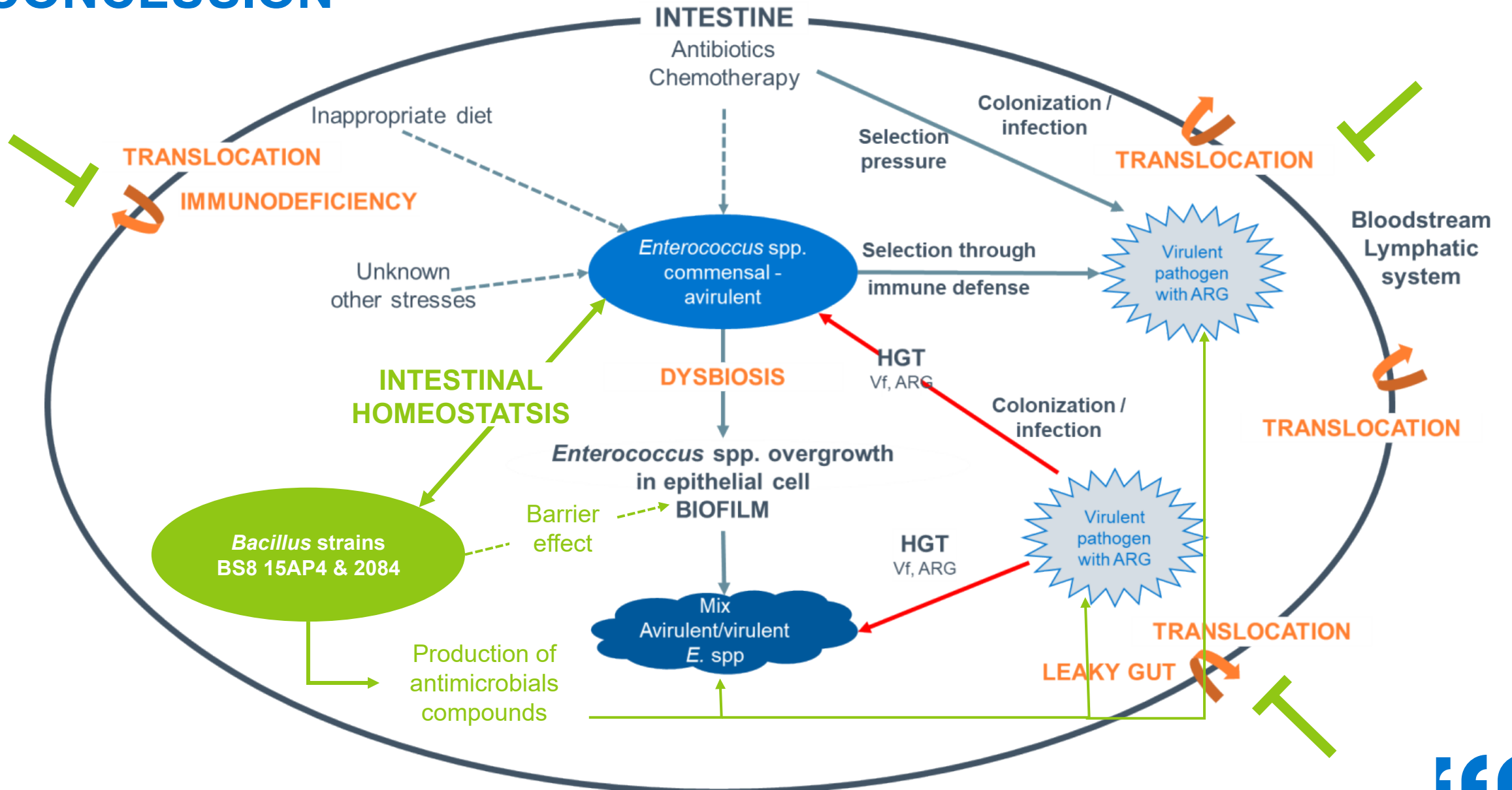


DELAYING THE GROWTH OF *E. CECORUM*

The lag phase of 40% of the tested *E. cecorum*, is extended by a minimum of 2 hours when in contact with *Bacillus* BS8, 15AP4 and 2084 CFSs.



CONCLUSION



ACKNOWLEDGMENTS



Kirsty Gibbs
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IFF Danisco Animal Nutrition & Health



Marina Cretenet
Scientist - Teacher
Normandy University, ABTE



Sara Medina Fernandez
Research Engineer
Normandy University, ABTE

THANK YOU



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