

# Unveiling the Immunomodulatory Potential of Probiotic *Bacillus* Strains in Chicken Peripheral Blood Mononuclear Cells

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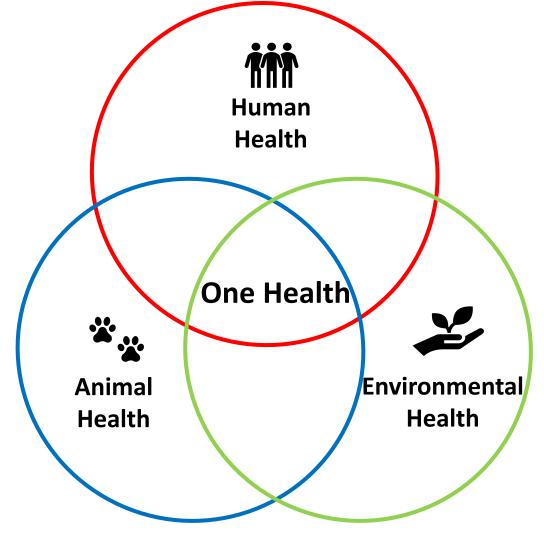


### Introduction

### The 'One Health' concept



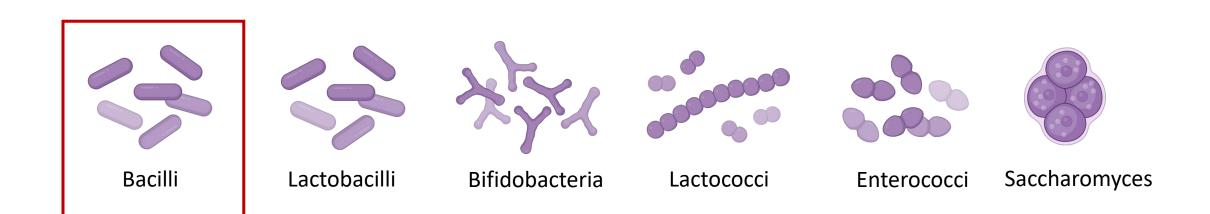
- Interdisciplinary approach
- Antimicrobial resistance
- Probiotics as alternative



## Probiotics as feed additives

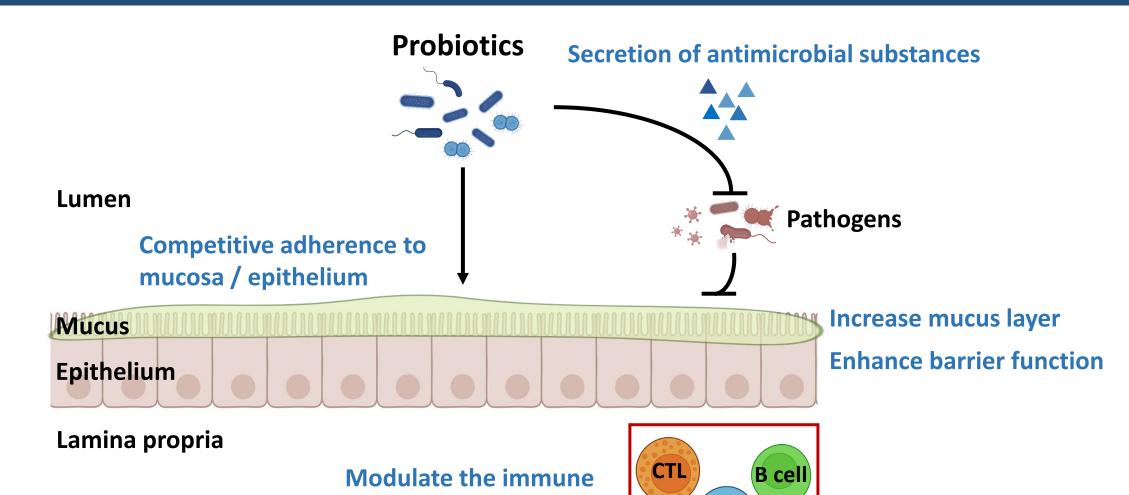


- **Probiotics:** Live microorganisms administered at appropriate amounts confer a health benefit to the host (FAO/WHO, 2002)
  - Species- and strain-specific



### Mechanisms of action of probiotics



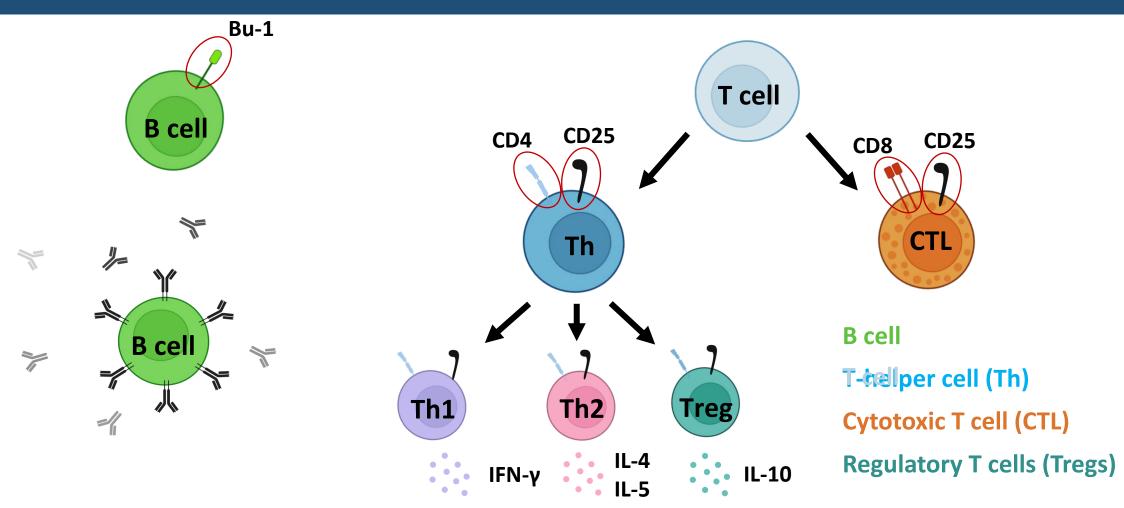


Th

system

### B and T cells – major players of the adaptive immune system





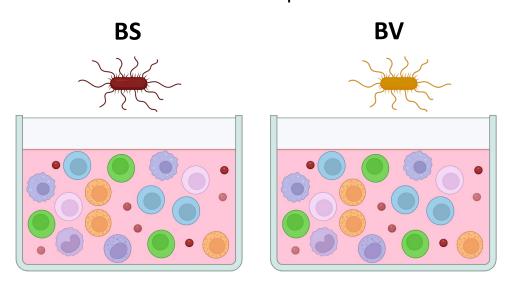
Do Bacillus strains modulate adaptive immune responses in chicken?

#### Material & Methods

### Investigation of the immunomodulatory effects of two Bacillus strains



#### PBMC treatment with probiotic bacteria



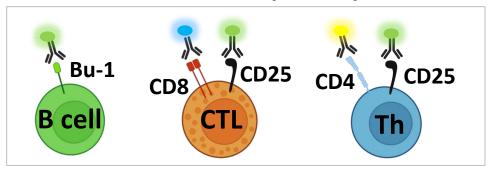
Peripheral blood mononuclear cellls (PBMCs)

B. subtilis (BS), B. velezensis (BV) (Evonik Operations GmbH)

Ratio: 1:3 (PBMC: Bacillus)

Cultivation: 24 h

#### Flow cytometry



Influence on immune cell differentiation and activation status

#### RNA-sequencing and expression analysis

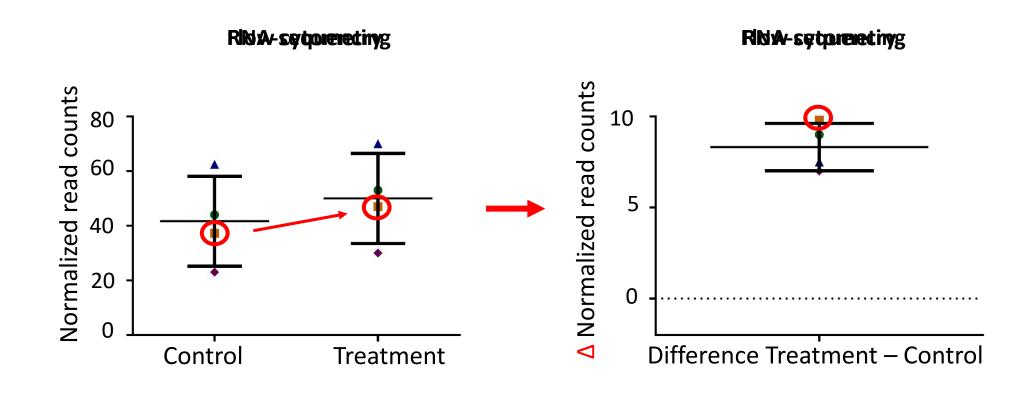


Influence on immune cell surface marker gene expression

6

### Data normalization on the respective negative control

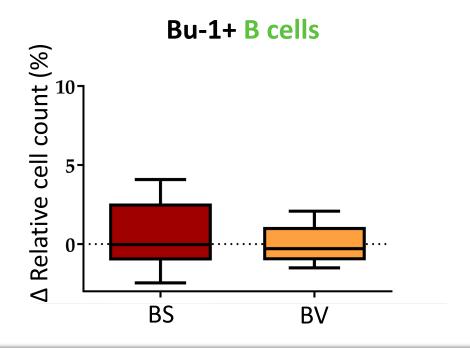




#### Results

### Effects of BS and BV on B cell surface marker expression





**BS** and **BV** did not affect the **B** cell count

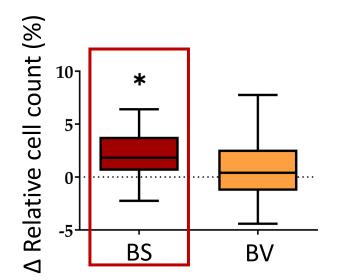
**B. subtilis (BS)**; n = 6 (FACS)

**B.** velezensis (BV); n= 9 (FACS)

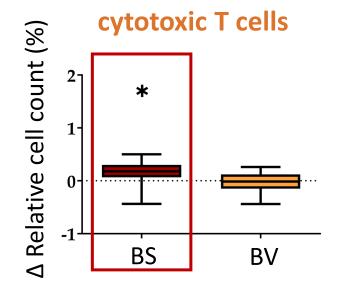
### Effects of BS and BV on cytotoxic T cell surface marker expression



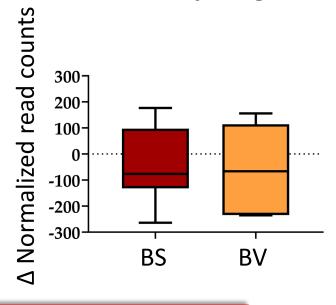
**CD8+ cytotoxic T cells** 



CD8+CD25+ activated



#### CD8 receptor gene



**BS** increased the count of cytotoxic T cells and activated cytotoxic T cells

Higher surface marker expression could not be validated by surface marker gene expression

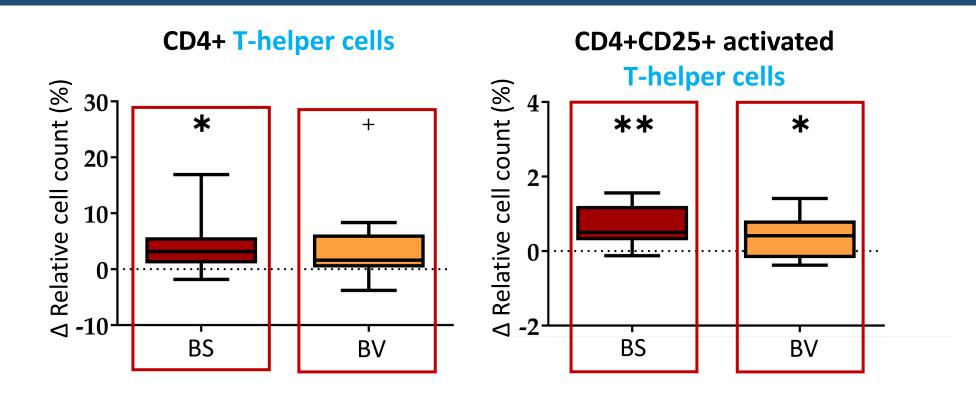
**B. subtilis (BS)**; n = 13 (FACS), n = 6 (RNA-Seq)

**B.** velezensis (BV); n= 11 (FACS), n = 6 (RNA-Seq)

<sup>\*</sup> p < 0.05

### Effects of BS and BV on T-helper cell surface marker expression





BS and BV elevated the count of T-helper cells and activated T-helper cells

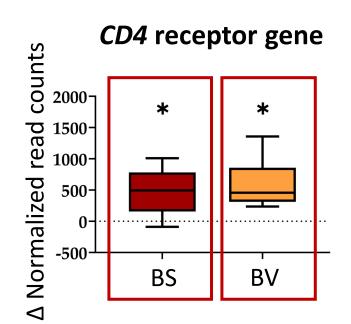
+ p < 0.1, \* p < 0.05, \*\* p < 0.01

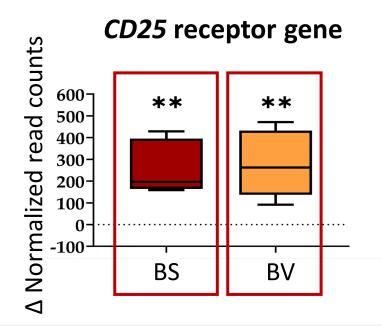
**B. subtilis (BS)**; n = 11 (FACS)

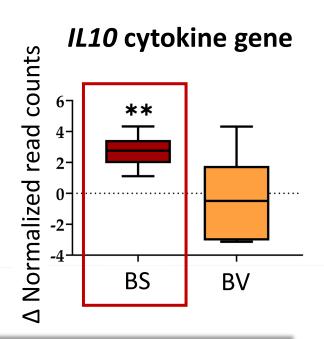
B. velezensis (BV); n= 11 (FACS)

### Effects of BS and BV on T-helper cell surface marker gene expression









**BS** increased the expression of Treg-associated surface marker and cytokine genes

BV elevated the expression of T-helper cell-associated surface marker genes

**B. subtilis (BS)**; n = 6 (RNA-Seq)

**B.** velezensis (BV); n = 6 (RNA-Seq)

<sup>\*</sup> p < 0.05, \*\* p < 0.01

### **Summary & Conclusion**



- BS and BV did not affect B cell surface marker expression
- **BS** stimulated surface marker expression of cytotoxic T cells
- BS and BV stimulated surface marker and surface marker gene expression of T-helper cells
  - BS most likely stimulated Tregs

BS and BV may have positive effects on chickens health by enhancing T cell immune responses

### Thank you for your attention!



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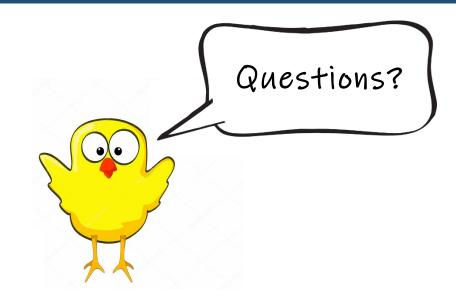
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