

monoguthealth

Optimal gut function in monogastric livestock

Prophybiotics, a novel approach for *in-ovo* gut microbiome reprogramming of broilers

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Lyon
31st August 2023

Content

01

- Background/ESR 3 project

02

- Screening for bioactives (*In-vitro*)

04

- Materials and Methods

05

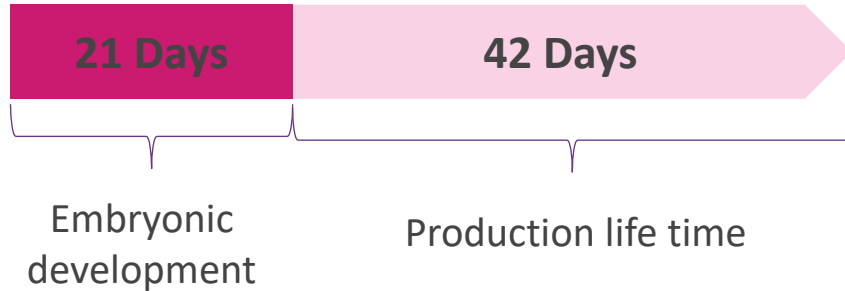
- Results and discussion

06

- Conclusion and future prospects

Background

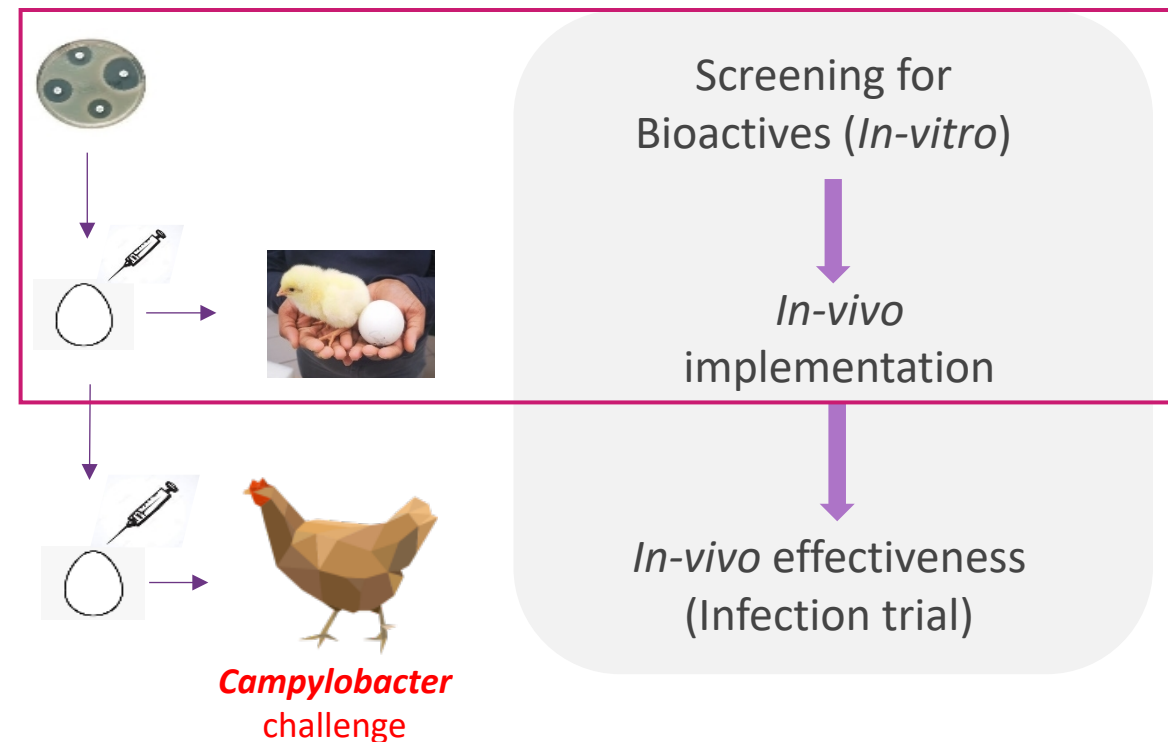
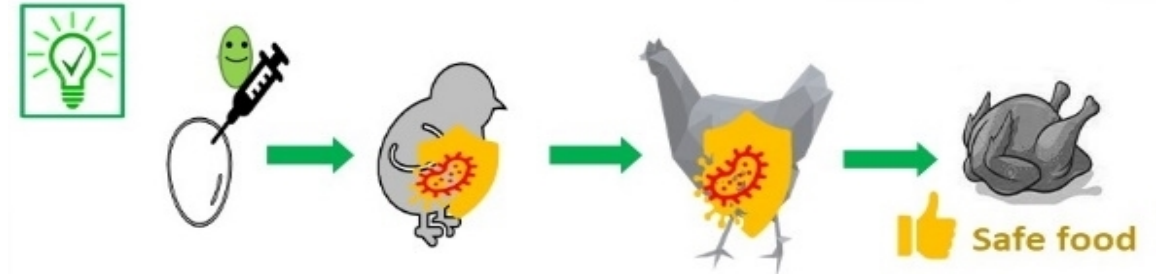
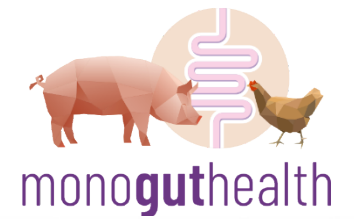
Broiler chicken lifespan



Key events related to gut health happening during embryonic development

- Development of **immune system**
- Development of **gastrointestinal tract**
- Development of **gut microbiome**

ESR 3 project



Screening for bioactives (*In-vitro*)

Probiotics + Phytochemicals = Prophybiotics



Probiotics



Plant extracts



Research questions

- What is the **best plant extract** to be used in **combination** with the probiotics and at what dose?
- What is the **best probiotic** in terms of *in-vitro* **anti-pathogenic** activity?
- Does *in-ovo* application of **prophybiotics** adversely affect the **hatchability and quality of chicks**?

Objectives:

- To determine effects of supplementation of different plant extracts in variable doses on *in-vitro* growth of probiotics
- To determine anti-*Salmonella* and anti-*Campylobacter* effects of probiotics *in-vitro*
- To determine the effects of *in-ovo* application of the selected pro/prophybiotic on the hatchability and chick quality parameters

Materials and Methods



Probiotic strains:

(JHJ Sp Zo.o, Poland)

1. *Lactiplantibacillus plantarum*
2. *Lacticaseibacillus casei*
3. *Limosilactobacillus reuteri*
4. *Lacticaseibacillus rhamnosus*
5. *Leuconostoc mesenteroides*
6. *Pediococcus pentosaceus*

Positive control: *Lactococcus lactis*



Plant supplements:

Aqueous extracts of

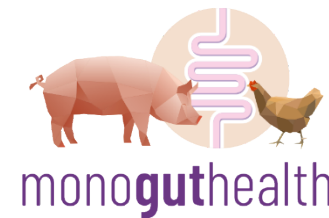
1. Turmeric
2. Green tea
3. Garlic



Pathogenic strains:

1. *Salmonella typhimurium* (DPC6463)
2. *Salmonella enterica* subsp. *Enterica* (ATCC 14028)
3. *Salmonella braenderup* (NRL-IE-22)
4. *Campylobacter jejuni* (DVI-SC181)

Materials and Methods



Selection of plant extracts

Methods:

- **Growth curve analysis** of probiotics supplemented with;
 - Turmeric (w/v: 0.06%, 0.1%, 0.6%)
 - Green tea (w/v: 0.06%, 0.1%, 0.6%)
 - Garlic (w/v: 0.25%, 0.5%, 1%)

Selection of best probiotic

Methods:

Anti- *Salmonella*

- Spot overlay assays
- Well diffusion assays
- Co-culture assays
- Co-aggregation assays

Anti- *Campylobacter*

- Well diffusion assays

Selection of best Prophybiotic

Methods:

Anti- *Salmonella*

- Co-culture assays with best probiotic + PEs

In-vivo implementation: Prophybiotics

Methods:

In-ovo injection of the selected **prophybiotic and probiotic alone** to **ROSS 308** chicken eggs at **12th Embryonic day**. Recorded;

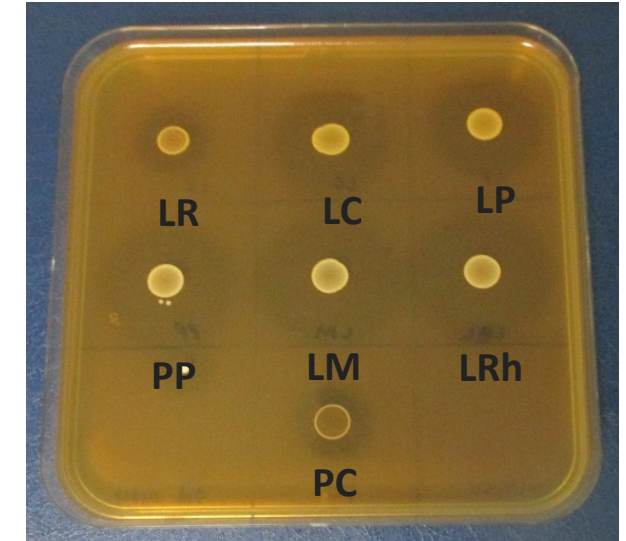
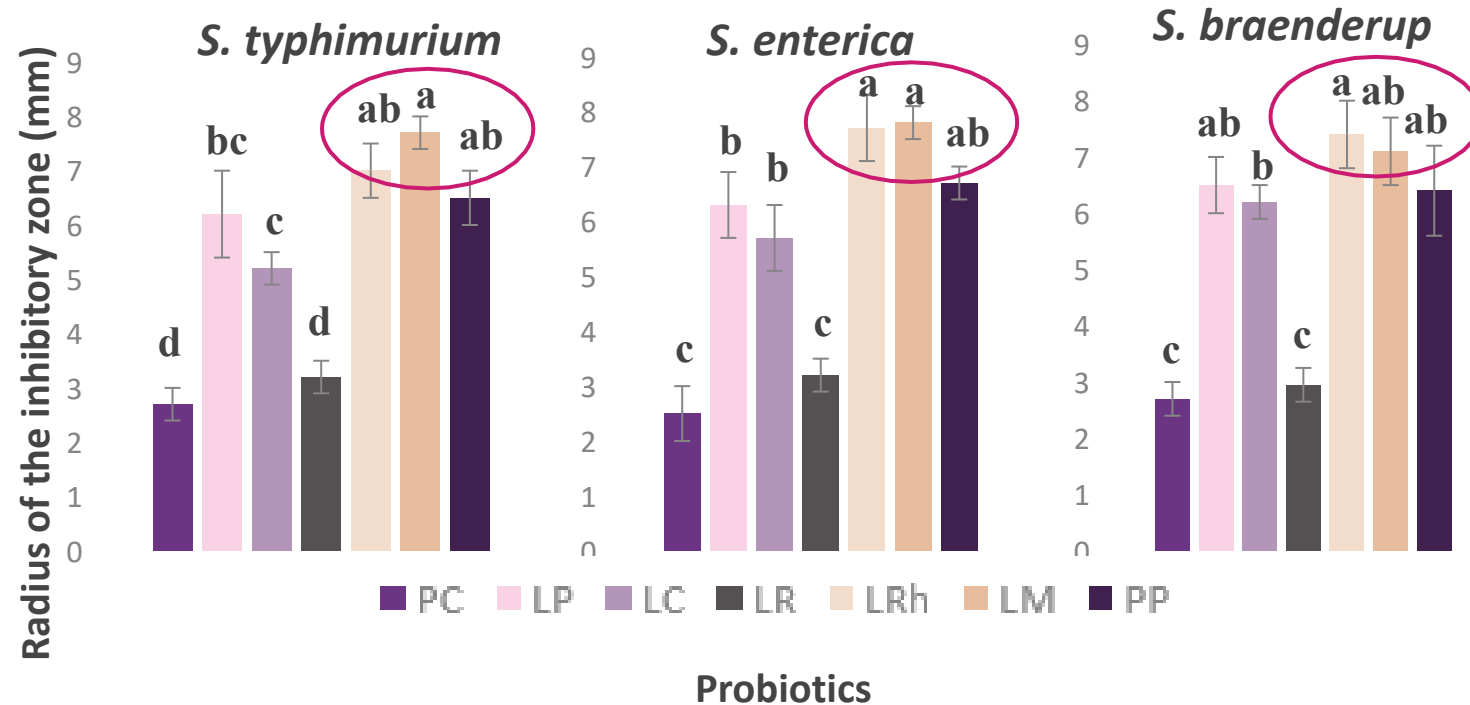
- Hatchability
- Chick quality
- Chick weight/length



Results (What's the best probiotic?)

Anti-Salmonella

Spot overlay assays



Inhibition of *S. typhimurium* in probiotic spot overlay assay

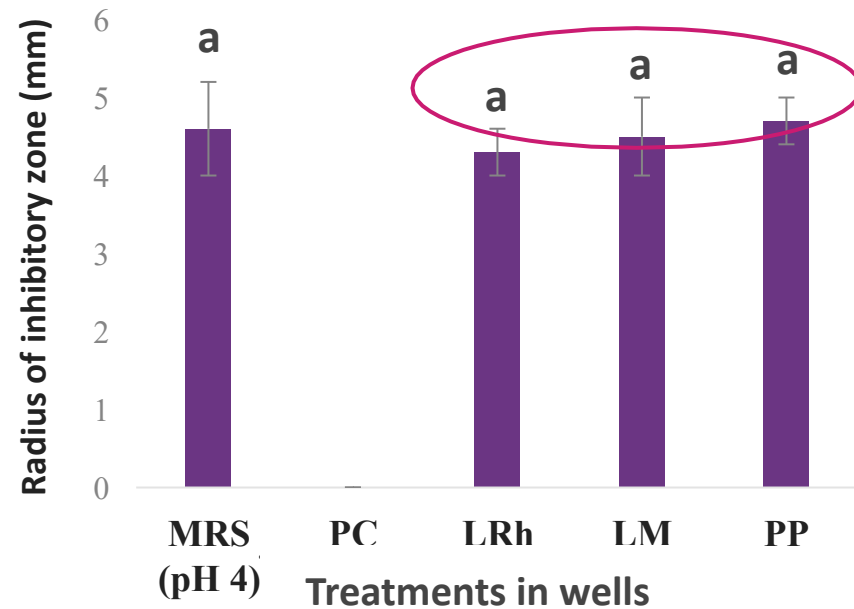
PC: *Lactococcus lactis* LP: *Lactiplantibacillus plantarum* LC: *Lacticaseibacillus casei* LR: *Limosilactobacillus reuteri*

LRh: *Lacticaseibacillus rhamnosus* LM: *Leuconostoc mesenteroides* PP: *Pediococcus pentosaceus*

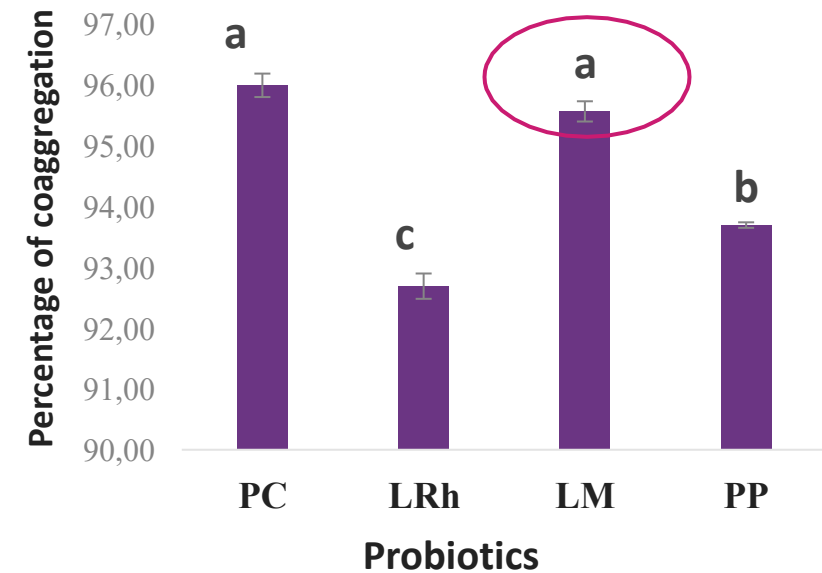
Results (What's the best probiotic?)

Anti-Salmonella

Well diffusion Assays



Co-aggregation Assay

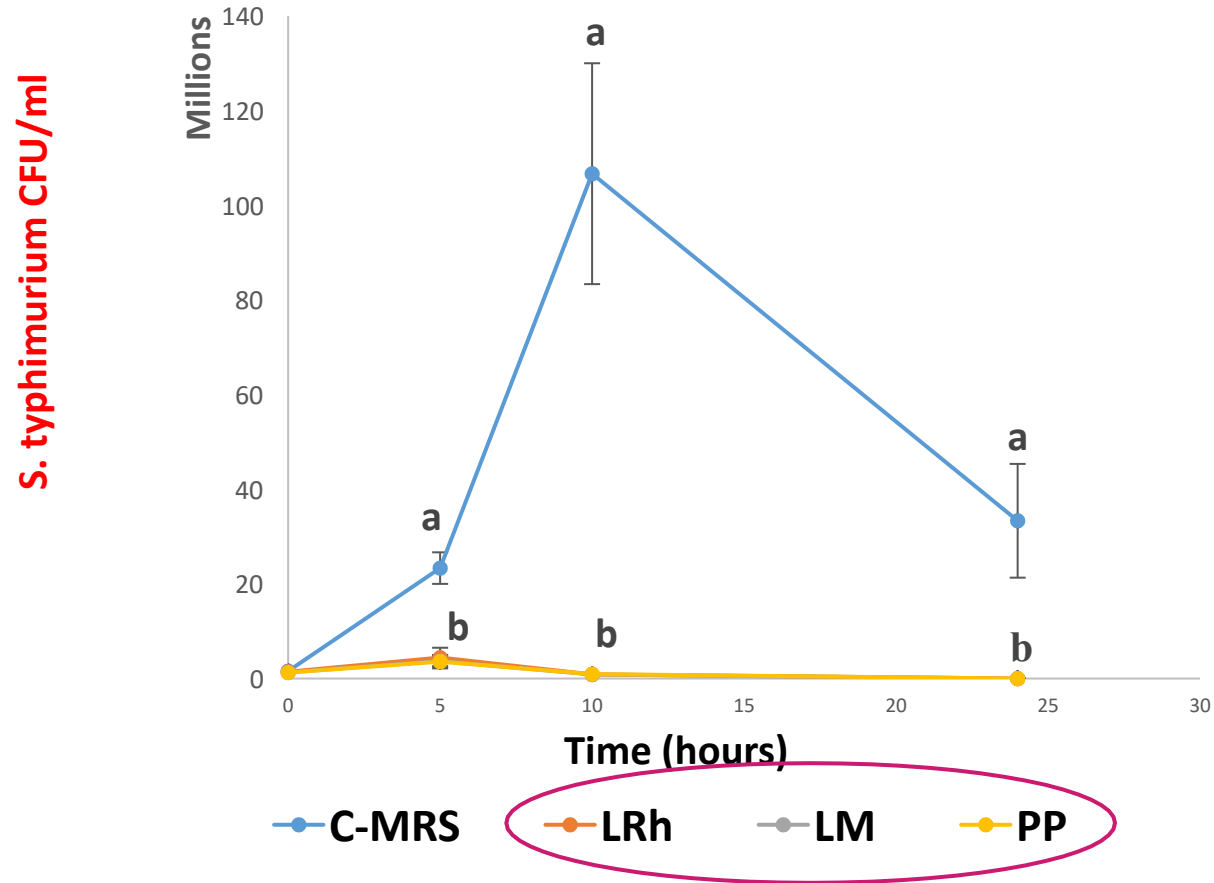


LRh: *Lacticaseibacillus rhamnosus* **LM:** *Leuconostoc mesenteroides* **PP:** *Pediococcus pentosaceus* **PC:** *Lactococcus lactis*

Results (What's the best probiotic?)

Anti-Salmonella

Co-culture Assay



C-MRS: Control medium **LRh:** *Lactobacillus rhamnosus* **LM:** *Leuconostoc mesenteroides* **PP:** *Pediococcus pentosaceus*



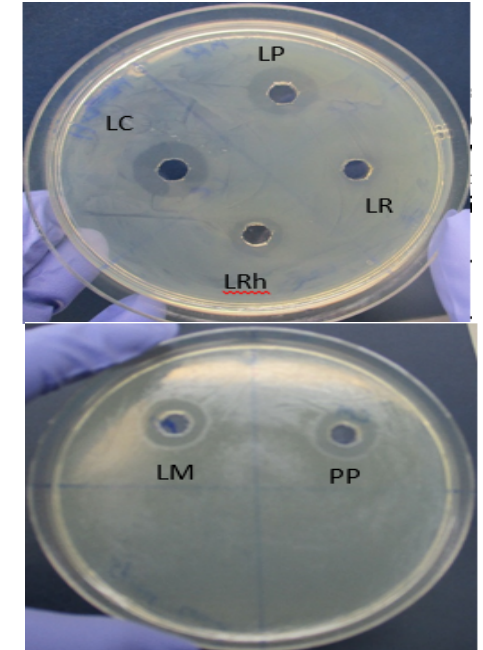
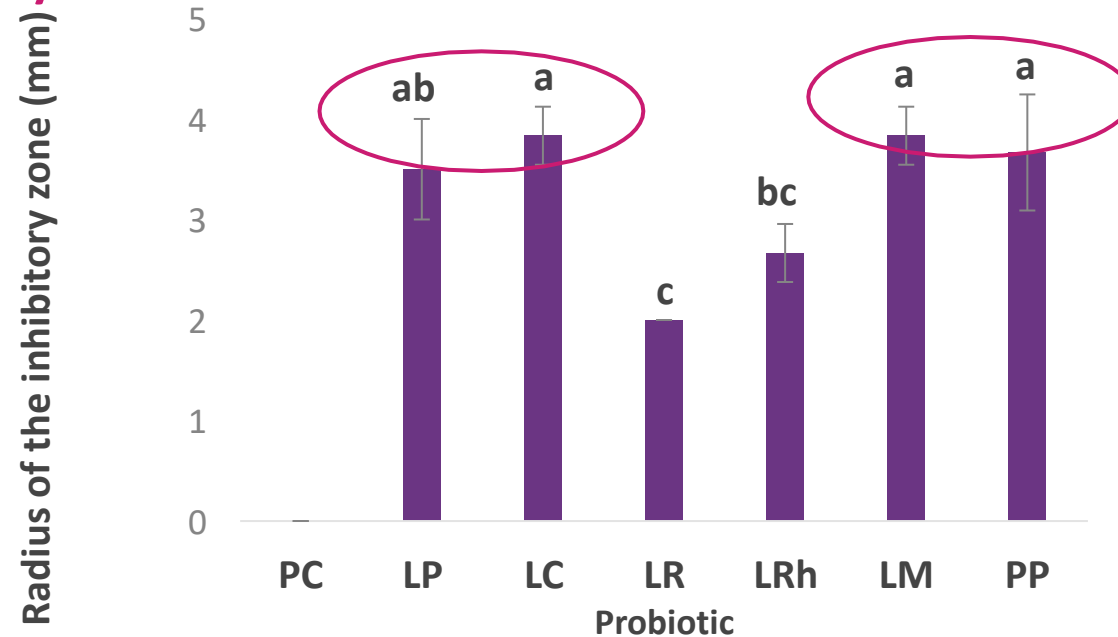
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EAAP Annual Meeting, Lyon
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Results (What's the best probiotic?)

Anti-Campylobacter

Well diffusion assays



Anti-Campylobacter well diffusion assay

PC: *Lactococcus lactis* LP: *Lactiplantibacillus plantarum* LC: *Lacticaseibacillus casei*
 LR: *Limosilactobacillus reuteri* LRh: *Lacticaseibacillus rhamnosus* LM: *Leuconostoc mesenteroides* PP: *Pediococcus pentosaceus*

* Best probiotic selected: *Leuconostoc mesenteroides*

Results (What's the best plant extract?)

<https://doi.org/10.1007/s10123-023->

Growth curve assays: *Leuconostoc mesenteroides*

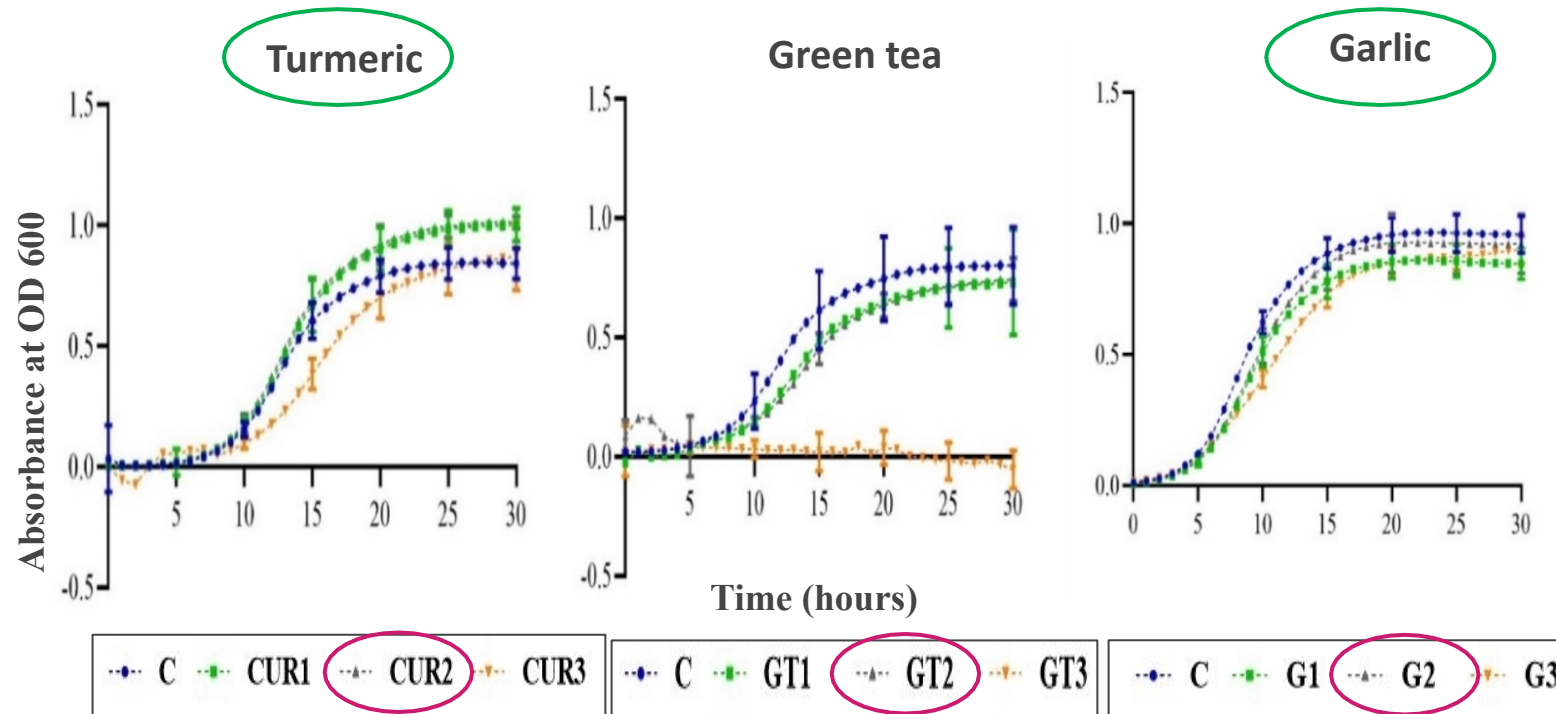
Home > International Microbiology > Article

In-vitro screening of compatible synbiotics and (introducing) “prophybiotics” as a tool to improve gut health

Research | Open Access | Published: 22 August 2023 | (2023)



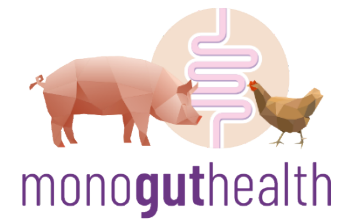
International Microbiology



* Best plant extracts for *L. mesenteroides*:

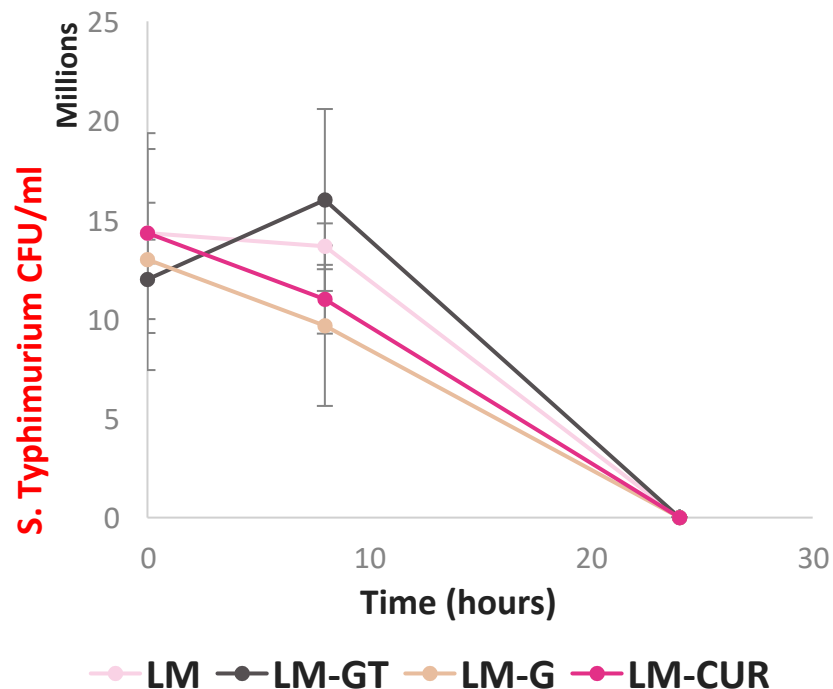
- Turmeric (0.1% and 0.06%)
- Garlic (0.5%)

Results (What's the best Prophybiotic?)

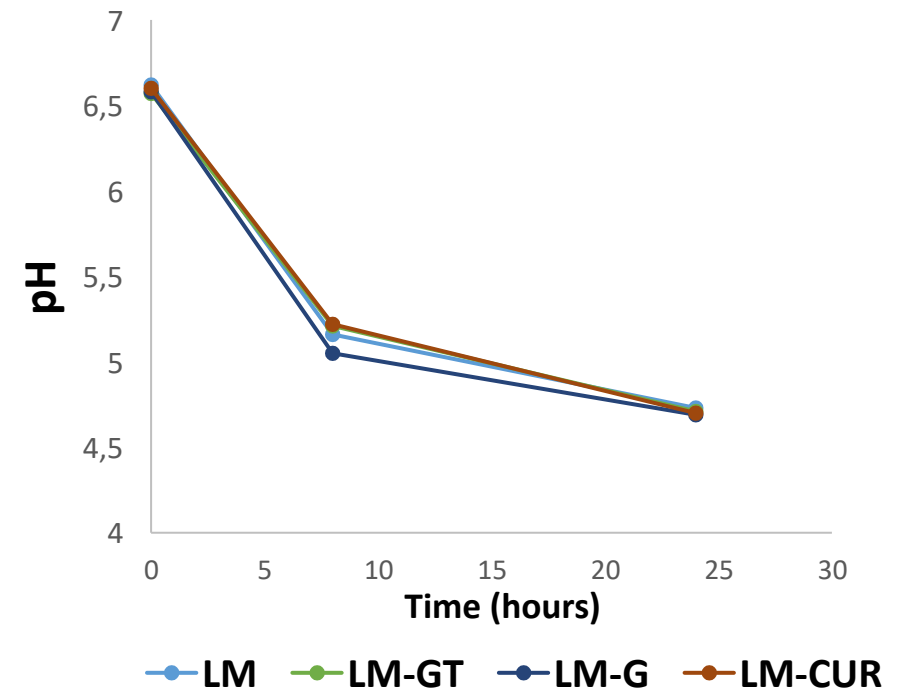


Prophybiotics = *Leuconostoc mesenteroides* + Plant extracts

Salmonella counts in co-culture with prophybiotics



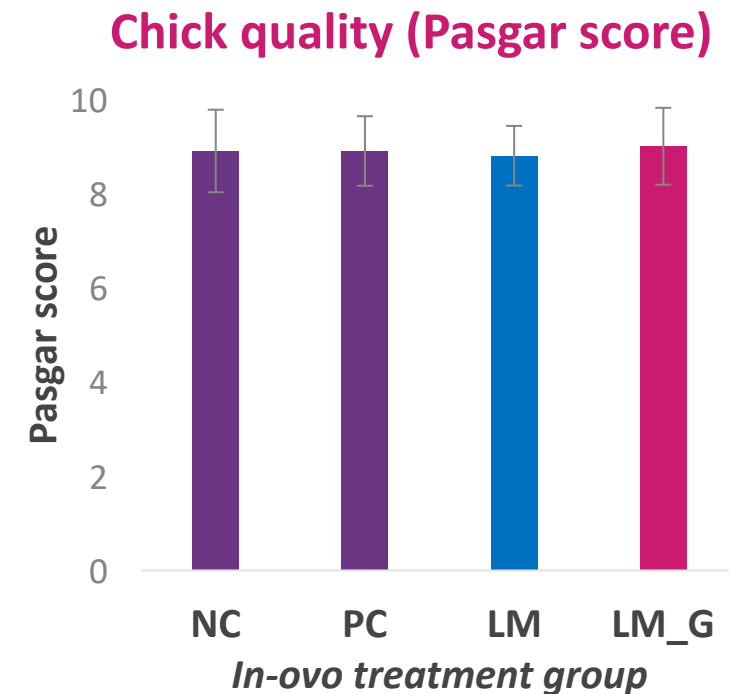
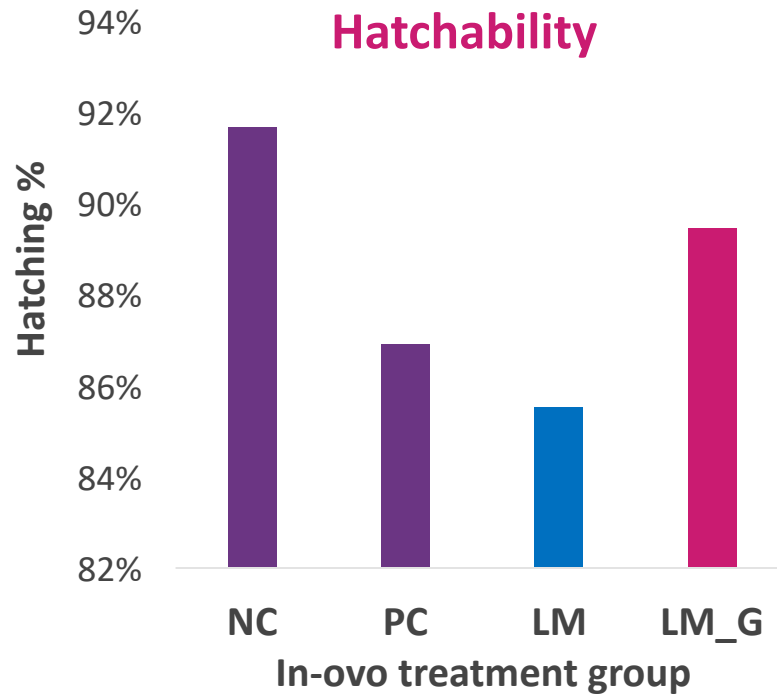
pH of the co-culture with prophybiotics



* Selected prophybiotic: *Leuconostoc mesenteroides* + Garlic (0.5%)



Results (*In-vivo* implementation)



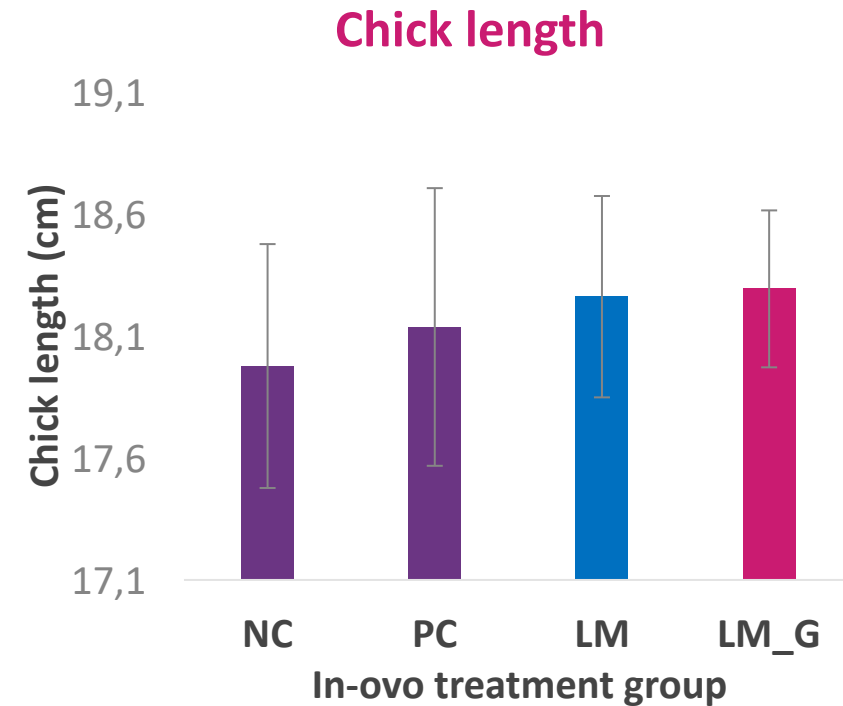
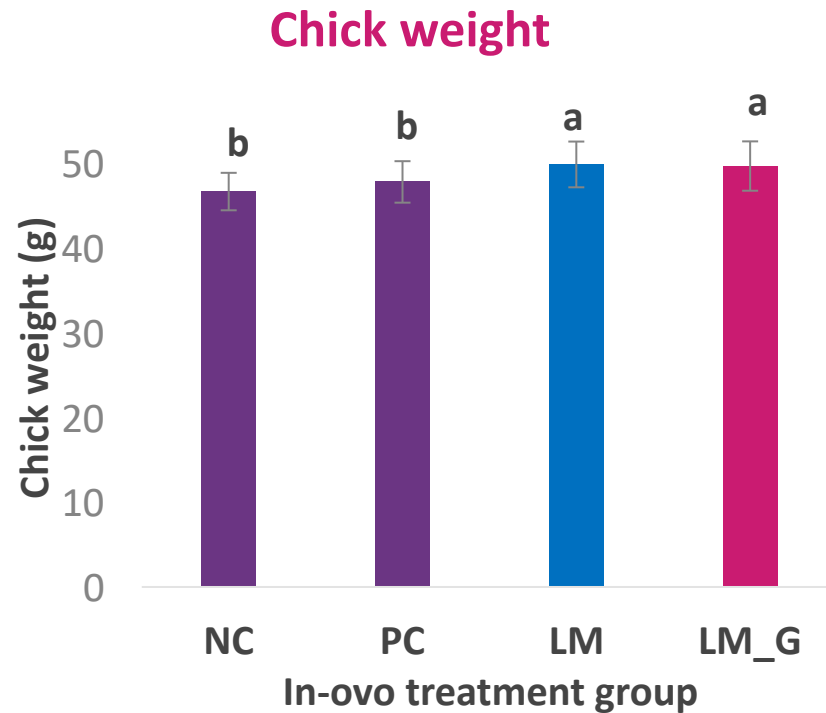
NC: Negative control (No *in-ovo* injection)

PC: Positive control (*In-ovo* injection with physiological saline - 0.2μl/egg)

LM: *In-ovo* injection with 10⁶ CFU of *L. mesenterodites* (0.2μl/egg)

LM_G: *In-ovo* injection with 10⁶ CFU of *L. mesenterodites* + 0.5% garlic (0.2μl/egg)

Results (*In-vivo* implementation)



NC: Negative control (No *in-ovo* injection)

PC: Positive control (*In-ovo* injection with physiological saline - 0.2μl/egg)

LM: *In-ovo* injection with 10⁶ CFU of *L. mesenterodies* (0.2μl/egg)

LM_G: *In-ovo* injection with 10⁶ CFU of *L. mesenterodies* + 0.5% garlic (0.2μl/egg)

Conclusion and Future prospects

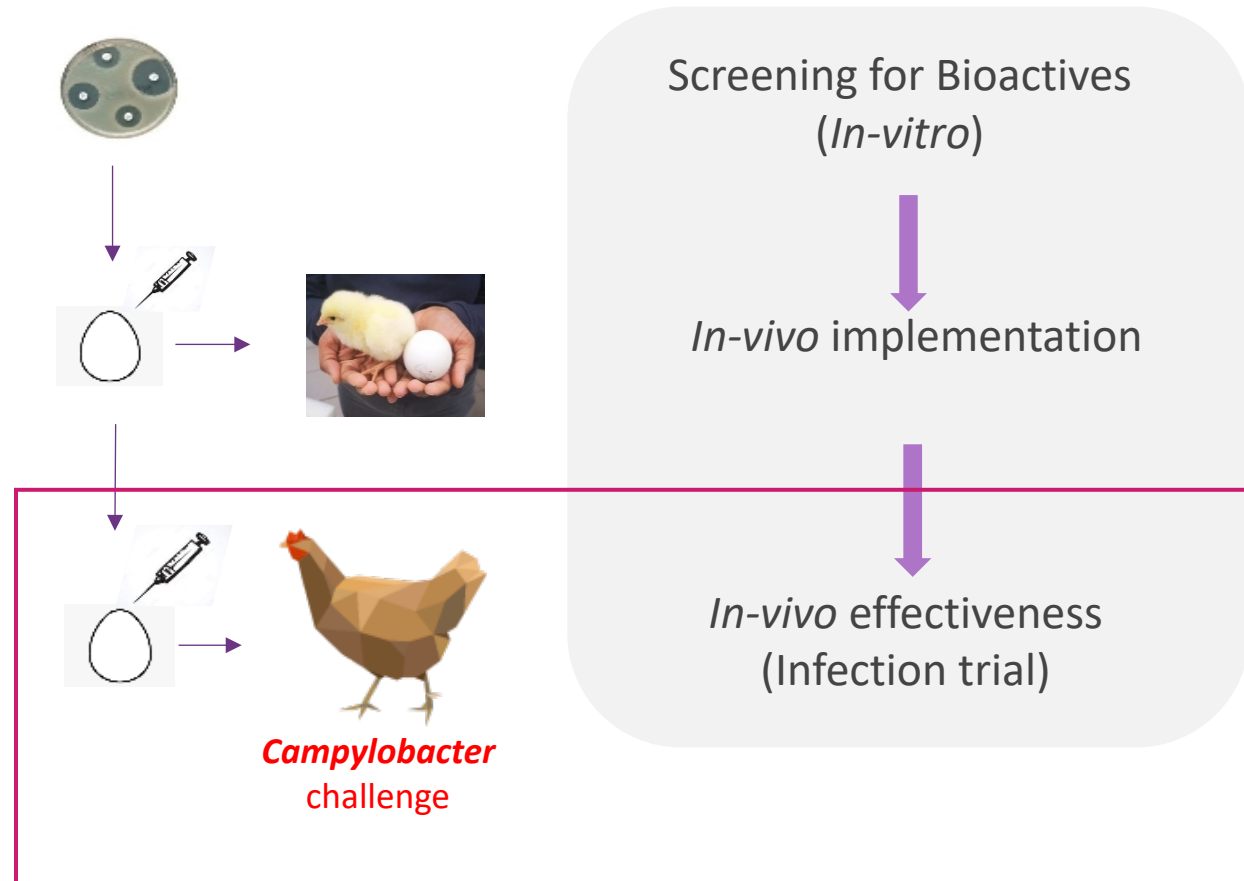
Conclusions

The selected **prophylactic** (*L. mesenteroides* + Garlic) displays;

a promising **antimicrobial potential** and

the **potential to be used in an *in-ovo* application without compromising the hatchability and affecting the chick quality parameters**

Further research is necessary **to validate the *in-vivo* effectiveness** of the protocol



THANK YOU

Do you have any questions?

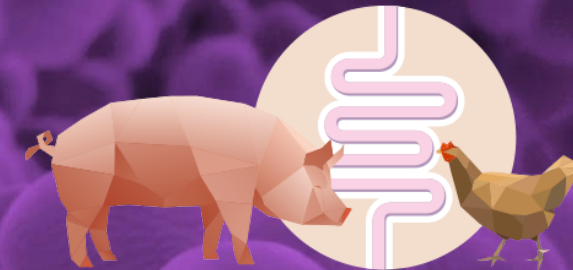
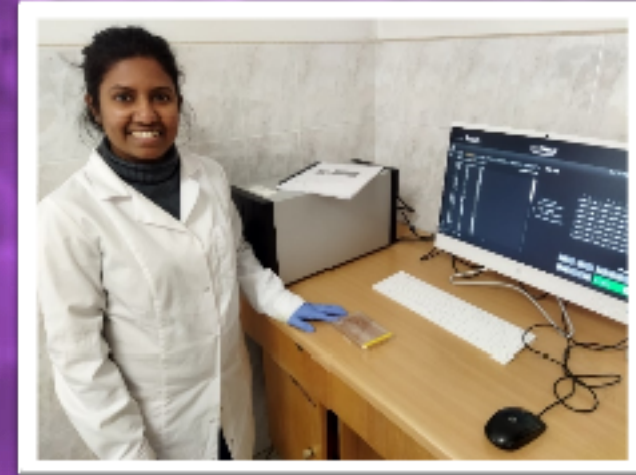
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Optimal gut function in monogastric livestock



Materials and Methods

Preparation of Garlic aqueous extract



+



- Garlic powder (Cultivar: **Thermodrome**)
- Add to sterile distilled water
- Vortex: 25 seconds
- Light shaking: 8 minutes
- Still: 2 minutes
- Centrifuge at 10000 rpm: 5 minutes
- Supernatant filter sterilized by 0.22um filters
- Supplement to media at;
G1: 0.25%
 G2: 0.5%
G3: 1%

Preparation of Turmeric and Green tea supplements



+



- Dissolved in MRS broth at;
CUR1: 0.06%
 CUR2: 0.1%
CUR3: 0.6%
- Filter sterilized by 0.22um filters

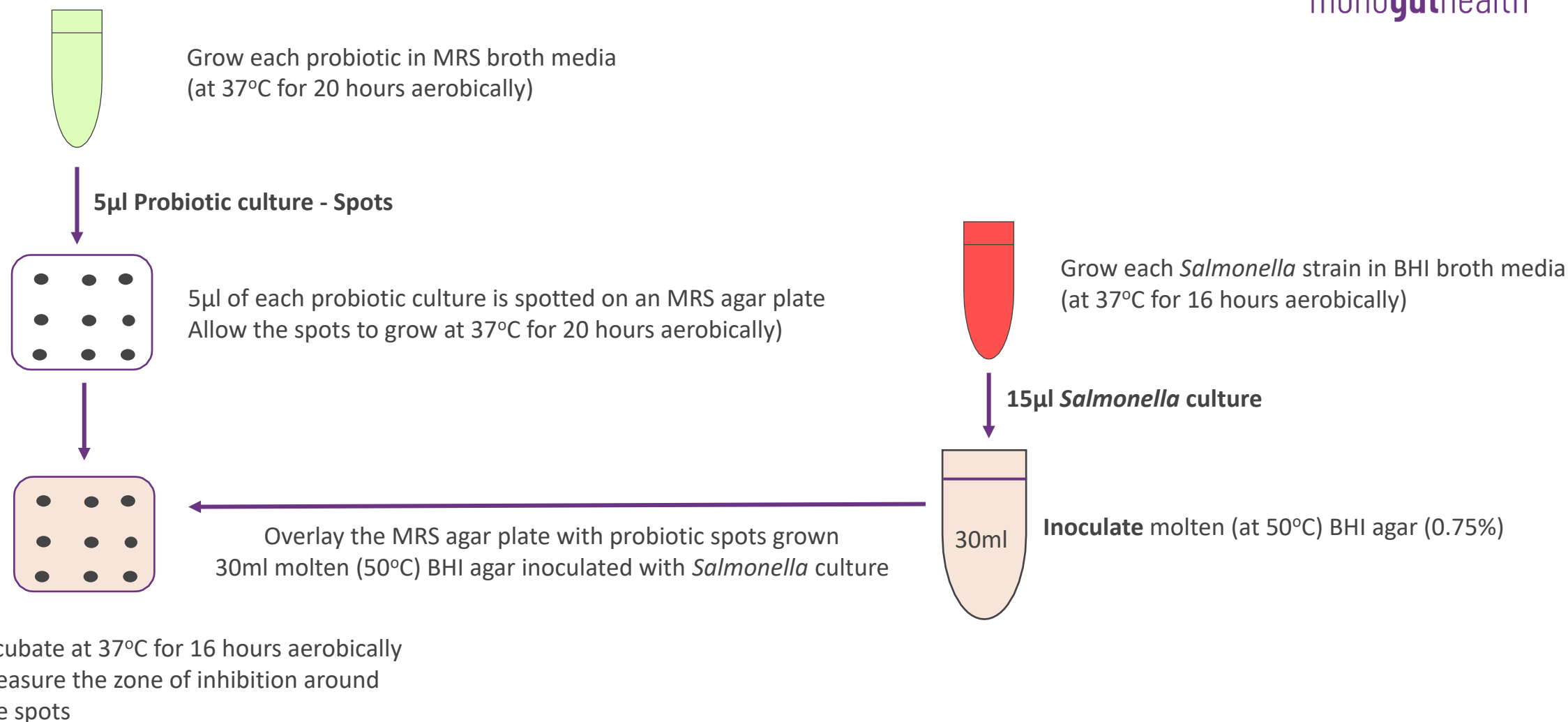


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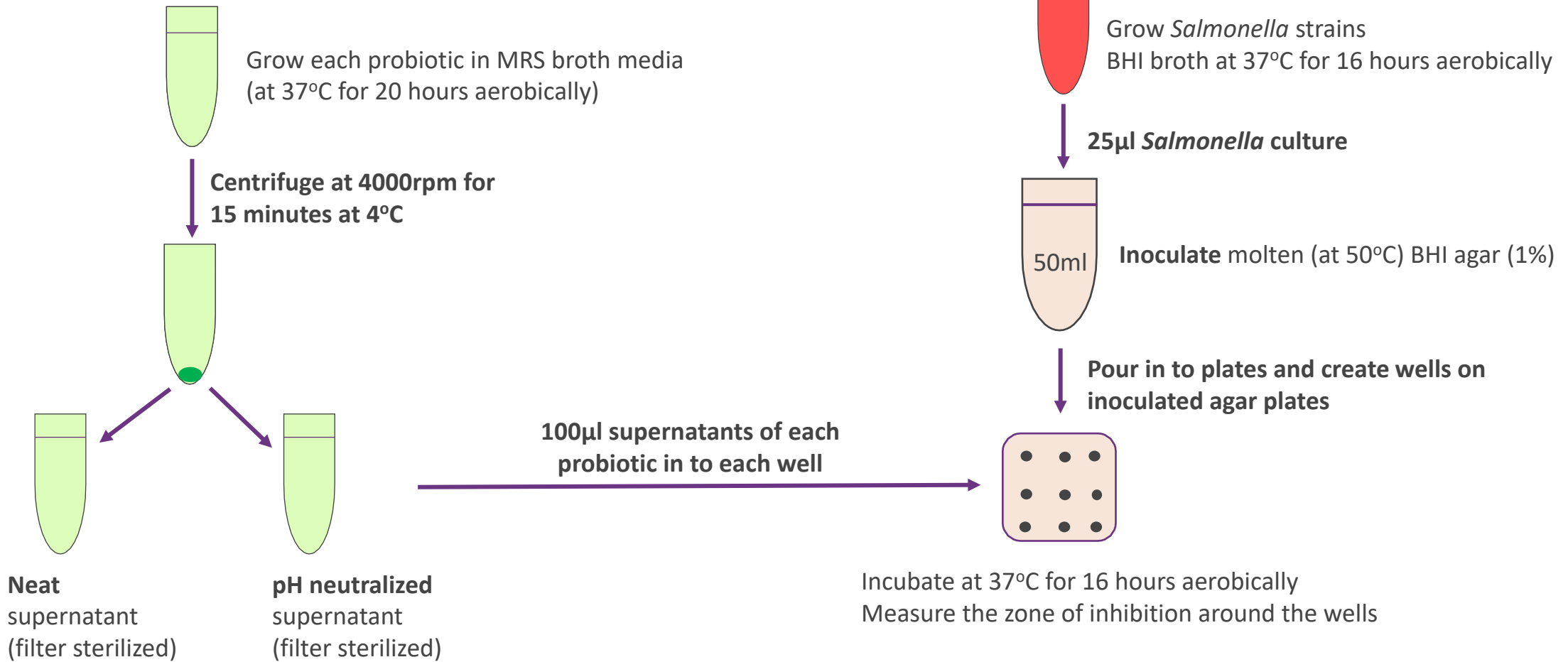


- Dissolved in MRS broth at;
GT1: 0.06%
 GT2: 0.1%
GT3: 0.6%
- Filter sterilized by 0.22um filters

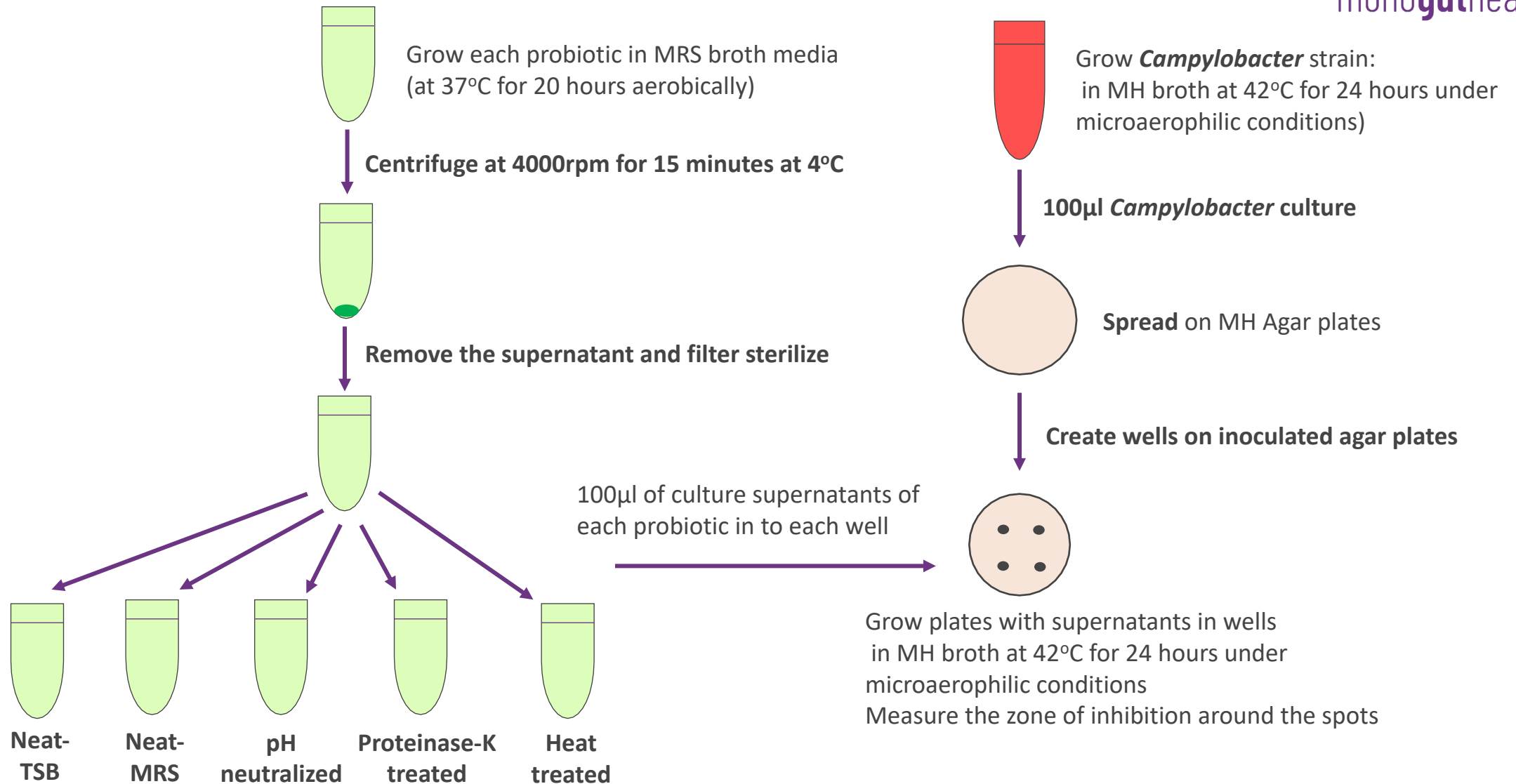
Spot overlay assays



Well diffusion assays – Anti-Salmonella

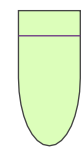


Well diffusion assays – Anti-*Campylobacter*

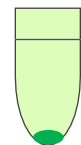


Co-aggregation assay

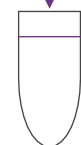
Grow each probiotic in MRS broth media at 37°C for 20 hours aerobically)



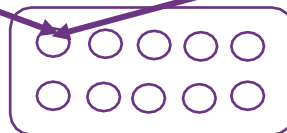
Centrifuge at 4000rpm for 15 minutes at 4°C
Discard the supernatant



Wash the pellet two times and re-suspend in PBS



Grow *Salmonella typhimurium* in BHI broth media at 37°C for 16 hours aerobically



$$\text{Coaggregation ability} = [1 - (2 \times A_{\text{mix}} / (A_{\text{prob}} + A_{\text{sal}}))] \times 100$$

- Transfer 250µl of each probiotic suspension and 250ul of *Salmonella* suspension to each well
- Incubate at 37°C for 20 hours aerobically
- Measure the OD600 (Optical density at 600nm)

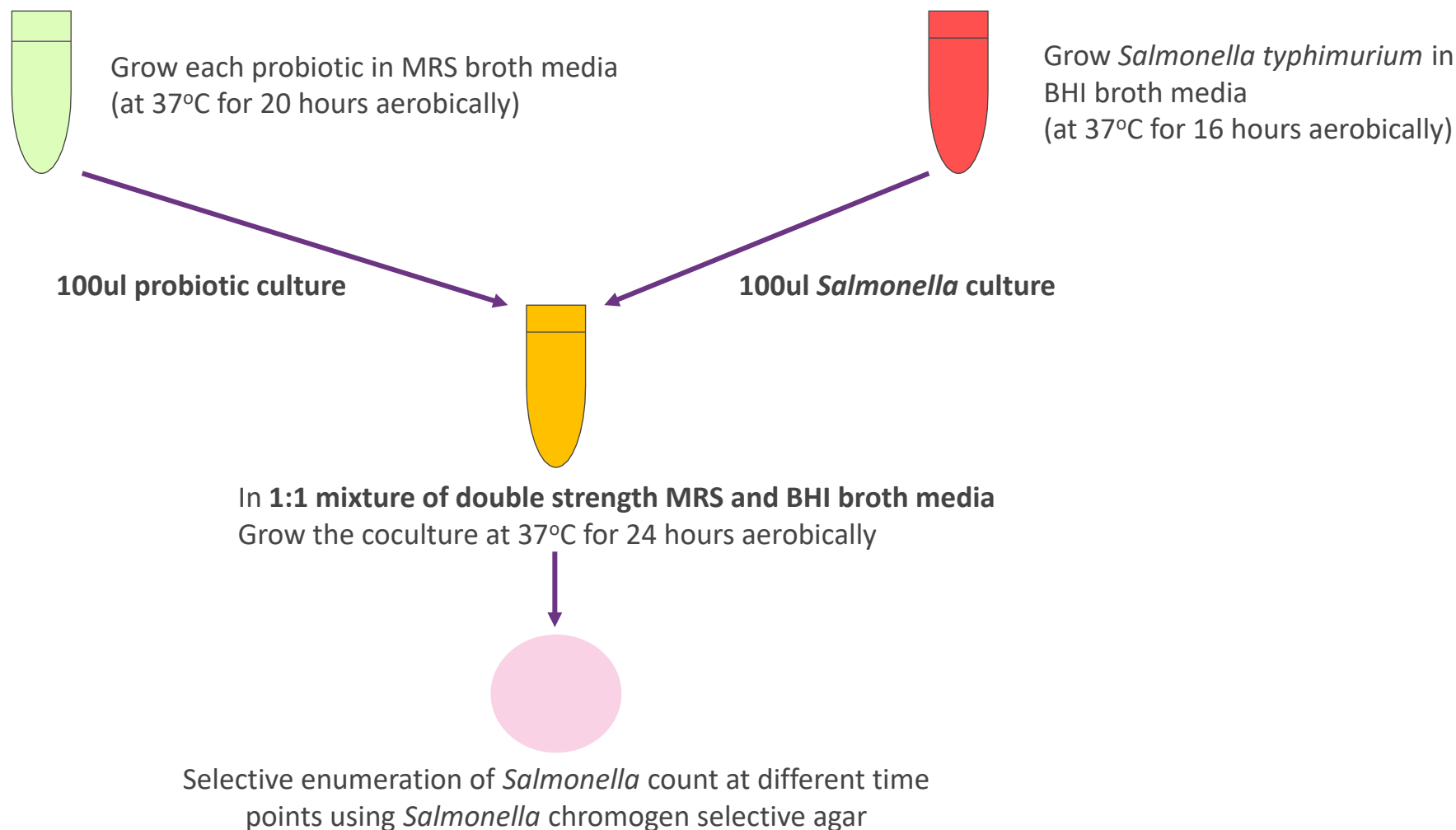
Where;

A_{mix} = OD₆₀₀ of mixture of probiotic and *Salmonella* suspensions

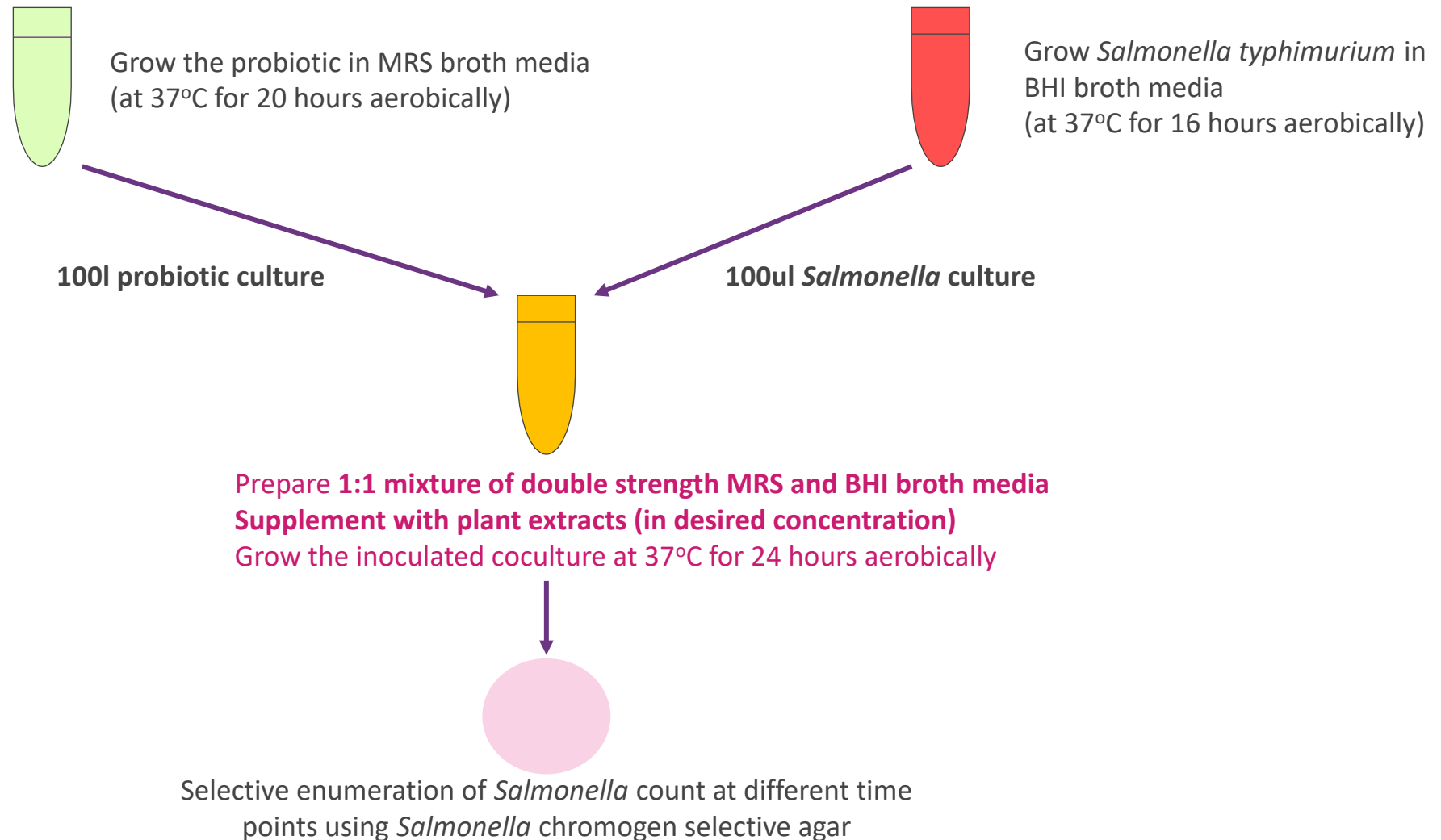
A_{prob} = OD₆₀₀ of probiotic suspension alone

A_{sal} = OD₆₀₀ of *Salmonella* suspension alone

Co-culture assay



Co-culture assay - with plant extracts



Scientific problem

