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Introduction

Buffered Peptone Water (BPW) is commonly used as a non-selective pre-enrichment broth improving the chances for recovering low numbers of sublethally injured *Salmonella* before being transferred to a selective enrichment. A high level of background flora in food can be a serious challenge for the successful detection of *Salmonella* due to an overgrowth of non-target bacteria that might interfere with the performance of the assays using RT-PCR and ELISA-based methods.

This study was aimed on the development of a selective supplement enabling an efficacious control of high levels of background flora without affecting the recovery and growth of low numbers of sublethally injured *Salmonella*.

Study Design

Selective Supplement Optimization



- Pre-study**
 - Using BPW as a base broth
 - Forecast of selective molecules
 - Selection of molecules
- Factorial Design**
 - Variation of molecules concentration
 - Media 1 to 11
 - In vitro* monocultures
 - In situ* matrix studies
- Steepest Ascent Method**
 - Optimization of molecules concentration
 - Media A to G
 - In vitro* monocultures
 - In situ* matrix studies

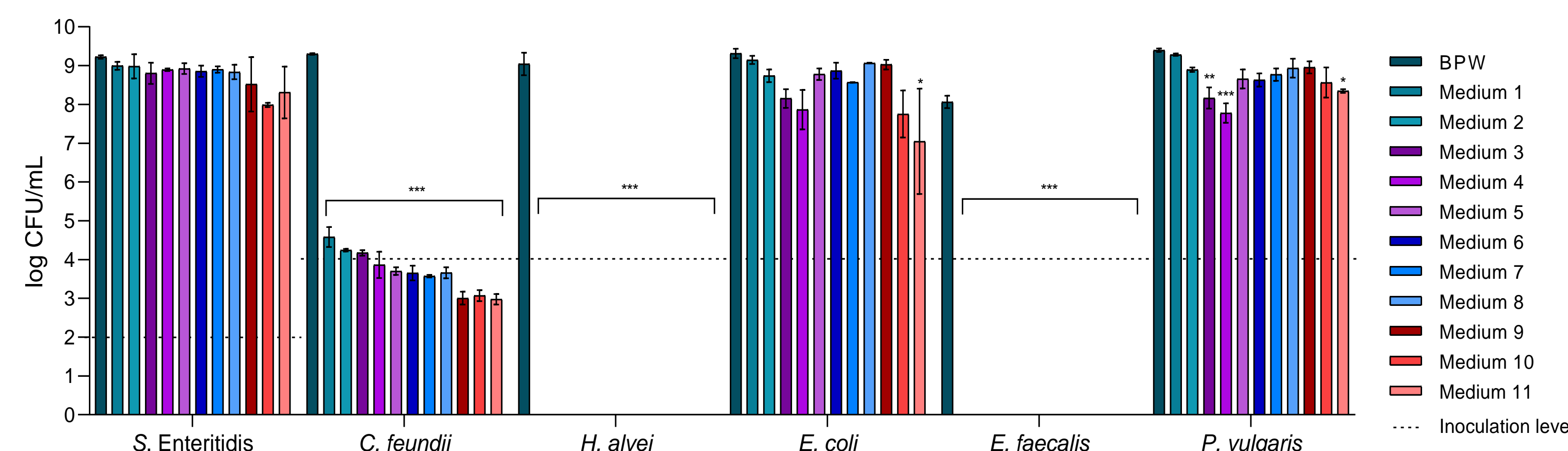
Effect of Selective Supplement Formulations on Growth and Recovery of *Salmonella*

Culture study

Experimental design. A factorial design (FD) with two levels was implemented to assess the effect of three selective molecules on the growth and recovery of sublethally injured *Salmonella*.

11 formulations corresponding to BPW supplemented with eight different combinations of the selective molecules were tested *in vitro*.

Fig. 1. Growth of Sublethally Injured *S. Enteritidis* and Competitive Bacteria for 16h at 35°C



Notes : *P<0.05 ; **P<0.01 ; ***P<0.001, as compared to BPW.

- Different formulations of selective molecules showed partial to complete inhibition of competitors growth without affecting sublethally injured *S. Enteritidis*.

Matrix study

Experimental Design. Those formulations were tested for the recovery of low number of *S. Enteritidis* from artificially contaminated raw ground beef.

Table 1. Enrichment Conditions and Inoculating Strain

Food Matrix	Strains (serogroup)	CFU / Sample	Sample Dilution	Sample Volume	Temperature	Time	Normal Flora (CFU/g)
Raw Ground Beef	<i>S. Enteritidis</i> (D1)	0,6	1:10	2 mL	35°C	16h	3.85 x 10 ⁴

Table 2. Recovery of *Salmonella* in Selective Formulations Designed by FD

Media	Positive Outcomes		Recovery level (log ₁₀ CFU/mL)
	direct plating	secondary enrichment	
1	6 / 16	8 / 16	6.34 ± 1.03
2	11 / 16	11 / 16	7.16 ± 1.43
3	6 / 16	11 / 16	5.32 ± 2.16
4	5 / 16	10 / 16	5.30 ± 2.34
5	7 / 16	9 / 16	6.85 ± 1.46
6	7 / 16	8 / 16	6.92 ± 1.56
7	10 / 16	11 / 16	7.04 ± 1.41
8	9 / 16	11 / 16	7.30 ± 0.85
9	5 / 16	7 / 16	7.00 ± 0.89
10	9 / 16	11 / 16	6.52 ± 1.81
11	6 / 16	7 / 16	7.19 ± 1.91
BPW	4 / 16	10 / 16	5.85 ± 1.45

Table 3. Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate
Intercept	35.94
Selective Molecule 1	1.56
Selective Molecule 2	-20.31
Selective Molecule 3	-4.69
Selective Molecules 1*3	-14.06

- The three selective molecules showed a significant effect on the recovery of *S. Enteritidis* (P<0.05).
- An interaction effect on the recovery of *S. Enteritidis* was observed between selective molecules 1 and 3.

Statistical Optimization of Selective Supplement Formulation

In situ study

Experimental design. Using the mathematical model developed in FD phase, new combinations of selective molecules, designed by steepest ascent method (SAM) were tested for the recovery of *S. Enteritidis* from raw ground beef.

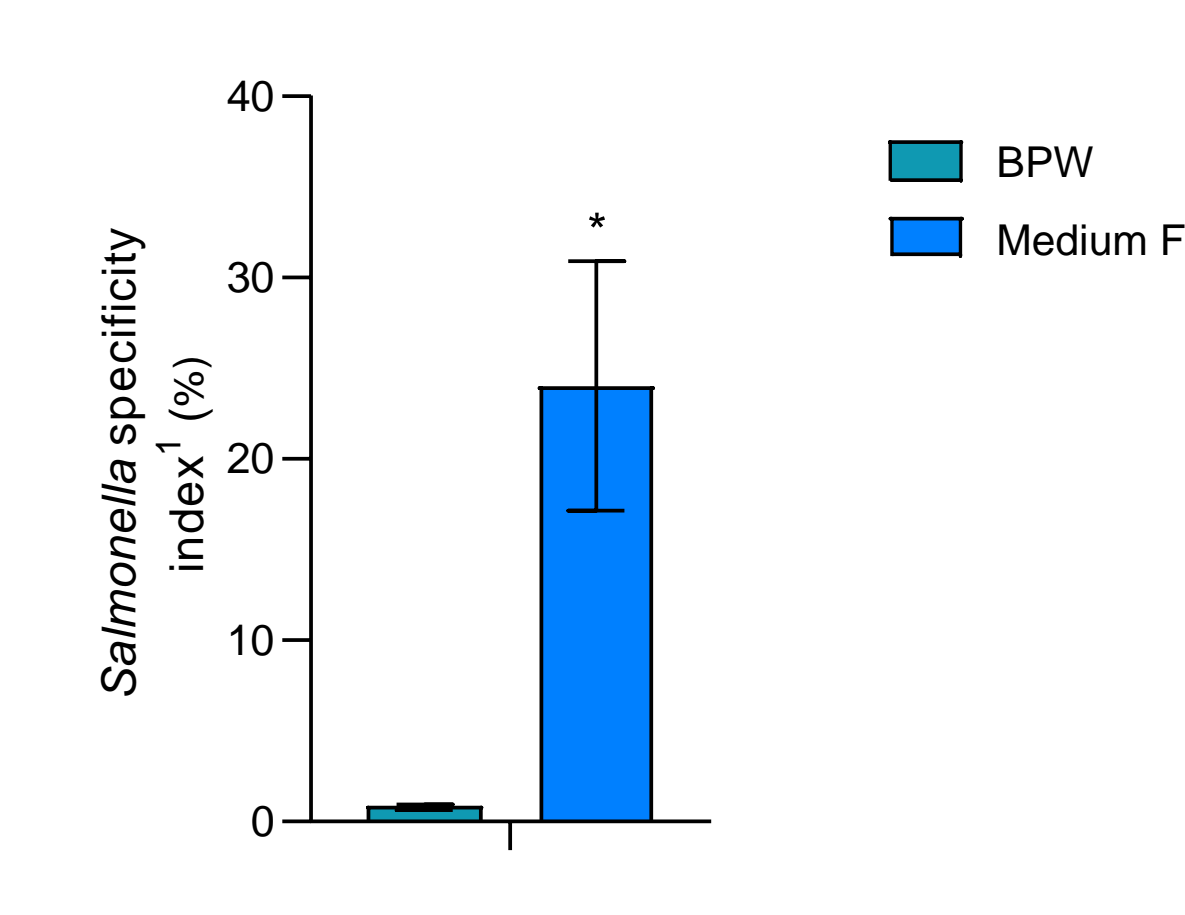
Table 3. Enrichment Conditions and Inoculating Strain

Food Matrix	Strains (serogroup)	CFU / Sample	Sample Dilution	Sample Volume	Temperature	Time	Normal Flora (CFU/g)
Raw Ground Beef	<i>S. Enteritidis</i> (D1)	0.7	1:10	2 mL	35°C	16h	1.58 x 10 ⁴

Table 4. Recovery of *Salmonella* in Selective Formulations Designed by SAM

Media	Raw Ground Beef		Recovery level (log ₁₀ CFU/mL)
	direct plating	secondary enrichment	
A	11 / 24	12 / 24	8.64 ± 0.70
B	10 / 24	10 / 24	8.71 ± 0.38
C	8 / 24	9 / 24	8.22 ± 1.12
D	9 / 24	9 / 24	8.55 ± 0.40
E	9 / 24	11 / 24	8.31 ± 0.97
F	13 / 24	13 / 24	8.21 ± 0.27
G	12 / 24	15 / 24	7.39 ± 0.41
BPW	12 / 24	13 / 24	8.03 ± 0.70

Fig. 2. Specific Growth of *Salmonella* in Raw Ground Beef Samples



Notes : *P<0.05, as compared to BPW.

¹Salmonella specificity index:

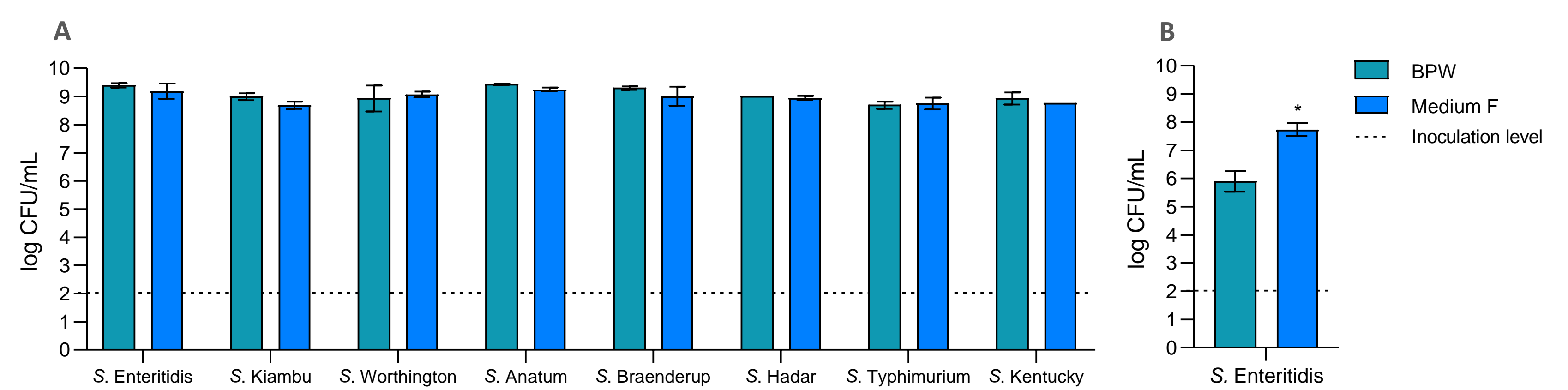
$$\frac{\text{Number of viable } Salmonella \text{ cells}}{\text{Number of total viable microbial cells}}$$

- Medium F showed no false negative after direct plating.
- Recovery level of *Salmonella* and inhibition of background flora growth were higher than BPW.

Inclusivity and Mixed Culture Study

Experimental Design. The most efficient formulation of selective molecules, medium F, was tested with monocultures of uninjured *Salmonella* strains and with *S. Enteritidis* cultured in the presence of high levels of competing bacteria including *E. coli*, *C. freundii*, *H. alvei*, *E. faecalis*, *P. vulgaris* (1:100 ratio).

Fig. 3. Growth of *Salmonella* in Monocultures (A) and Mixed Culture (B) for 16h at 35°C



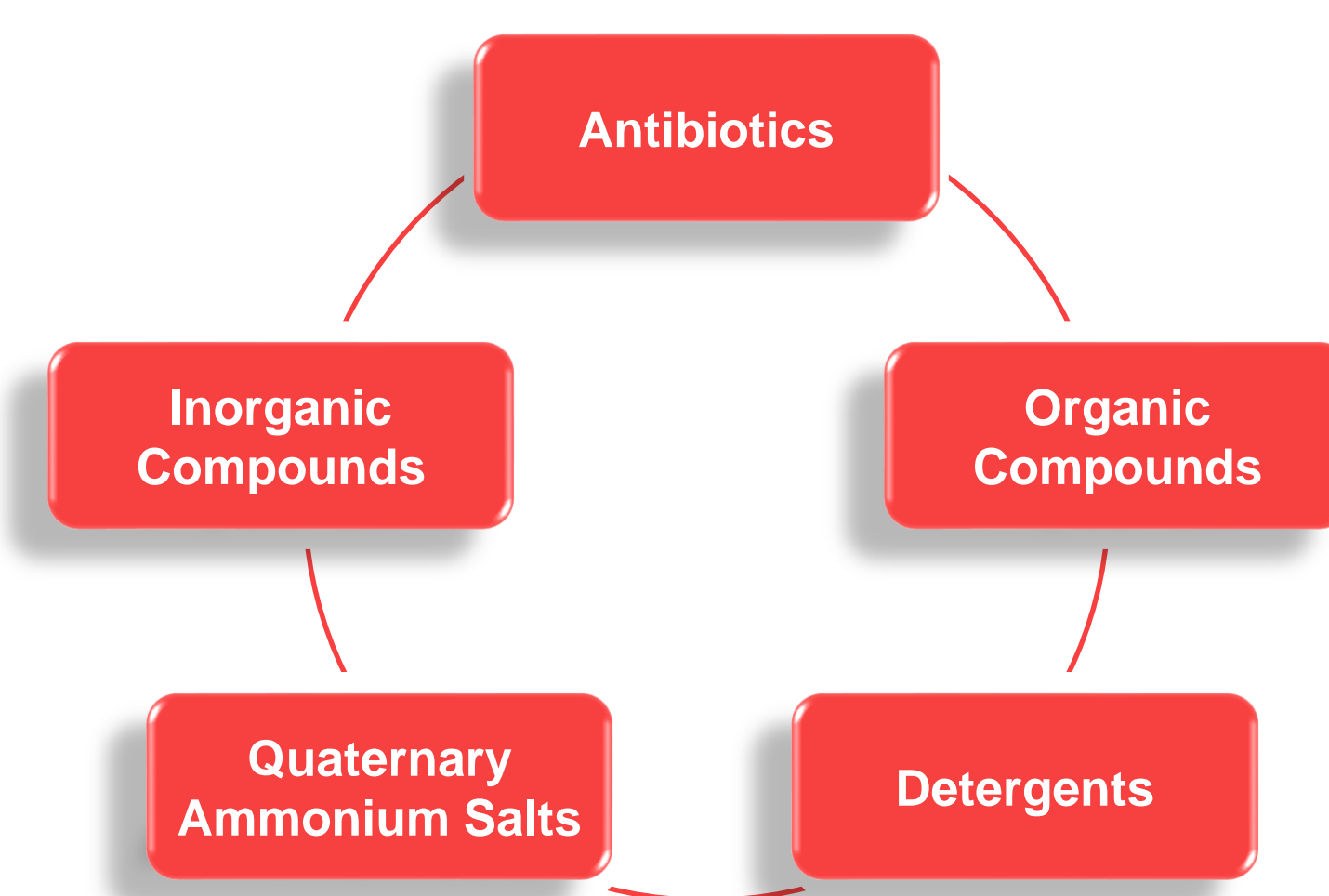
Notes : *P<0.05, as compared to BPW.

- Selective supplement formulation F did not affect growth of different *Salmonella* serovars in monocultures.
- Growth of *S. Enteritidis* in the presence of competitive bacteria was significantly better when selective supplement formulation F was used.

Conclusion

- Three selective molecules were combined together to develop the selective supplement allowing the control of competing bacteria without affecting growth of injured and uninjured *Salmonella* strains.
- BPW, supplemented with the optimized selective formulation, resulted in an improved growth of *Salmonella* in food with high levels of background flora when compared to the un-supplemented BPW.

Nature of Tested Selective Molecules



Matrix Methodology Design

