Probiotics for cattle reared in extreme climate

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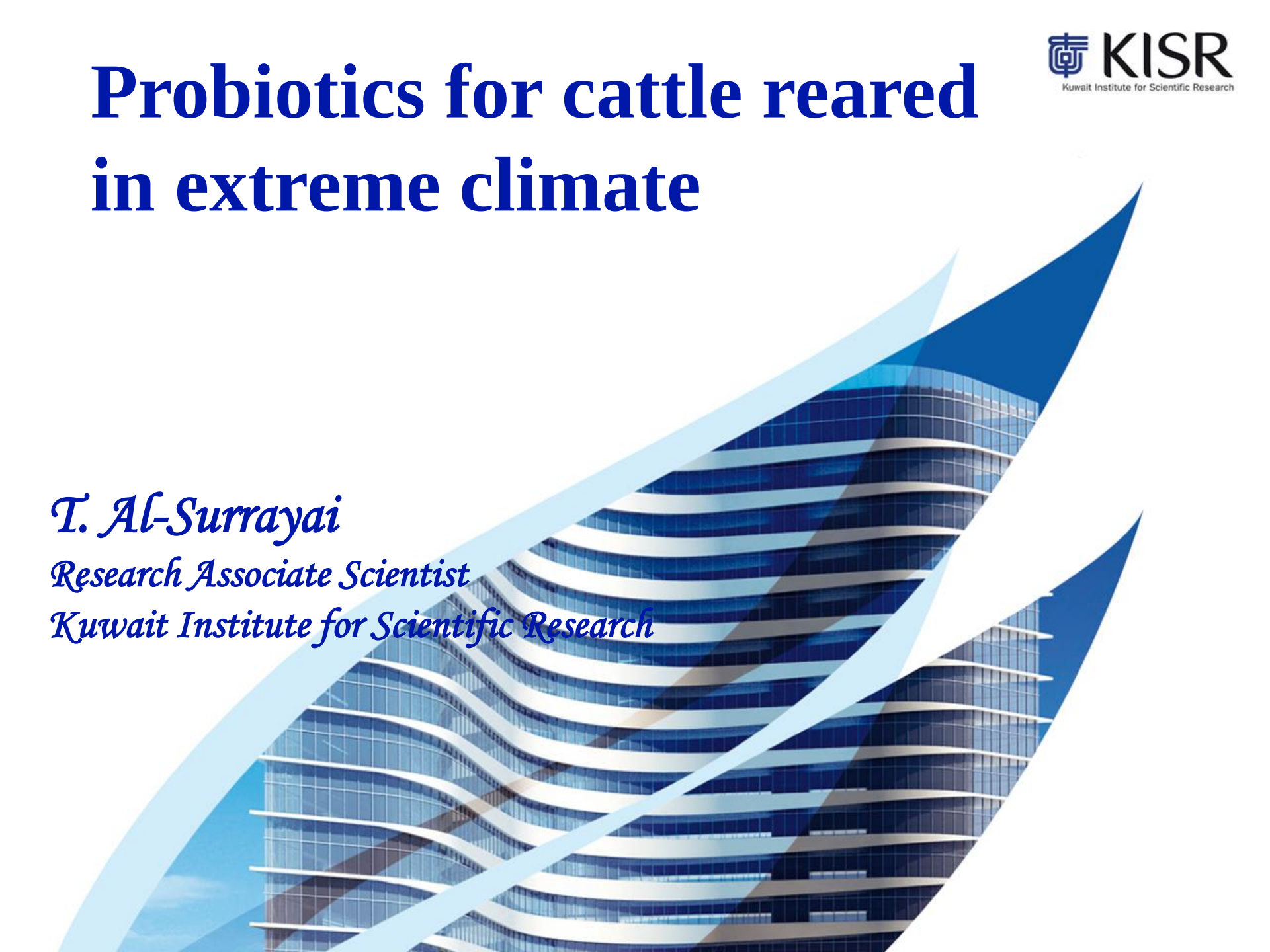


Introduction

- Kuwait has an extreme climate:
  - In summer > 50^{o}C
  - In winter < 4^{o}C
  - Dusty throughout the year.
- Grazing is impossible due to the lack of vegetation.
- Dairy farmers in Kuwait use closed-type housing.
- Stress on the animal

Introduction

- Dairy farmers in Kuwait are facing the problem of a high calf mortality rate.
- Major cause of mortality are:
  - Enterotoxaemia
  - Diarrhea
  - pasteurella pneumonia.
- Diarrhea is still the most common and costly disease affecting calf.

Introduction

- Large quantities of antibiotics are fed to animals to control diseases.
- Antibiotics are killing all bacteria (harmful and useful).
- Producing antimicrobial resistant bacteria that can cause disease in both animals and humans.

What is Probiotics?

- A Probiotic is a living microbial feed supplement, which beneficially effect the host animal by improving its intestinal microbial balance (Fuller et al., 2004).
- Probiotics as supplements are used widely in farm livestock associated with reducing pathogen load and increasing growth rates (Collins et al., 2009).

Cattle probiotics

The Fast growing demands for probiotics in production was due to:

1. The urgent need for safe and cost effective alternatives to antibiotic growth promoters.
2. The stricter regulation controlling the use of antibiotics.
3. The increase in cattle production due to the real need of meat consumption.

Claimed benefits of probiotics

- Increased growth rate and meat production.
- Protection against infectious diseases due to stimulation of immunity.
- Reduction of mortality rate.
- Improved milk yield and quality.
- Improved food utilization.

Methods

- Sample Collection Preparations.
  - Four different seasons.
  - Samples collected from intestinal tissue, milk, and feces of cows
- Enrichment Experiments.
- Isolation, Characterization, and Identification of Lactic Acid Bacteria (LAB).

Methods

- Biochemical Evaluation Tests.
  - Antagonistic Activity
  - Tolerance to Acidic pH
  - Bile Resistance
  - Resistance to Antibiotics
  - Bacterial Attachment Measurements
  - Quantitative Determination of Bacteriocin
  - Aggregation and Co-Aggregations Tests
    - Adhesion Activity of LAB Strains to Caco-2 and HT-29 Cells
    - Mucus Adhesion Assay

Results and Discussions

- Effect of Seasonal Changes on LAB Strains.
  - LAB strains belong to 17 strains of *Lactobacillus*, *Weisella*, *Enterococcus*, and *Pediococcus*.
  - *Lactobacillus plantarum* is the most dominant LAB isolate from the four seasons ≈ 40% from the total isolates.
  - Ten different species of isolated bacterial strains were chosen for the evaluation

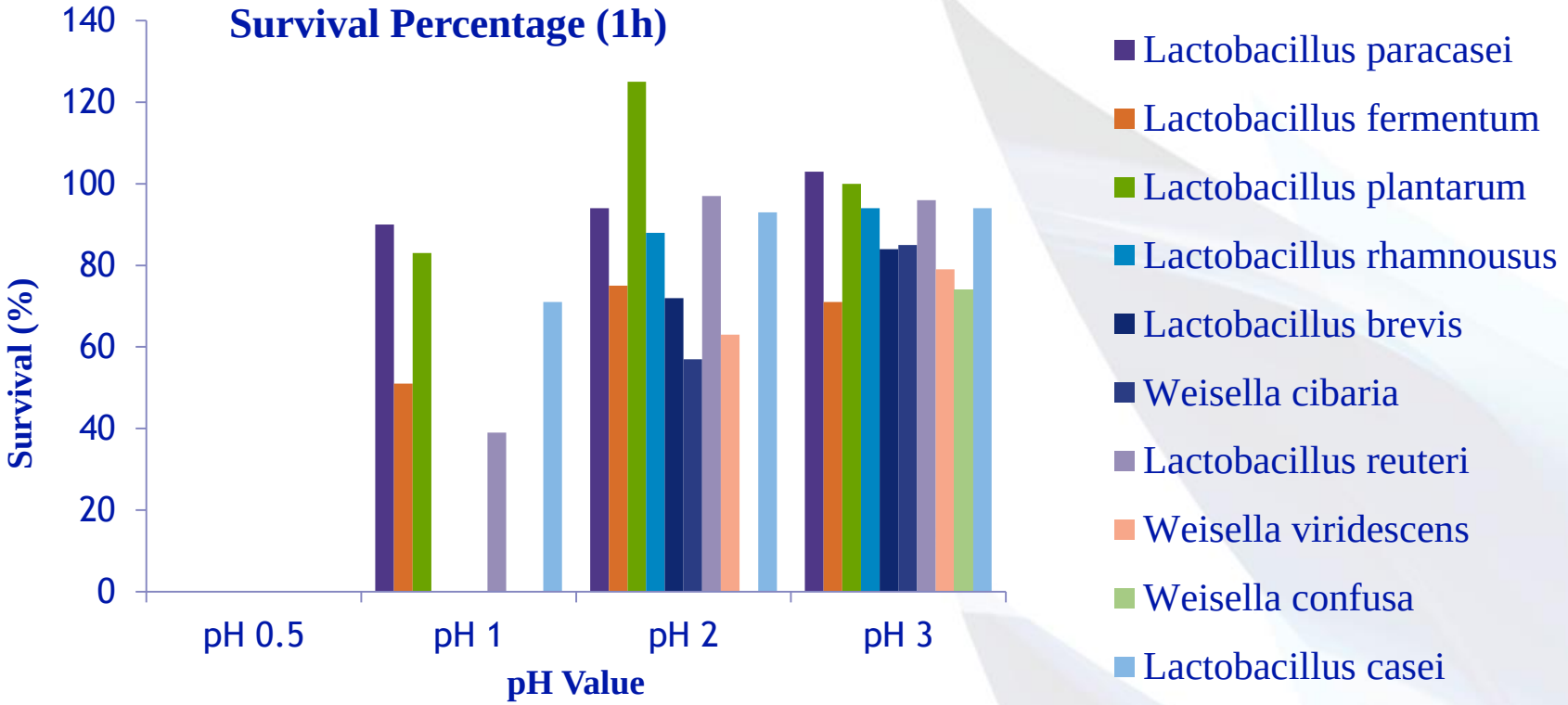
Selected Bacterial Strains Isolated from All Seasons

Strain Code	Strain Name	Strain Season
W5	*Lactobacillus paracasei*	Winter
W26	*Lactobacillus fermentum*	Winter
W32	*Lactobacillus plantarum*	Winter
W37	*Lactobacillus rhamnousus*	Winter
W44	*Lactobacillus brevis*	Winter
W40	*Weisella cibaria*	Winter
SP1	*Lactobacillus reuteri*	Spring
SP7	*Weisella viridescens*	Spring
SP19	*Weisella confusa*	Spring
S24	*Lactobacillus casei*	Summer

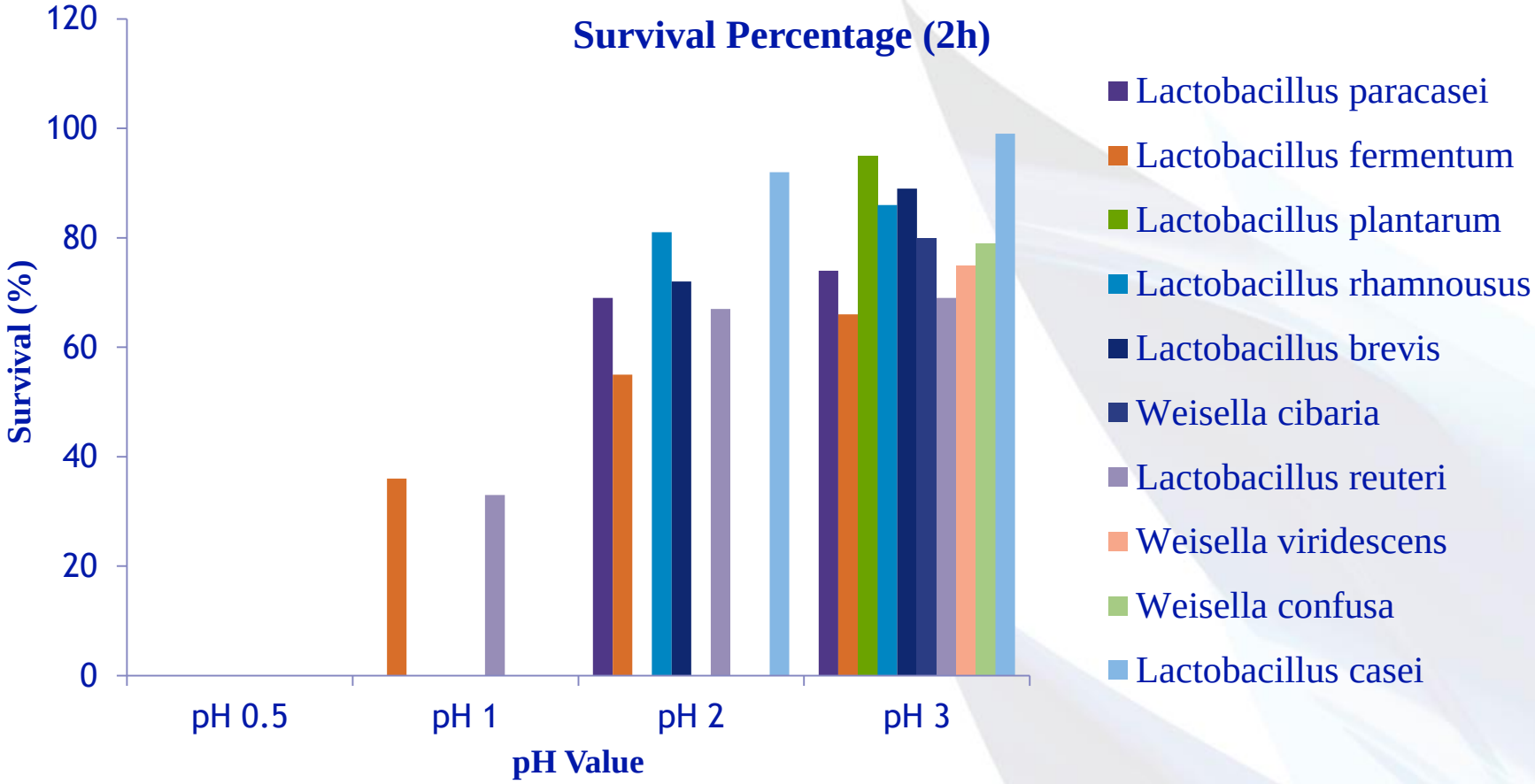
Antagonistic Activity of LAB (Lactic acid bacteria) Against Selected Pathogens

Strain Name	*Escherichia coli*	*Salmonella enterica*	*Salmonella spp.* (from cattle tissue)
	Diameter of Inhibition Zone (cm)		
L. paracasei	1.5	2.45	1.9
L. fermentum	0.7	2.15	2.05
L. plantarum	2.55	3.0	2.25
L. rhamnousus	1.75	3.4	3.0
L. brevis	1.05	2.0	1.45
Weisella cibaria	1.15	2.15	1.6
L. reuteri	2.0	0.9	1.75
Weisella viridescens	0.5	1.85	1.75
Weisella confusa	1.3	2.0	2.2
L. casei	1.4	2.5	2.35

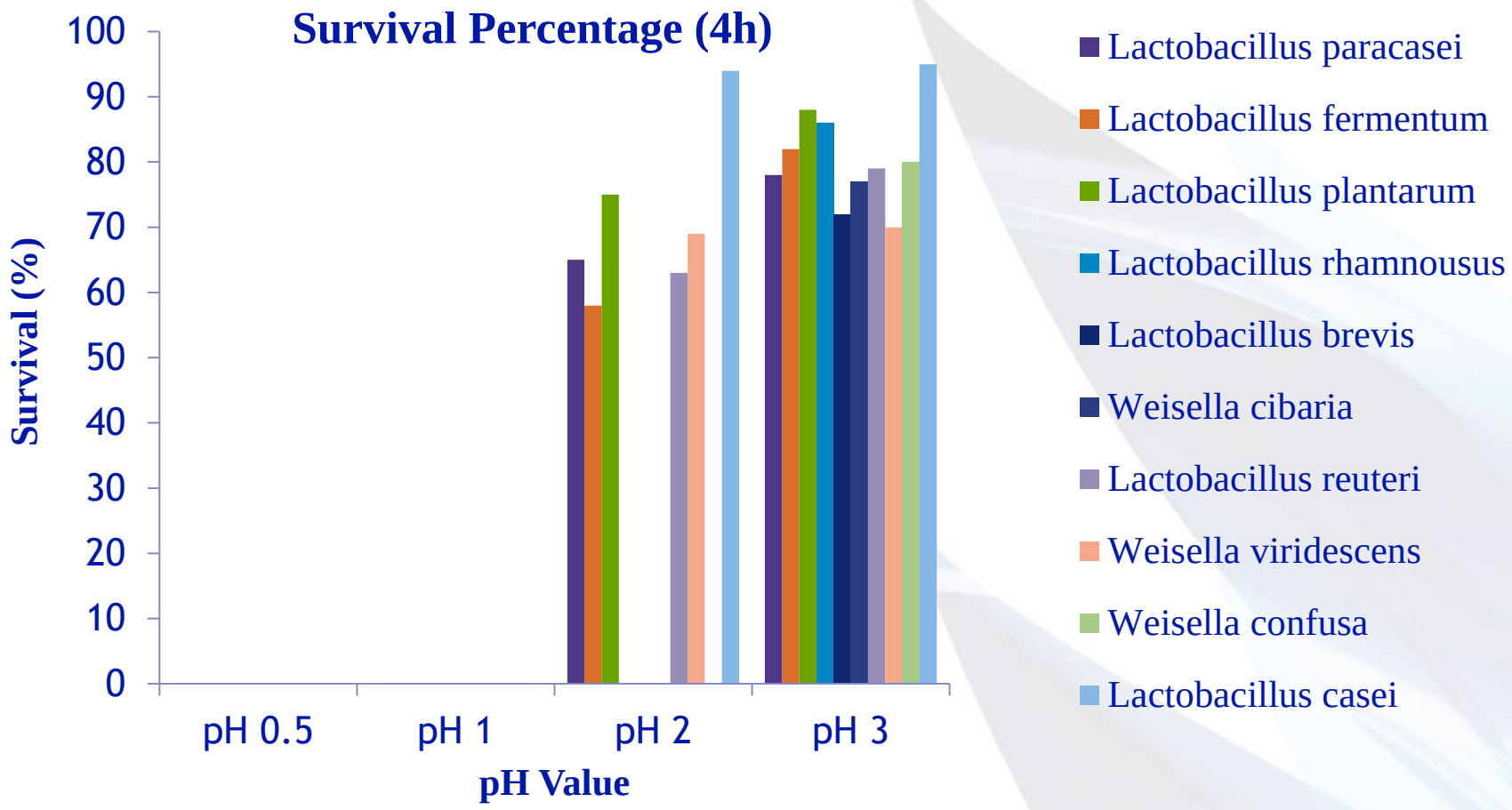
Survival percentage for selected LAB at different pH values after 1 h of incubation



Survival percentage for selected LAB at different pH values after 2 h of incubation



Survival percentage for selected LAB at different pH values after 4 h of incubation



Tolerance of LAB Isolates to Bile Salts

Strain Name	Survival Percentage *								
	0 h			2 h			24 h		
	0%	0.5%	2.0%	0%	0.5%	2.0%	0%	0.5%	2.0%
Lactobacillus paracasei	100	92	0	102	101	0	149	99	0
Lactobacillus fermentum	100	94	91	100	83	63	113	89	90
Lactobacillus plantarum	100	73	85	100	0	0	119	0	0
Lactobacillus rhamnousus	100	0	0	106	0	0	115	0	0
Lactobacillus brevis	100	101	98	101	127	97	115	104	103
Weisella cibaria	100	100	97	108	125	107	134	122	115
Lactobacillus reuteri	100	97	92	99	102	96	153	117	100
Weisella viridescens	100	108	0	100	95	0	117	80	0
Weisella confusa	100	110	93	101	79	86	121	107	87
Lactobacillus casei	100	97	96	123	107	99	133	138	124

Microbial Inhibitory Concentration of LAB Strains on MRS Agar

Strain ID	Erythromycin (15 µg)	Streptomycin (300 µg)	Chloramphenicol (30 µg)	Neomycin (5 µg)	Penicillin G (10 µg)
Lactobacillus paracasei	S	R	S	R	S
Lactobacillus fermentum	S	R	S	R	MS
Lactobacillus plantarum	S	R	S	R	R
Lactobacillus rhamnousus	S	R	S	R	S
Lactobacillus brevis	S	R	S	R	R
Weisella cibaria	S	R	S	R	R
Lactobacillus reuteri	S	R	R	R	R
Weisella viridescens	S	R	MS	R	MS
Weisella confusa	S	R	S	R	R
Lactobacillus casei	S	R	S	R	R

S : Sensitive; MS: Moderate Susceptible; R: Resistant; LAB: Lactic Acid Bacteria

Adhesion and Attachment Test of LAB

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Strain Name	Adhesion to HT-29	Adhesion to Caco-2	Attachment to Mucin
Lactobacillus paracasei	+	+	++
Lactobacillus fermentum	++	+	+++
Lactobacillus plantarum	+	++	+++
Lactobacillus rhamnousus	++	++++	-
Lactobacillus brevis	-	++	-
Weisella cibaria	-	+	+++
Lactobacillus reuteri	-	++	+
Weissella viridescens	+++	++	-
Weissella confusa	++	+	-
Lactobacillus casei	+++	++++	-

*+++ = highly adhesive (compared to the control); ++ = moderate adhesion; + = low adhesion;
- = very low or non-adhesive

Discussion and Conclusion

- In this study, the probiotic potential of some LAB isolates in controlling enteropathogens, such as *Salmonella spp* and *E. coli*, was investigated.
- It was strongly suggested that the strain of *L. fermentum* is the most probable candidate for probiotic use, possibly coupled with *L. casei*. Also, *L. plantarum* and *L. rhamnosus* could be included in the list of potential probiotic.

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