Probiotics for the Use in the Development of cattle production in Kuwait

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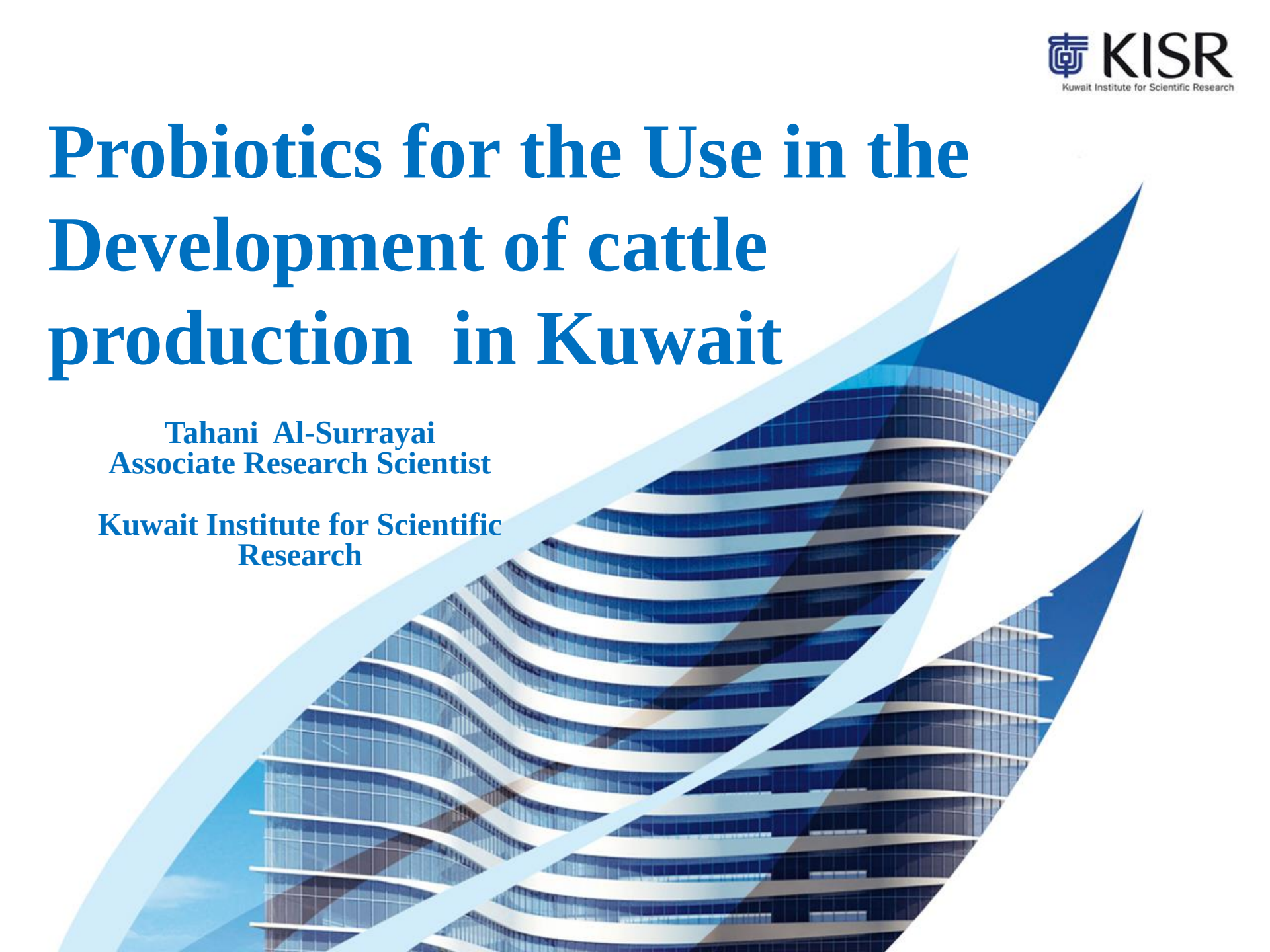


Introduction

Dairy farms in Kuwait are managed in an intensive system due to:

1. **An extreme arid environment.**
2. **Closed-type housing environment**

The unnatural rearing conditions of calves & diets

⇓

induce stress

⇓

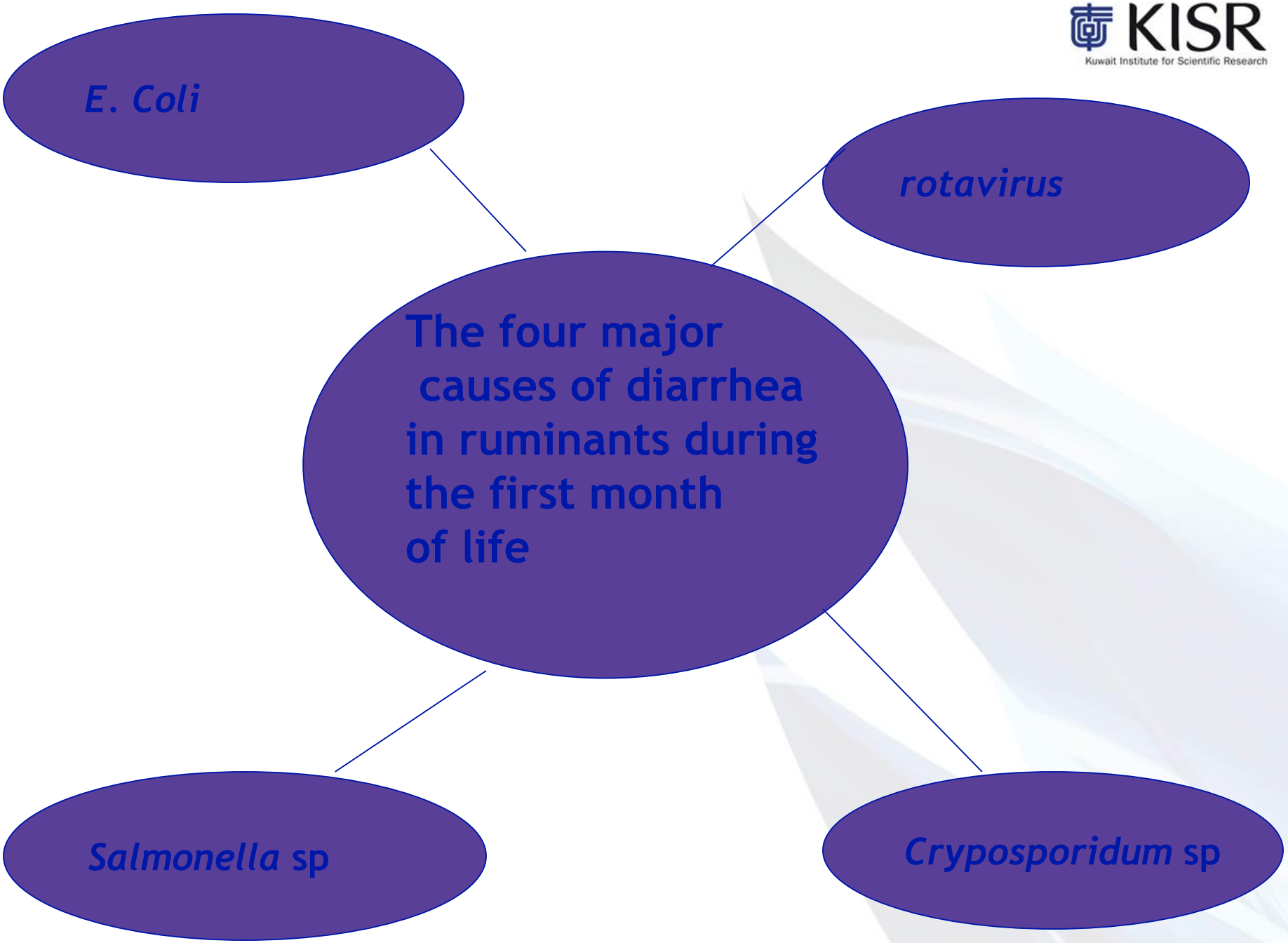
changes in the composition of the gut micro flora

⇓

affect the animals' resistance to infection

- **Major cause of mortality are:**
  - **Enterotoxaemia,**
  - **diarrhea and**
  - **pasteurella pneumonia.**

- **Diarrhea is still the most common and costly disease affecting ruminants.**


E. Coli
rotavirus
The four major causes of diarrhea in ruminants during the first month of life
Salmonella sp
Cryposporidum sp

- **Large quantities of antibiotics are fed to animals to control diseases.**

- **Antibiotics are killing all bacteria (harmful and useful) affecting the digestion of grain and nutrient absorption.**

- **There is real danger that is producing antibiotic resistant bacteria that can cause disease in humans and animals.**

What is probiotic?

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- **A Probiotic is a living microbial feed supplement, which beneficially effect the host animal by improving its intestinal microbial balance" Fuller, 2004.**

- **Probiotics can replace the chemical growth promoters for farm animals and increase resistance to disease.**

- **Commercial probiotic products typically contains *Lactobacillus* species, *Bifidobacterium* species and yeast.**

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.**

Objectives

- **The Isolation and evaluation of probiotic lactic acid bacteria (LAB) during four seasons for utilization as an alternative to antibiotics in cattle production to control enteric pathogens, enhance productivity and improve food safety.**

Methodology

I. Sample Collection and Microbial Isolation, Characterization and Identification of LAB

1. Seasonal collection of healthy samples from the Ruminant Digestive System including gut content and also cattle bi-product (30 samples per season).
2. Microbial isolation using selective media (MRS) and characterization of the intestinal LAB.
3. Identification of the isolated LAB strains by 16S rRNA -Polymerase Chain Reactions (PCR)-sequencing).

II. Screened The probiotic potential in vitro for selected strains by the following biochemical analysis:

1. Antagonistic Activity Assessment.
2. Assessment to tolerate Acidic pH (1-3).
3. Assessment to Bile Resistance (0.2, 0.3, 0.4, 2.0% Oxgall).
4. Assessment to tolerance to Antibiotics (neomycin, erythromycin, penicillin G, streptomycin and chloramphenicol).
5. Aggregation Assessment.
6. Bacteriocins Assessment.
7. Bacterial hydrophobicity, attachment to epithelial cells.

Results

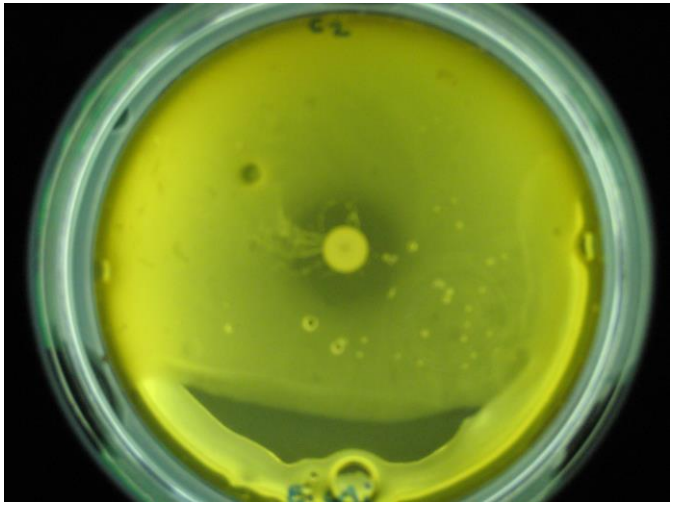
1. **Isolation of 263 presumptive lactobacilli and the effect of seasonal changes on LAB strains was considered.**
2. **80 were confirmed to belong to the lactic acid bacteria group by means of molecular tools.**
3. **Ten representative strains were chosen and screened for their probiotic potential in vitro.**

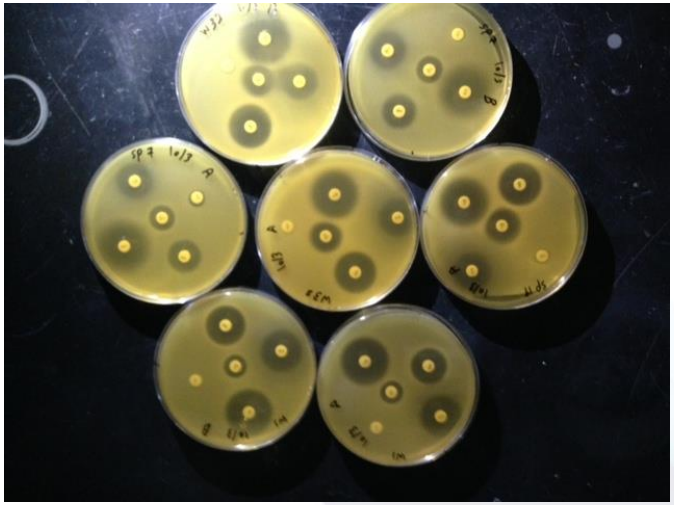
Phylogram tree

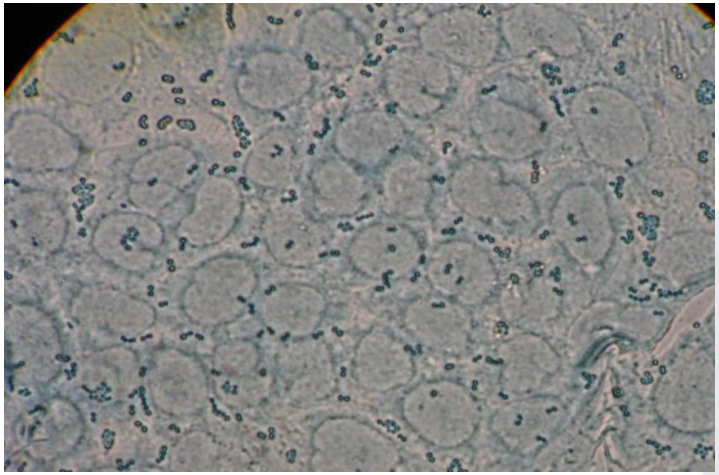
The selected strains

- ***Lactobacillus paracasei***
- ***Lactobacillus fermentum***
- ***Lactobacillus plantarum***
- ***Lactobacillus rhamnousus***
- ***Lactobacillus brevis***
- ***Weisella cibaria***
- ***Lactobacillus reuteri***
- ***Weissella viridescens***
- ***Weissella confusa***
- ***Lactobacillus casei***

The biochemical analysis results







1. Antagonistic Activity of LAB Against Selected Pathogens

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

Table 2. 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	0
Weisella cibaria	100	100	97	108	125	107	134	122	103
Lactobacillus reuteri	100	97	92	99	102	96	153	117	115
Weisella viridescens	100	108	0	100	95	0	117	80	100
Weisella confusa	100	110	93	101	79	86	121	107	0
Lactobacillus casei	100	97	96	123	107	99	133	138	87

Table 3. Microbial Inhibitory Concentration of LAB Strains on MRS Agar

Strain Name	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

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

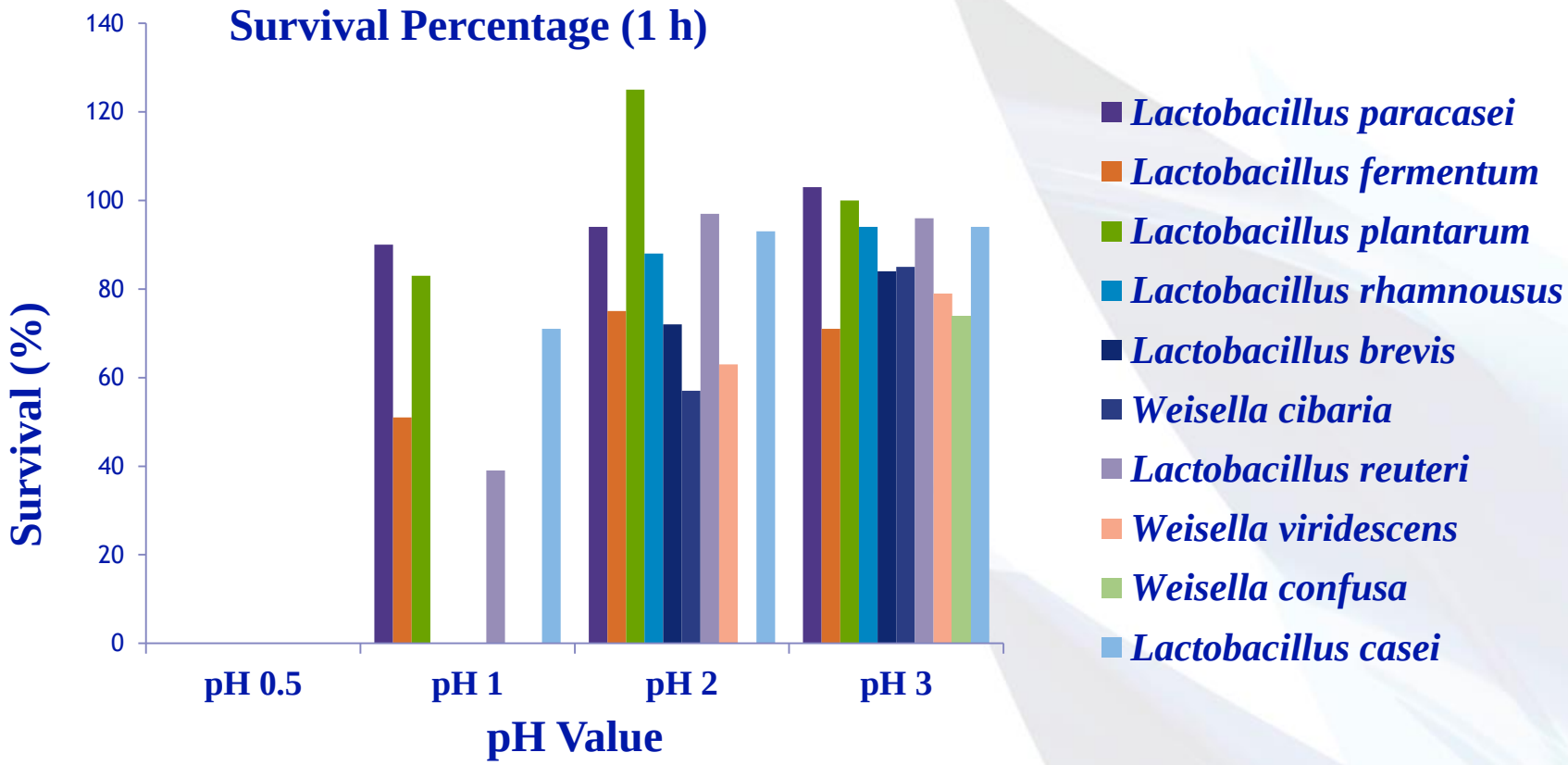


Fig 2. Survival percentage for selected LAB at different pH values after 2 h of incubation

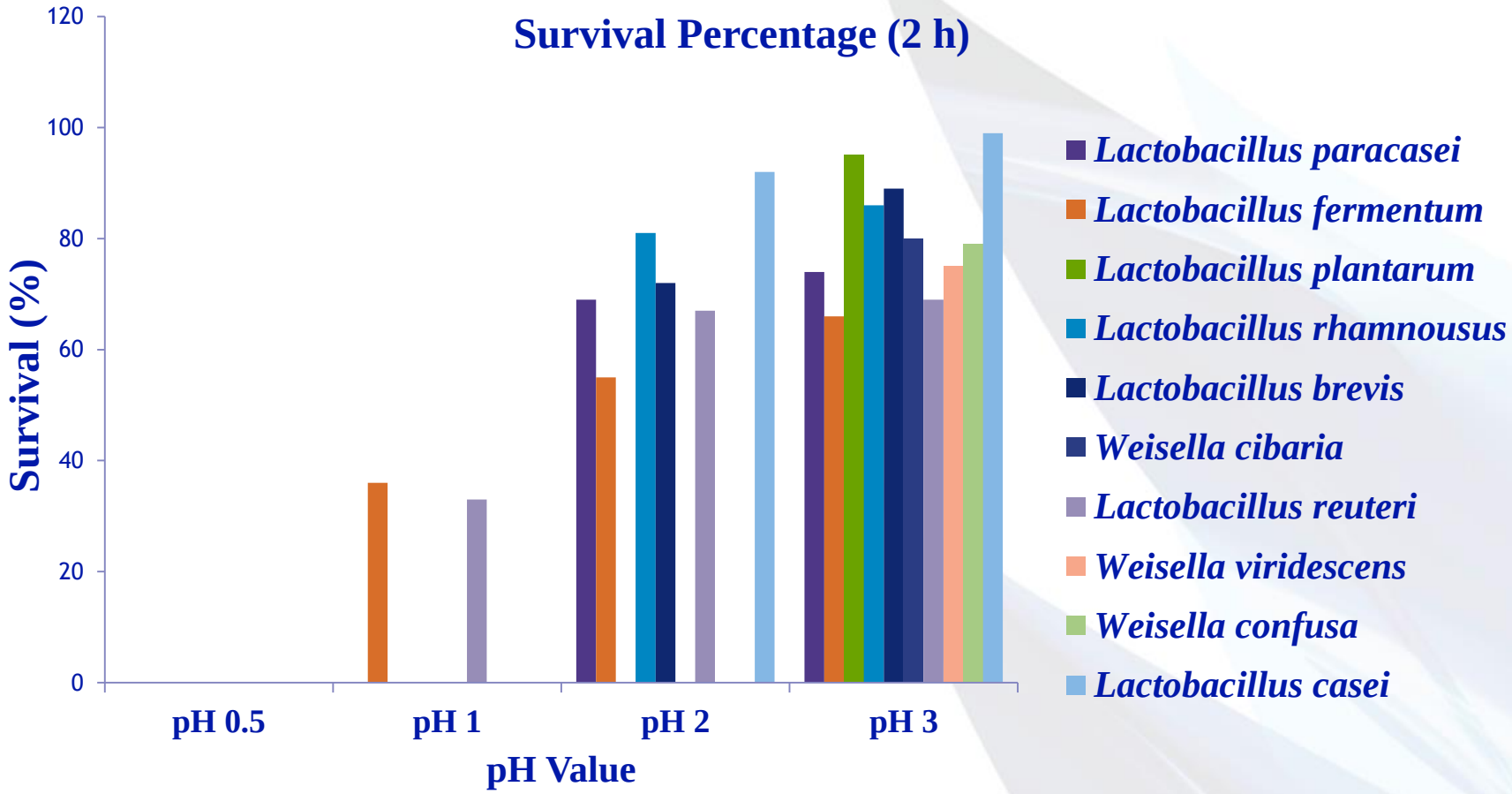
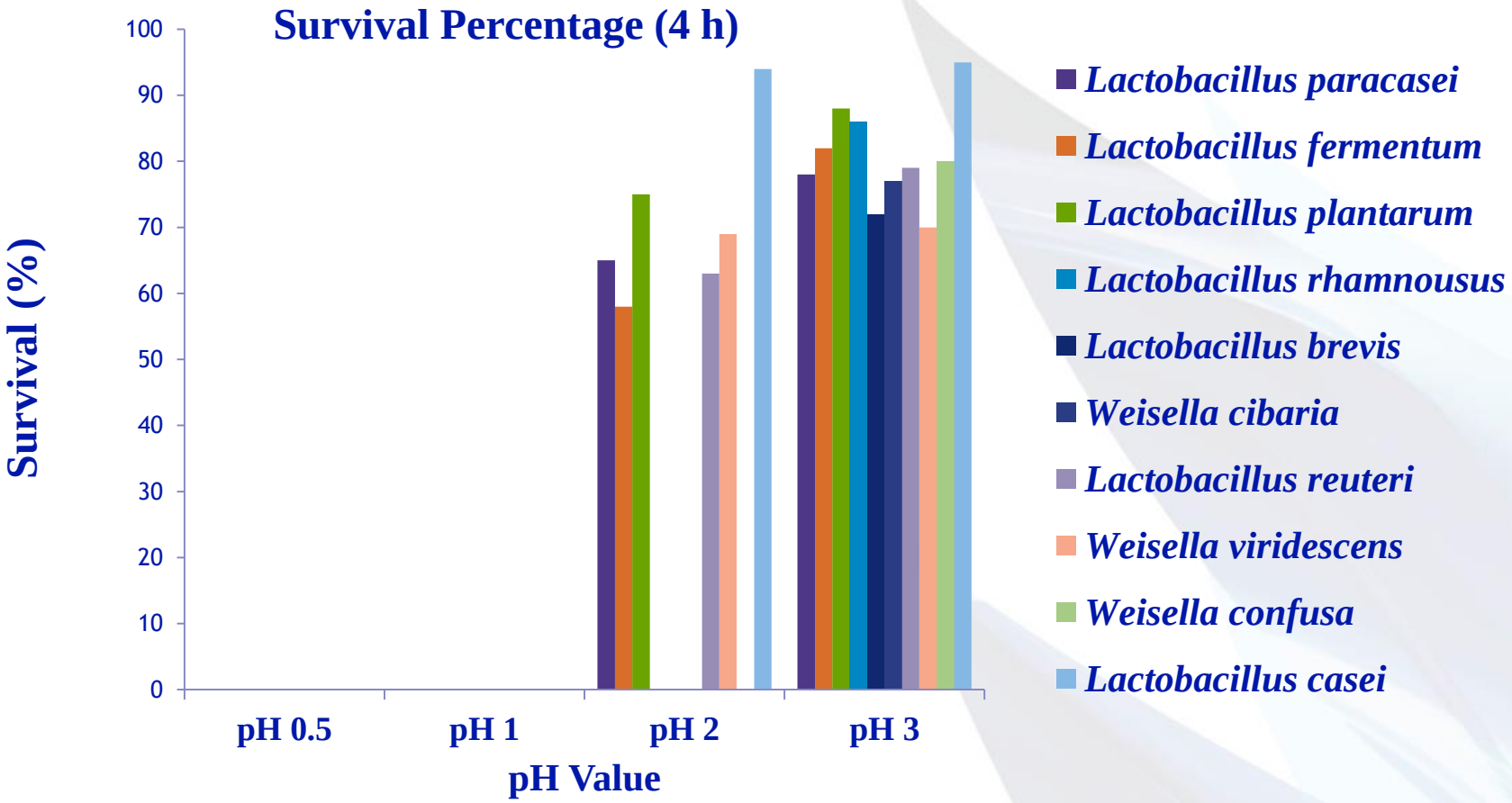


Fig 3. Survival percentage for selected LAB at different pH values after 4 h of incubation.



Conclusion

- **Active LAB strains were isolated such as *L. fermentum, L. rhamnousus* and *L. reuteri.***
- **The selected strains can strongly inhibit the growth of some effective pathogen (*Salmonella* spp *and E. coli*), can tolerate low pH and the bile salts and had highest percentage of adhesion that similar to the condition in the animal stomach.**

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Thanks