

**Isolation and Characterization of  
Lactic Acid Bacteria  
for Use in the Development of  
Probiotics for Sheep Production in  
Kuwait**

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# Introduction

**Mortality rate in the commercial farms:**

❖ **12.2 % for adult sheep**

❖ **32.4 % young lamb**

❖ **Major cause of mortality are:**

- 1. Enterotoxaemia,**
- 2. diarrhea and**
- 3. pasteurella pneumonia.**

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

**The four major  
causes of diarrhea  
in ruminants during  
the first month  
of life**

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graph TD; A([The four major causes of diarrhea in ruminants during the first month of life]) --- B([E. Coli]); A --- C([rotavirus]); A --- D([Cryposporidium sp]); A --- E([Salmonella sp]);
```

*E. Coli*

*rotavirus*

*Salmonella sp*

*Cryposporidium 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?

- ❖ **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.**



# Materials and Methods

- **LAB Isolation and Enrichment**
- **Characterization and Identification of LAB.**
- **Determination of Antagonistic Activity of LAB Using In Vitro Tests .**
- **Determination of LAB Tolerance to Acidic pH**
- **Bacterial Adhesion to hydrocarbons.**
- **Quantitative Evaluation of Bacteriocin by Lactic Acid Bacteria against *Salmonella enteritidis*.**

# LAB Isolation and Enrichment

## Sample Collection and Preparations.

Sample No	Code	Sample Source	Sample Description
1	A1	Rumen	Five- month old
2	A2	Abomasum	Five -month old
3	A3	Reticulum	Five -month old
4	A4	Duodenum	Five -month old
5	A4S	Duodenum	Five -month old
6	A5	Jejunum	Five -month old
7	A5S	Jejunum	Five -month old
8	A6	Caecum	Five -month old
9	A7	Rectum	Five -month old
10	B1	Rumen	One- year old
11	B2	Abomasum	One -year old
12	B3	Reticulum	One -year old
13	B6	Caecum	One -year old
14	B7	Rectum	One -year old
15	E M	ewe Milk	One -week ewe breed
16	F	Feces	five -month sheep

# Results and Discussion

- **Characterization and Identification of LAB**

Sample No	Code	Shape	Gram Stain	Bacterial ID	16S rRNA Sequence I.D.
1	A5	Oval Shape	G +ve	Lactobacillus acidophilus	Enterococcus faecium
2	A5S	Oval Shape	G +ve	Lactobacillus brevis	Pedicoccus pentosaceus
3	A7	Rodo Cocci	G +ve	Lactobacillus salivarius	Pediococcus pentosaceus
4	E M	Oval Shape	G +ve	Lactobacillus plantarum	Enterococcus faecium

# Antagonistic Activity of LAB Using *In Vitro* Tests:

- . Isolated salmonella strains was *Salmonella enterica*.
- . Clear zone < 1 mm scored as positive.

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Sample No	Code	Bacterial ID	Salmonella enteritidis	Salmonella enterica	E. coli
1	A5	Enterococcus faecium	1	1	0
2	A7	Pediococcus pentosaceus	1.5	2	1

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# LAB Tolerance to Acidic pH

**pH  
Value**

**Incubation Time in hrs**

Enterococcus faecium

Pedicoccus pentosaceus

0hr

1hr

2 hrs

4 hrs

0hr

1hr

2 hrs

4 hrs

pH 0.5

OG

0

0

0

OG

12

0

0

pH 1

OG

25

16

0

OG

OG

OG

125

pH 2

OG

363

OG

OG

OG

OG

OG

OG

pH 3

OG

OG

OG

OG

OG

OG

OG

OG

# Bacterial Adhesion to hydrocarbons

<b>Bacterial ID</b>	<b>A0</b>	<b>A</b>	<b>Percentage</b>
Enterococcus faecium	0.834	0.212	74.5%
Pedicoccus pentosaceus	0.842	0.820	2.61 %

# Discussion and Conclusion

- In this study, two active strains were isolated *Enterococcus faecium* and *Pedicoccus pentosaceus*. This strain can strongly inhibit the growth of some effective pathogen (*Salmonella* spp and *E. coli*). At the same time they can tolerate low pH that similar to the condition in the animal stomach.

# THANKS

