Vitro Evaluation of Probiotic Bacteria

Universita Cattolica Del Sacro Cuore

Italy

19th – 30th June 2011

Tahani Al-Surrayai

Batla Al-Mutairi

Course Program

The course was covered:

1. Identification of LAB using RAPD test.
2. Evaluation of attachment to crop, cecuim and ileum tissues.
3. Measurement of hydrophobicity by hydrocarbon partition.
4. Electron microscopy observation of colonized tissues.
5. Adhesion test of bacteria to pig mucin.
6. Coaggregation with Pathogens.

Objectives

- To learn the latest techniques applied in vitro evaluation of probiotic bacteria.
- To evaluate the isolated LAB strains for their potential probiotic.

LAB isolates were sent to Italy for evaluation test:

1. *Lactobacillus plantarum*
2. *Pediococcus acidilactic*
3. *Lactobacillus coryniformis*
4. *Lactobacillus farciminis*
5. *Lactobacillus parabuchneri*
6. *Pediococcus pentosaceus*
7. *Lactobacillus reuteri*
8. *Lactobacillus brevis*
9. *Pediococcus lolii*
10. *Lactobacillus johnsonii*
11. *Lactobacillus salivarius*

Laboratory Experiments

- Identification of LAB using RAPD test.

RAPD is a PCR test used one primer that is very short, synthesized in both directions foreword and reverse in order to obtain overlapping sequences, and can recognize randomly.

LAB Selected for the evaluation test

1. *Lactobacillus plantarum*
2. *Pediococcus acidilactic*
3. *Lactobacillus coryniformis*
4. *Lactobacillus parabuchneri*
5. *Pediococcus pentosaceus*
6. *Lactobacillus reuteri*
7. *Lactobacillus brevis*
8. *Pediococcus lolii*
9. *Lactobacillus salivarius*

Adhesion assay of LAB to crop, cecuim and ileum tissues

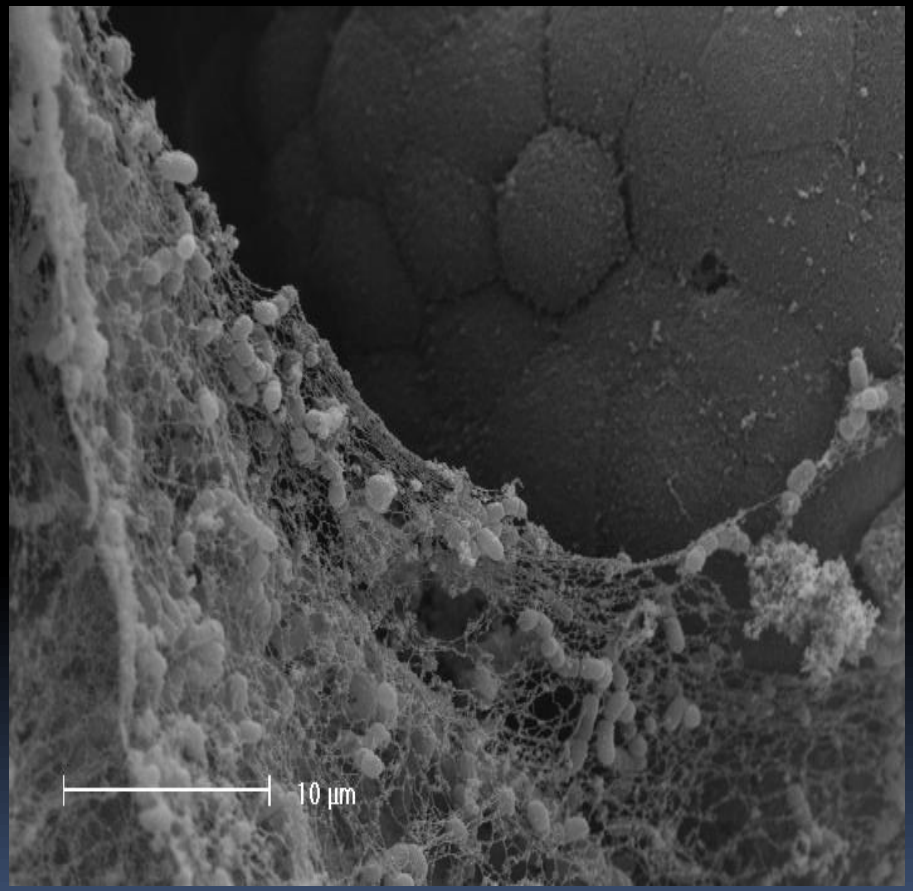
Dehydration of the chicks tissue using ethanol

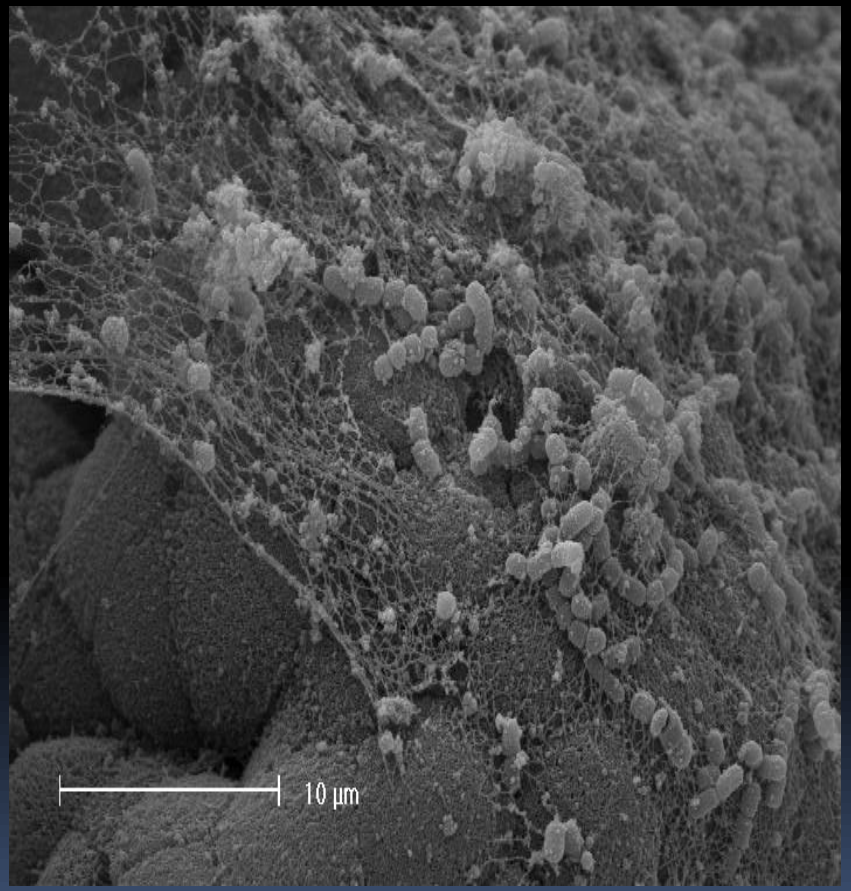
Electron microscopy observation of colonized tissues.

No.	Bacterial strain	Different Animal tissue		
		crop	illeum	cecum
1	*Lactobacillus plantarum*	+	++++	-
2	*Pediococcus acidilactic*	Not done	++	+++
3	*Lactobacillus coryniformis*	++++	Not done	-
4	*Lactobacillus parabunchneri*	-	++	+++
5	*Pediococcus pentosaceus*	+++	++	-
6	*Lactobacillus reuteri*	+	+	+
7	*Lactobacillus brevis*	+++	++	+++
8	*Pediococcus lolii*	Not done	Not done	++
9	*Lactobacillus salivarius*	-	-	+++

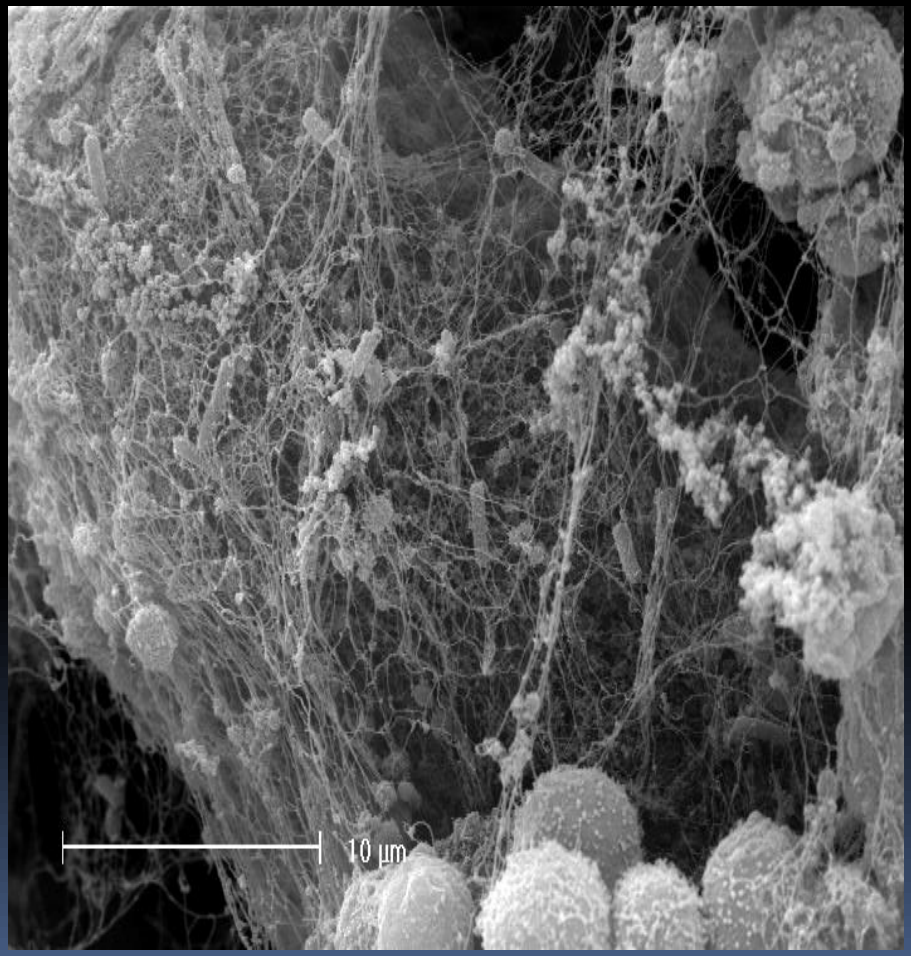
++++ attached to both tissue and mucus
+++ attached to tissue
++ attached to mucus
+ few cells attached
-negative in attachment

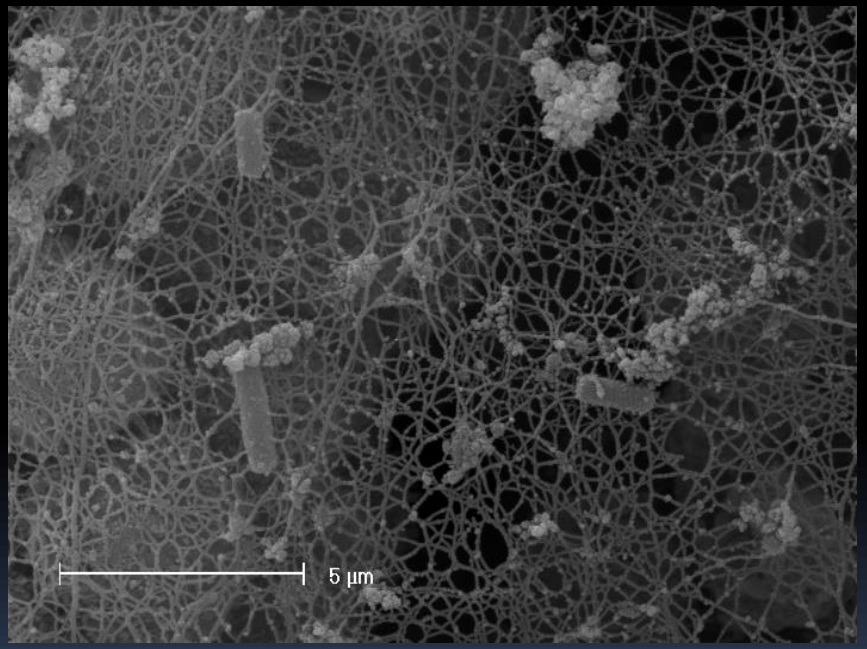
Attached *Lactobacillus parabunchneri* on illeum tissue



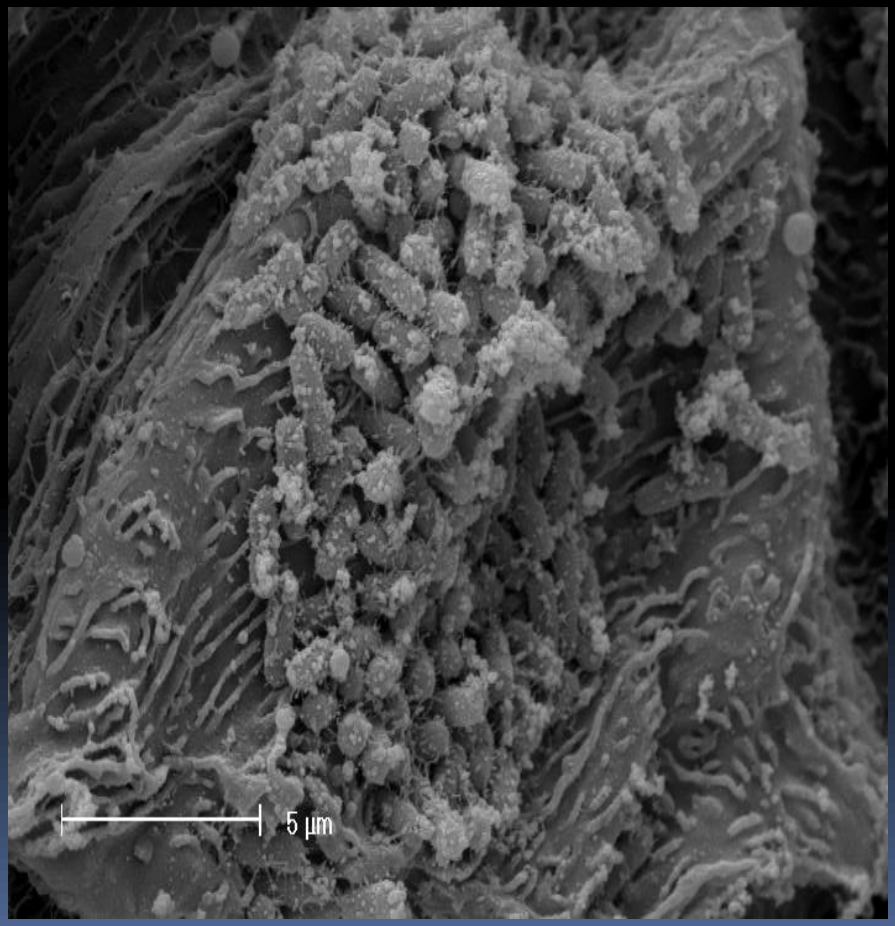


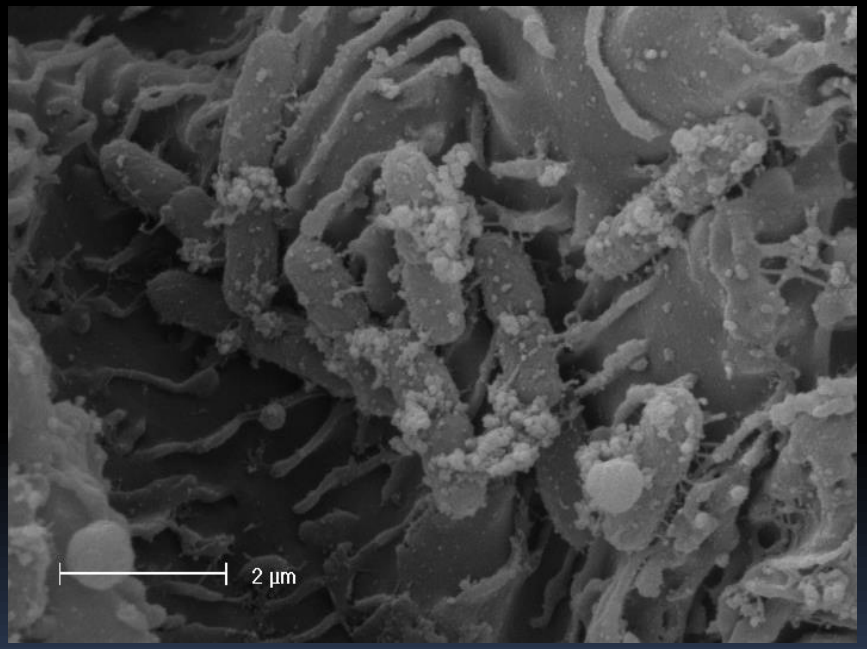
Attached *Lactobacillus brevis*crop on illeum tissue.



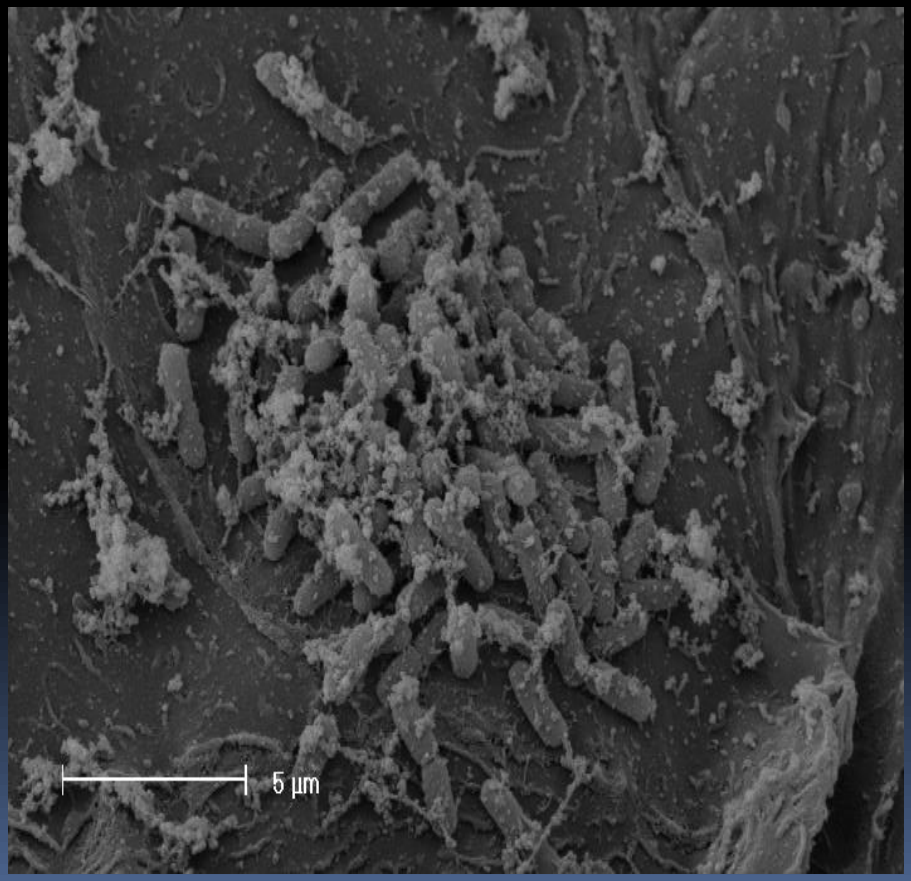


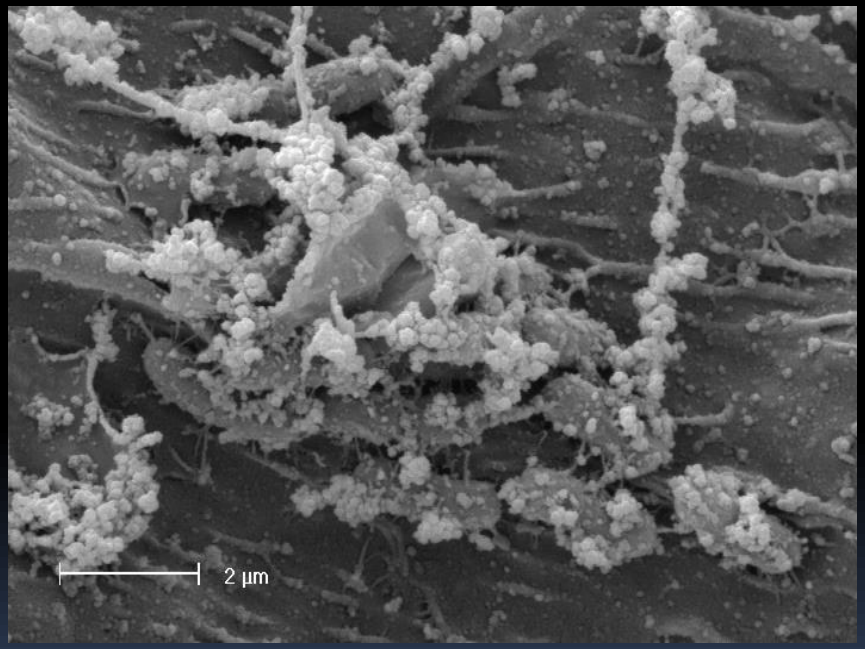
Attached *Lactobacillus brevis* on crop tissue.



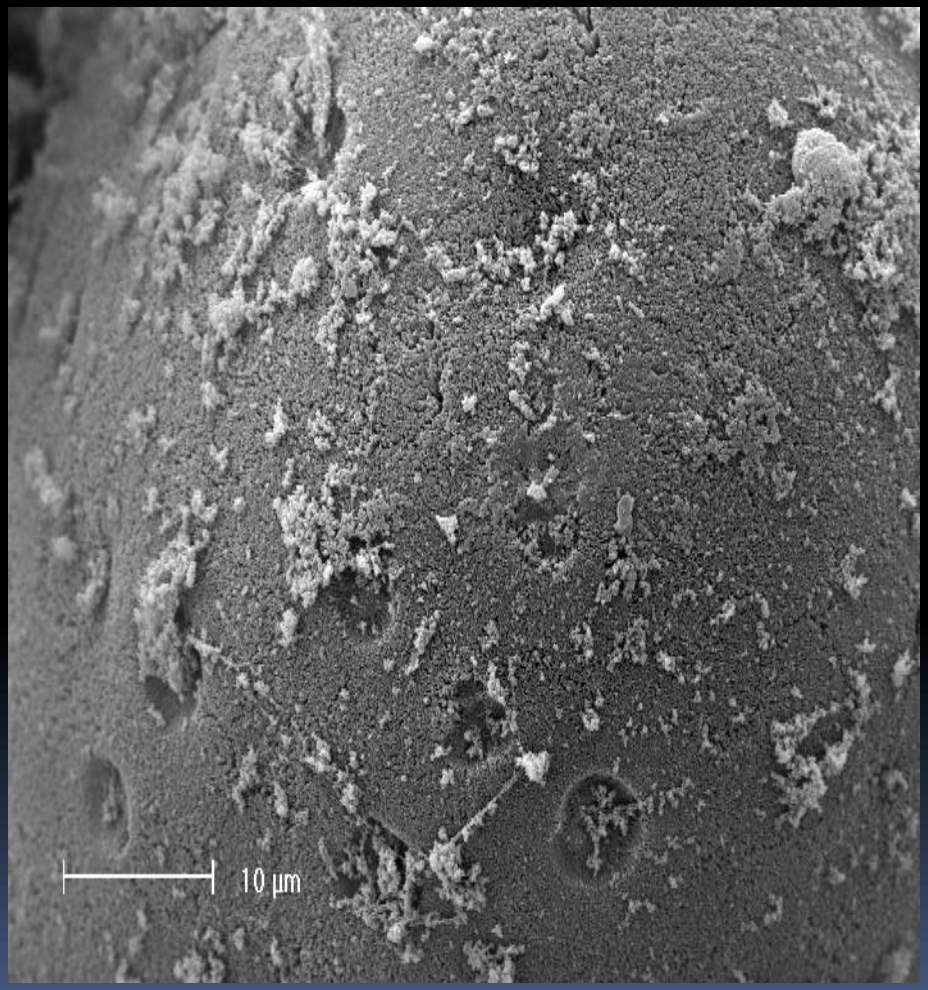


Attached of *Lactobacillus coryniformis* on crop tissue.

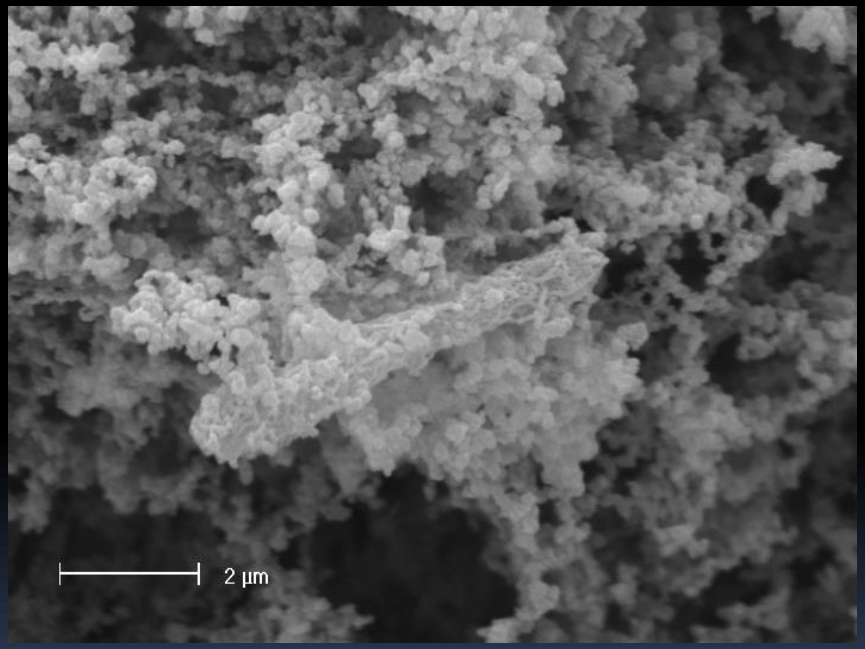




Unattached *Lactobacillus Salivarius* on illeum tissue



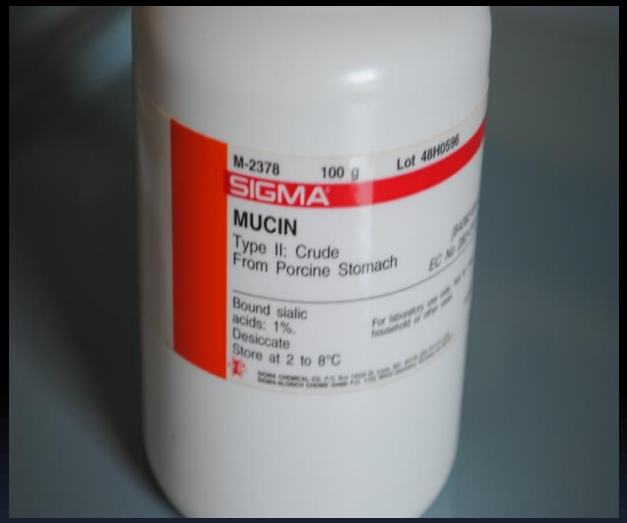
Unattached *Lactobacillus Salivarius* on cecum tissue



Hexadecan assay of putative probiotic strains

Bacterial strains	O.D before the addition of Hexadecane	O.D after the addition of Hexadecane	Percentage of hydrophobicity
Lactobacillus salivarius	0.8	0.49	38.75
Lactobacillus parabunchneri	0.93	0.57	38.71
Lactobacillus plantarum	0.95	0.57	38.71
Lactobacillus brevis	0.92	0.87	5
Lactobacillus coryniformis	0.84	0.82	5
Lactobacillus reuteri	0.83	0.76	5

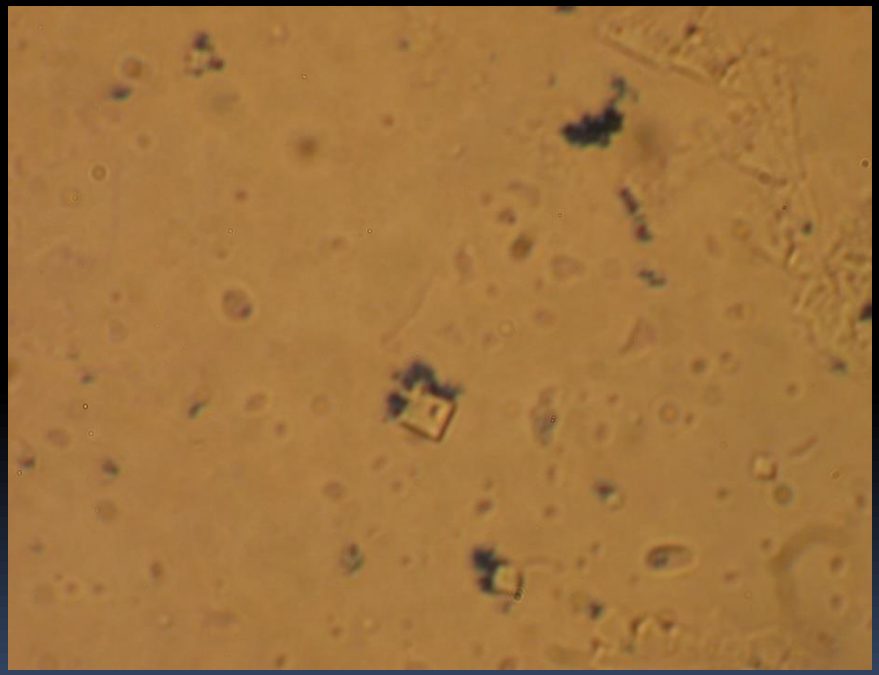
Adhesion test of bacteria to pig mucin



Bacterial strains	Attached to mucus
Lactobacillus salivarius	Positive
Lactobacillus parabunchneri	Positive
Lactobacillus plantarum	Positive
Lactobacillus brevis	Positive
Lactobacillus coryniformis	Positive
Lactobacillus reuteri	Negative
Pediococcus lolii	Positive
Pediococcus pentosaceus	Positive
Pediococcus acidilactic	Positive

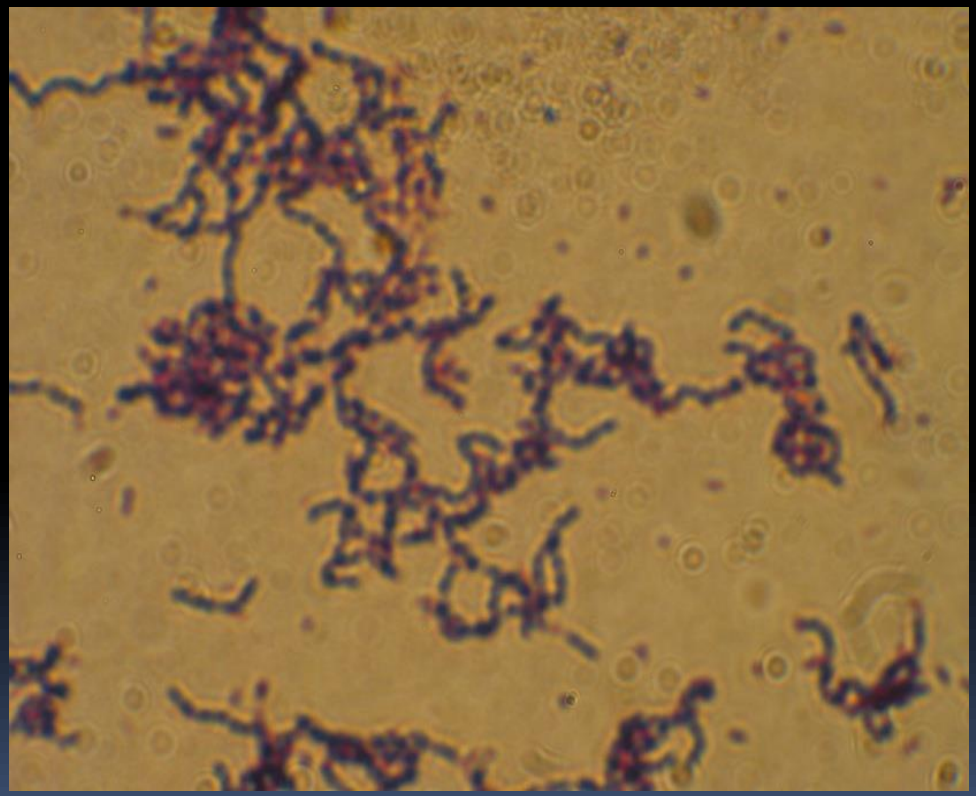
Adhesion test of bacteria to pig mucin

Positive adhesion test of bacteria to pig mucin Under Microscope



Negative adhesion test shows only pig mucin cell

The aggregation test of Lactobacillus strains with Salmonella and Campylobacter



Conclusion

- *Lactobacillus plantarum*
- *Lactobacillus parabuchneri*
- *Lactobacillus reuteri*
- *Lactobacillus brevis*

Thanks