

# Screening and Binding Characterization of Lactic Acid Bacteria Using Small Intestine Membrane Components from Swine

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## Abstract

Probiotic bacteria are defined as a food ingredient or dietary supplement that provides well being to the consumer when they are live, active cultures. In recent years, they have gained more attention because of their known health benefits such as gastrointestinal health, enhancement of the immune system and their ability to inhibit pathogenic bacteria. Unfortunately, there is still disagreement in defining the activity of probiotics as well as methods of assessing them. We focused on developing a non radioactive assay for lactic acid bacteria (LAB) that gives a quantitative measure of their affinity to various regions of the small intestine. LAB strains used in this study were genetically characterized, isolated and typed using pulse field electrophoresis at the Dairy Products Technology Center, Cal Poly San Luis Obispo. Bacteria were grown anaerobically in MRS broth, Cells were used in their exponential and stationary phase of growth for each experiment. A dot blot assay, adapted from an immunoblotting technique was used as a quantitative measurement of the binding ability of the bacteria to various compounds from regions of the mini-pig small intestine (ileum, duodenum, jejunum). Intestinal membrane compounds (proteins and lipids) were blotted onto a nitrocellulose membrane and inoculated with a known concentration of bacteria. The bacteria/protein interaction was detected using Avidin-HRP and Diaminobenzidine for a visual color reaction. These results will help the dairy industry create probiotic containing dairy foods that are optimized for various regions of the small intestine.

## Introduction

One of the main criteria for selecting probiotic strains is their ability to adhere to intestinal surfaces (Rinkinen et al. 2003). Attachment to mucosa prolongs the time time probiotics can influence the gastrointestinal immune system and microbiota of the host (Kirjavainen et al. 1998) Much of the in vitro and in vivo functional testing of probiotic strains has focused on acid and bile resistance, lactase production, antimicrobial activity, and survival in dairy products, but only a few commercial strains have been tested for membrane-binding activity (Bernet et al. 1993; Smit et al. 2001; Johnson et al. 2001). Most researchers agree that to achieve colonization in the intestinal tract, binding activity is essential (Sanders, 1998). In our efforts to understand the role that probiotic bacteria may play in human health, we must justify our health claims with good research and statistically significant data. The research characterizing the health effects of probiotic bacteria is not sufficient, although hundreds of publications address the topic. Mechanisms of actions are not thoroughly established; results for some endpoints are vague; and properly controlled human intervention trials are limited. Yet the emergence of new public health risks even in industrialized nations suggests that we can find ways in which effective probiotic bacteria may play an important role in maintaining human health (Sanders, 1999). Our overall objective is to understand the membrane-to-surface interactions of the bacteria. **By focusing on the binding mechanisms in which bacteria interact within small intestine as well as on our previous work with probiotics in dairy products we hope to learn how to enhance our processing systems in order to optimize the conditions needed by the bacteria maintain their peak performance capabilities.** Therefore this assay can be used to determine which strains have affinity to the various regions of the small intestine and their suitable dairy product as a delivery system.

## Materials and Methods

### BACTERIA PREPARATION (LABELLING)

The lactic acid bacteria that used for this study were obtained from the DPTC library. Mutant strains were also used and were donated by Dr. T. Klaenhammer (North Carolina State University). Bacterial cells that were grown overnight (12 -15hr) in MRS (with 5% cysteine) media were washed twice with PBS buffer and then diluted using a spectrophotometer to an OD of 1.0 (+/- .01) at 450nm. 2.5mL of cells were spun down to form a pellet and then reconstituted in 1mL PBS.  $\alpha$ -lactose was then added to a concentration of 100mM and then 0.5 mg of NHS-biotin (Pierce) was added and allowed to incubate at room temperature for 30 minutes. Excess biotin was removed by centrifugation of the cells, resuspending them in TBS buffer, centrifuging again and resuspending in PBS buffer.

### INTESTINAL CELL LINING ISOLATION

Porcine intestines from six week old piglets were donated by Dr. Odle (North Carolina State University). Intestine sections were divided into duodenum, jejunum and ileum and were kept at -60C. Intestines were thawed on ice and then cut using scissors. A plastic spatula was used to gently scrape the inner portion of the intestinal wall. The scrapings were diluted in HEPES-Hanks buffer. Epithelial cells were removed by spinning at 12000 g for 10 minutes at 4C.

### DOT-BLOT ASSAY

The methodology for the dot blot assay was adopted from Laboratory Manual (Goers, J., 1993). The dot blot assay gives a visual representation of the binding abilities of the bacteria to various intestinal sections (duodenum, jejunum, ileum).

The crude mucin extraction was blotted onto nitrocellulose membrane (pore size .45 $\mu$ m) with the use of a membrane blotter (BioRad)at 25 $\mu$ L and 50 $\mu$ L increments. The membrane was allowed to dry and then blocked with 3% H<sub>2</sub>O<sub>2</sub> for 10 minutes at room temperature. The membrane was rinsed twice with DI water and allowed to dry. The membrane was then blocked with a 2% BSA solution and again allowed to air dry. The biotinylated bacteria were then allowed to incubate with the membrane overnight (-15hr @ 25C)

Nitrocellulose strips were washed and then incubated in 1:2000 dilution of Immunopure® Streptavidin Horseradish Peroxidase Conjugate (Pierce) for one hour and then rinsed. The rinsed strips were then developed using 3,3'-Diaminobenzidine tetrahydrochloride (Sigma). BioRad's Quantity One software was used to acquire the intensity readings of the blots. Based on the intensity of the blots, we analyzed the binding ability of the bacteria.

## References

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## Results

Dot blot results showed that different strains of probiotic bacteria had varying degrees of binding affinity. Figure1 shows a strain that has varying degrees of binding based on the intestinal section. The results showed that although there was some variation in binding amongst the three intestinal samples for each isolate, those bacteria that had higher binding were consistent in all three intestinal samples. Three of the isolates were consistently low in binding in comparison to the rest of the samples (see Table 1). It was also noted that the binding sensitivity differed within a single strain (see Table 2). Mutant strains were consistent amongst each other in the Duodenum and Jejunum. Two of the strains in the Ileum fraction had markedly low binding which is mostly likely due to the surface proteins that were knocked out in their genetic sequence. These proteins may be needed in this part of the intestine in order to aid in proper binding.

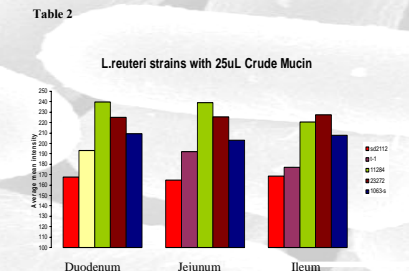
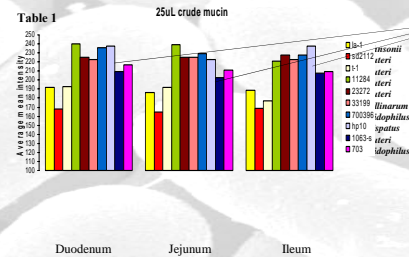


Table 3  
25 $\mu$ L Crude Mucin with Mutants

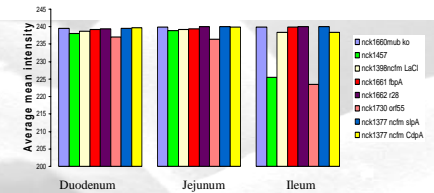
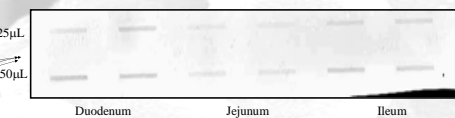


Figure 1 Dot Blot Assay Example (*L. crispatus*)



## Results Summary

- 1) The bacteria tested showed in general similar binding ability to the different sections of the intestine (slight variation tendencies were noted in some strains with the different intestinal sections)
- 2) Significant variation on binding ability was detected among strains of *L. reuteri*, but similar affinities for the three intestinal sections
- 3) Deletion mutants NCK1457 and NCK1730 had dramatically decreased binding affinity to Ileum, however, they presented similar binding ability to duodenum and jejunum.

## CONCLUSION

Our assay is sensitive enough to measure small changes in binding affinity of LAB to substrates.

## Acknowledgments

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