Researchers seek link between probiotic organisms and human health

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Executive Summary

Probiotics are live microbial food ingredients that benefit the host’s health, specifically, the gastrointestinal (GI) tract. Mucin, a highly glycosylated protein, is a major constituent of mucosal membranes and is present in the GI tract. To be effective, probiotic organisms must compete efficiently for adhesion to intestinal cells. One specific aspect of adherence to intestinal cells is the ability to bind to mucin.

The overall objective of this work was to contribute to finding a link between probiotic organisms, dairy products, and human health by developing an assay based on mucin binding that can be used for primary evaluation of probiotic strains. Specific objectives were focused in three areas: 1) isolating lactic acid bacteria from feces where they were presumably exposed to intestinal mucins; 2) using the test developed on commercial and newly isolated bacteria.

Probiotic organisms can be very beneficial, but must compete efficiently for adhesion to intestinal cells.
for comparison of mucin-binding ability; and 3) contributing to the understanding of the mechanisms of binding by assessing the possible use of mucins as nutrients by the probiotic strains.

To work with bacteria directly from the intestine, bacteria were isolated from baby feces and identified using API 50 CH carbohydrate fermentation kits. Five isolates were obtained. Although they were inconsistently identified by the API tests, two isolates, DPTC 100 and DPTC 101, were used in subsequent experiments as a comparison against commercial strains.

Screening the bacteria for mucinase activity involved binding mucin to a 96-well microtitre plate. A purified mucin source is necessary for this procedure. However, the objectives of this project were more completely met using a crude mucin preparation.

To test for binding, a non-radioactive dot blot method was used to test various Lactobacillus strains for the ability to bind bovine sub maxillary mucin. Bacterial cells were biotinylated using EZ-Link™ Sulfo-NHS-LC Biotin and were then used to probe immobilized mucin on a membrane. An avidin-alkaline phosphatase conjugate was used to develop the dot blot. A ratio of the experimental mucin value to positive control was calculated and averaged for each strain, with a minimum N = 3 and a maximum N = 15. Forty-six strains were screened, and 13 demonstrated a binding ratio of binding to biotinylation of > 0.2.

To further characterize the binding abilities of the positive bacteria, Western blots were performed. Bovine mucin and bovine serum albumin were run on 10% SDS polyacrylamide gels and electrophoretically transferred to a membrane. The membrane was probed with biotinylated bacterial cells and developed using an avidin-alkaline phosphatase conjugate. Information regarding which protein portion of the mucin the bacteria were binding was lacking, possibly due to the occurrence of β-elimination of the O-linked oligosaccharides on the mucin rendering it unrecognizable to the bacteria.

The bacteria were also mildly sonicated to allow comparisons of the protein profiles between the strains that bound mucin and strains that did not. Differences between the two groups were not seen. However, protein profiles suggested that binding is strain specific and not species specific. A sequenced and identified surface layer (S-layer) protein was isolated from NCFM strain. The single-step conditions under which this protein was isolated were not repeatable in all the other strains tested, although a guanidine hydrochloride extraction yielded S-layer proteins from two more strains in addition to NCFM: MR 220 and BG 2F 04.
To test the bacteria's ability to use mucin as a carbon source, growth curves in media that contained mucin were performed. Two strains, NCFM and Yakult, demonstrated growth in this media. Additional growth studies in a minimal media with nitrogen supplemented with mucin were also performed. Five strains—Actimel, LA-1, NCFM, 53103, and 700396—demonstrated some growth in this media during a 24-hour test period. Growth appeared to be the result of an older mucin solution. These experiments laid the foundation for additional studies involving mucin degradation over time and how this affects bacterial growth.

Major Accomplishments

The most important accomplishment of this work was to develop a rapid and non-radioactive procedure for reliable screening of lactic acid bacteria binding to mucin. This is of major importance in industrial settings because one link between dairy products and intestinal health may be found in the lactic acid bacteria-mucin interactions. Milk contains a mucin that closely resembles those found in the lining of the intestine. Therefore, this test is a very practical way to discriminate between the different potential benefits to health of probiotic strains.

- Part of this work was presented in the National Meeting of the American Dairy Science in Quebec, Canada.
- A manuscript is being prepared for publication in the *Journal of Dairy Science*.
- This test is simple and will be included in the laboratory practices in our course of Dairy Microbiology.

Impact Statements

1) Development of a technique practicable in industry and research laboratories everywhere.

2) Identification of four potential strains from the DPTC collection of probiotic strains that have demonstrated a binding ability to mucin five times above the average. (These strains will be continue to be studied).

3) Establishment of a new possible parameter for evaluation of nutritional enhancement of dairy foods.
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