

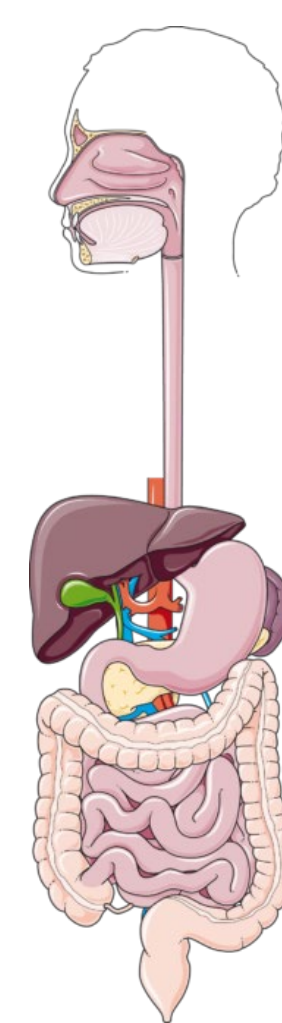
Development of a pH reactive fecal sampling molecule for in vivo sampling of murine small intestine

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Background Information

- Pronounced regional differences between the microbiota of the upper and lower gastro-intestinal tract (GIT) in both humans and mice reinforce the importance of analyzing the complete gut microbiota when sampling
- No commercial system currently exists to survey upper GIT contents without requiring highly invasive or post-mortem sampling
- The goal of this project is to develop a pH sensitive in situ sampling nanoparticle to allow for capture, recovery, and subsequent analysis of upper GIT microbiota



The principal objective of this study was to visualize bacterial association of *L. lactis* with a poly(amino acid) using Fluorescence Microscopy.

Methods

- Creation of a pH sensitive polymer attached to a metallic nanoparticle
- Poly-histidine side chain pKa value correlates with the pH change of the upper and lower GIT
- Polymer is positively charged with extended arms at the pH of the duodenum (upper GIT)
- When the pH increases in the jejunum and ileum (lower GIT) the polymer collapse as the charge becomes neutral

Amino Acid	Side chain pKa value
Cysteine	8.33
Tyrosine	10.07
Aspartic Acid	3.86
Glutamic Acid	4.25
Lysine	10.79
Arginine	12.48
Histidine	6.04

Area	pH
Stomach	1.5-3.5
Duodenum	5.5-6.0
Jejunum	6.0-7.4
Ileum	7.4

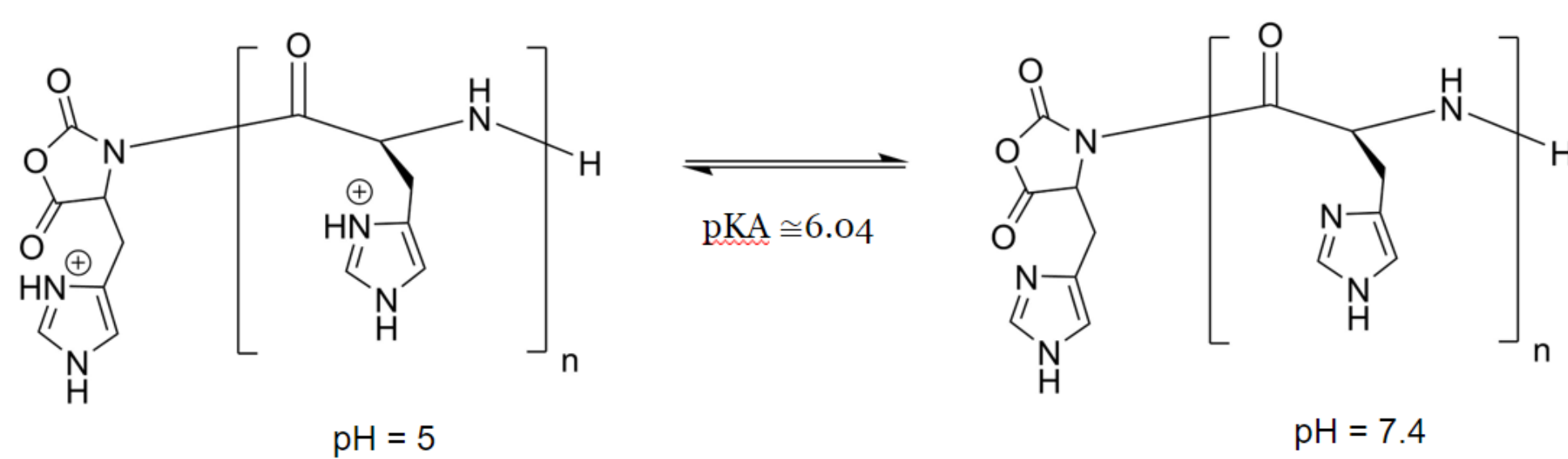


Figure 1. Poly-histidine charge states. The dominant form at the Duodenum pH of 5 is positively charged. The dominant form at the ileum pH of 7.4 is neutral.

Poly-histidine Charge Collapse

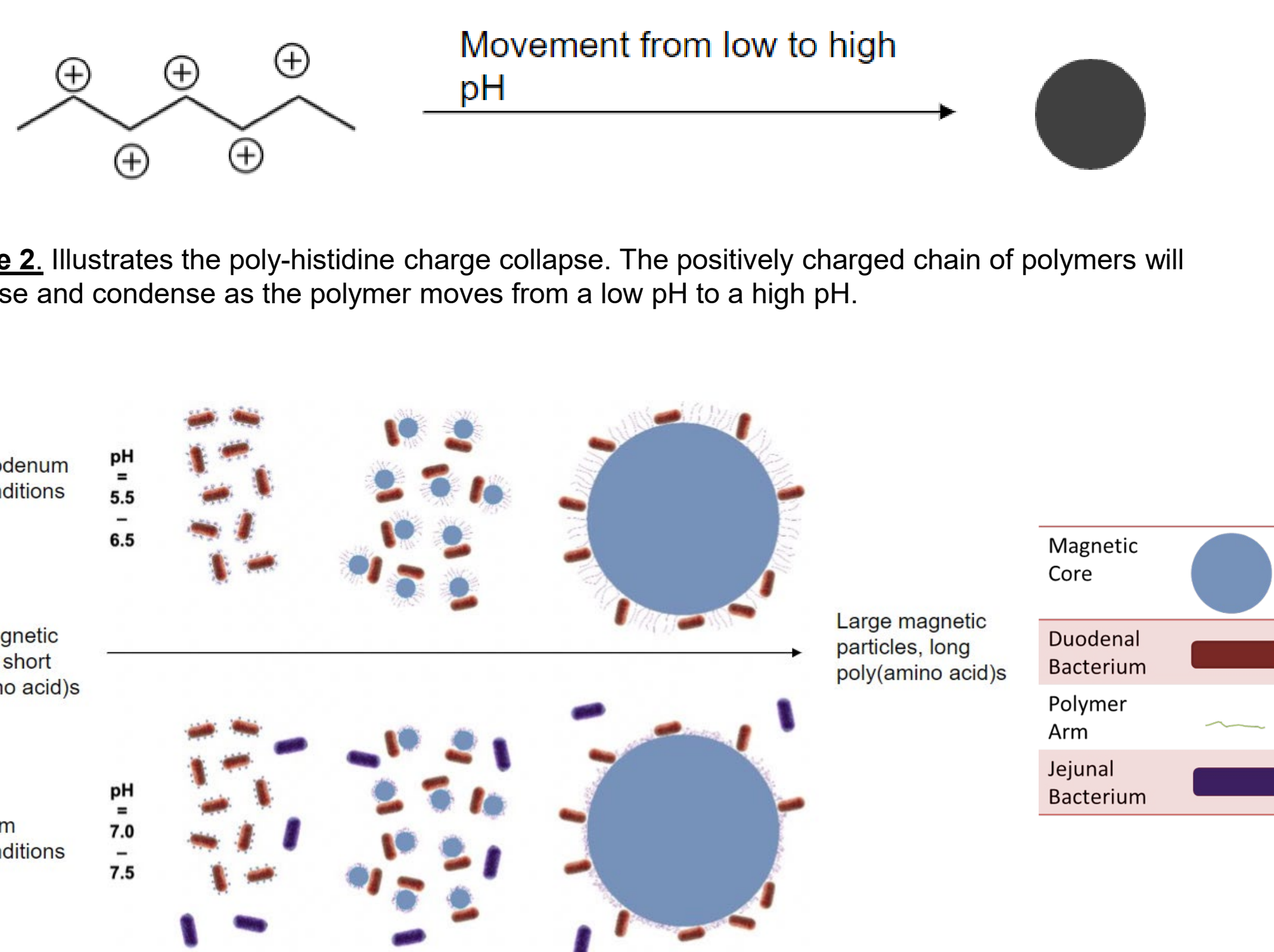


Figure 2. Comparison of the nanoparticle in duodenum conditions and ileum conditions.

- Metallic nanoparticle core attached via click chemistry to poly-histidine arms
- At the pH of the duodenum (5.5-6.5), a histidine-containing polymer arm will be net positively charged and polymers arms will be extended
- The polymer arms will collect bacteria in the upper GIT
- At the pH range of the jejunum and ileum (7.0-7.5), the polymer arm is expected to be neutrally charged and collapse
- As a result of the polymer arm collapse, little to no association is expected between the polymer arms and bacteria in the lower small intestine
- After exiting the GIT, the nanoparticle can be recovered from the feces and upper GIT microbiota analyzed

Future Goals

- Explore additional variables:
 - Microbial specificity
 - Enzyme degradation of polymer
 - accuracy
- Mouse model

GFP Induced *L. lactis*

- L. lactis* was genetically engineered to express green fluorescent protein (GFP)
- Reporter genes are regulated by the nisin-controlled gene expression (NICE) system
- Nisin induced *L. lactis* will fluoresce when visualized under fluorescent microscopy

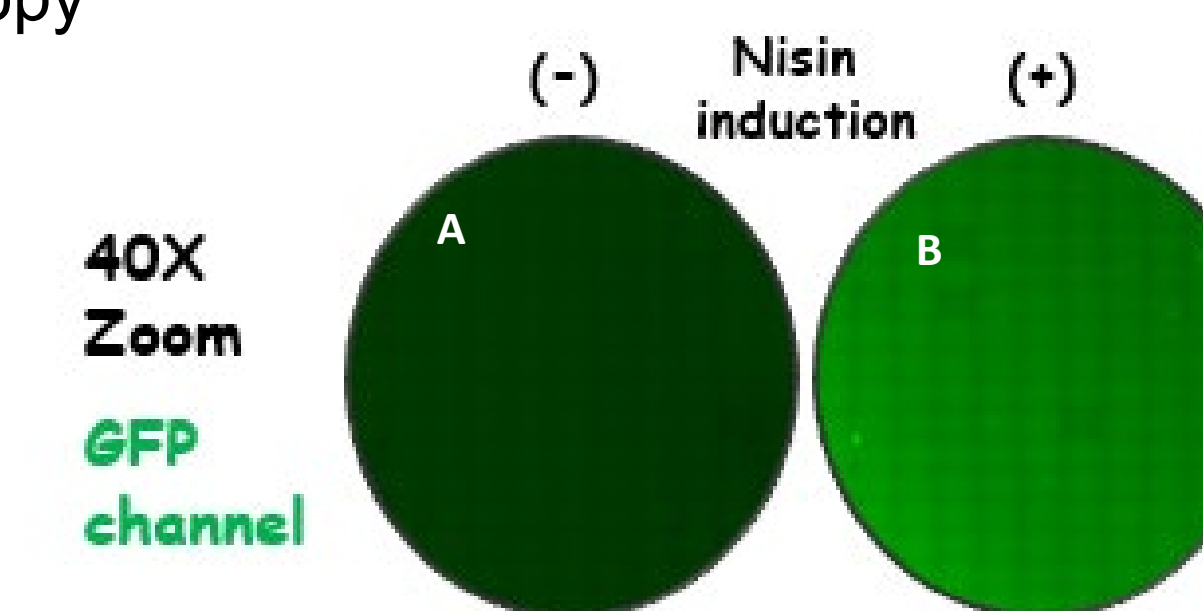


Figure 3. *L. lactis* wells observed under 40X utilizing a GFP channel A. Negative control, *L. lactis* is not induced with Nisin B. *L. lactis* is induced with Nisin, media fluoresces

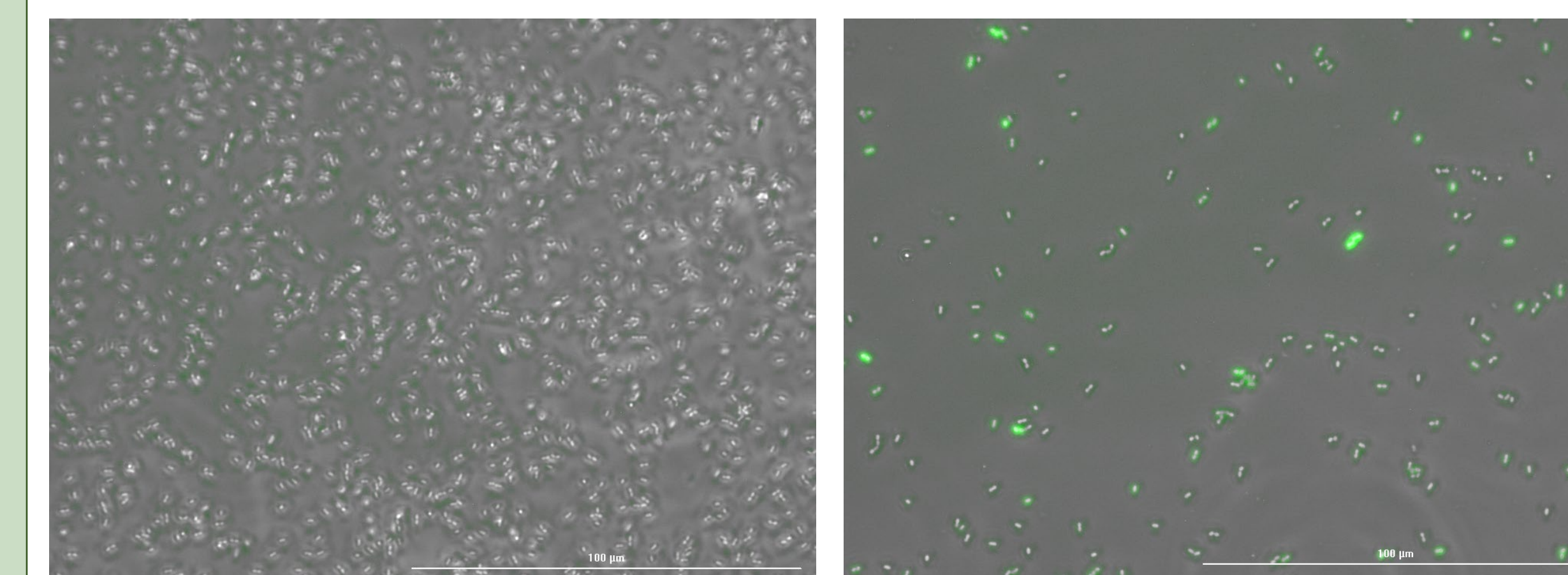


Figure 4. *L. lactis* slides observed under 40X utilizing a GFP channel A. Negative control, *L. lactis* is not induced with Nisin B. *L. lactis* induced with Nisin, fluorescent cocci visible

Conclusions

- GFP fluorescent induced *L. lactis* can be used to visualize the association between the bacteria and the polymer.
- Further plans to test the association between *L. lactis* and the poly-histidine polymer are in progress.

Acknowledgments

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