Acquisition of Morphological Variations of Mutant Lactobacillus acidophilus NCFM Strains by Means of Atomic Force Microscopy

Daniel Ruiz*, Derek Gragson, and Rafael Jiménez-Florez

Department of Chemistry and Biochemistry, California Polytechnic State University
Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo

Abstract

Atomic force microscopy has been an important tool in the study of atomic orientation, macromolecular structure, surface structure, morphological structure of living/nonliving tissues and synthesized materials. Although ten modes of imaging are routinely used, all information on surface structure and forces are obtained from the oscillation of the cantilever at its resonance frequency which is induced by the piezoelectric element. The photodetector senses deviations of the laser reflected off the surface of the cantilever from the normal resonance caused by attractive and repulsive forces between the tip of the cantilever and the sample. All oscillations and torsions of the cantilever that do not correspond to the sinusoidal wave produced can be converted to topographic images and force curves of the sample.

In reference to the project, AFM technology aids in the visualization of important physicochemical characteristics of lactic acid bacteria (LAB) including lactobacillus acidophilus NCFM strains. LAB has important immunomodulatory effects that induce the gastrointestinal tract to host and maintain health. Single bacteria strains and combination of strains adhered to milk fat globule membranes (MFGM) introduced to gastrointestinal tract (GIT) via milk fat globules (MFG) have been found to prevent and reduce illness by providing improved mucosal immunity of the GIT. MFG allow adhered LAB increased probability of subsisting through bile, stomach acid and other autoimmune defenses. Central to this function is the composition and structure of the surface of these important microorganisms. We have some single gene deletion mutants of genes important for the outer surface of NCFM. DNA analysis also yields good quantification of observed binding dependent upon mutation type and expression of surface proteins. Therefore, it is crucial to study the morphology in LAB that contribute to adherence to MFGM. Using milk fat could be a means of supplying the world with probiotics that maintain a healthier lifestyle.

Methods

Figure 1: Asylum Research Atomic Force Microscope used for imaging (A); Representation of vital informational gathering components (B); and Mechanism for acquiring images (C).

Motivational Interest

− Probiotic effectiveness
− Delivery of probiotic LAB to host via dairy products
− Relationship between dairy product and microbial fitness

Sucrose Density Gradient (SDG) Separation

Bacterial DNA recovered in bottom fraction of SDG after incubation with Ultrafiltration/Diafiltration BMP

Processed AFM Images

<table>
<thead>
<tr>
<th>Motivational Interest</th>
<th>Sucrose Density Gradient (SDG) Separation</th>
<th>Bacterial DNA recovered in bottom fraction of SDG after incubation with Ultrafiltration/Diafiltration BMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ceruminous component (bottom)</td>
</tr>
</tbody>
</table>

Conclusions

- Clear morphological changes can be observed between SlpA and all imaged LAB.
- Flagella become evident in NCFM mutant SlpA.
- Flagella could be a major factor in increased binding of SlpA to MFGM.
- L. acidophilus NCFM and mutants show differential binding to UF/DF BMP, dependent upon mutation type and expression of surface proteins.
- Mutation in the gene coding for SlpA surface protein expression greatly enhances binding of L. acidophilus NCFM to MFGM.
- DNA analysis yields good quantification of observed binding.
- Procedures are easily replicated and produce consistent results.

Acknowledgments

Research supported by the Science Teacher and Researcher Program, Robert Noyce II Scholarship Teacher Program, and the California State University System. This work was funded by the National Science Foundation.

Request for copies, references, and further question: danielruiz@mail.com