Dynamic of Biofilm Formation by *Lactobacillus plantarum* in Continuous-Flow Culture System

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER at the Permian Basin

Jonathan Gomez Garcia[1,2], Roy Williams[1,2], Stacy Martinez[1], Andrew West[3] (MS III) , Dr. Natalia Schlabritz-Lutsevich[1] and Dr. Gary Ventolini[1]

[1]Department of Obstetrics and Gynecology, [3]Texas Tech University Health Sciences Center at the Permian Basin, Odessa, TX
[2]University of Texas of the Permian Basin, Odessa, TX

INTRODUCTION

Traditional methods of evaluating and visualizing, with the naked eye, bacterial biofilm formation have been limited by small quantity of the biofilm and are further hindered by the ability to fully visualize and record the dynamic phases of the biofilm cycle: attachment, colony formation, structural formation growth, and detachment in a real-time setting. Probiotic bacteria, including the lactic acid-producing *Lactobacillus plantarum,* are important species for real-time biofilm formation investigation. The visualization and real-time documentation of the dynamic phases of growth of this lactic acid-producing species pose an important subject for evaluation in the continuous-flow culture system.

OBJECTIVE

To visualize and document in real-time, with the naked eye, the dynamic phases of growth of *Lactobacillus plantarum* utilizing the continuous-flow culture system method (Louis Pasteur Institute, Paris, France).

MATERIALS & METHODS

L. plantarum (Louis Pasteur Institute, Paris, France) was plated on MRS (De Mann, Rogosa, and Sharpe) for 24 hours at 37°C. After 24 hours, a sample was taken for later DNA isolation using Mo-Bio PowerLyzer Power Soil kit (Carlsbad, CA, USA) and Q-PCR analysis for the detection of *L. plantarum*, Eubacteria universal primers, *E. coli*-specific primers, and RecA primers. The remaining lawn was transferred to 50ml of MRS media and a spatula was submerged for a period of 1.5 hours. After 1.5 hours, the spatula was removed and placed in the continuous-flow culture system for periods of 24 hours and 48 hours. A peristatic pump was utilized at 10 rpm to push fresh MRS media through the system, and a mix of 95% O_2, 5% CO_2 was used to pressurize the system to cycle the media through the biofermenter. Real-time documentation of the dynamic phases of biofilm growth and development were captured with a Nikon D3200 digital camera, and a Panabhi digital timer remote took time-lapse captures at intervals of 2.5 minutes. Final biofilm growth was collected, photographed, weighed, flash frozen, and stored at -80°C.

RESULTS

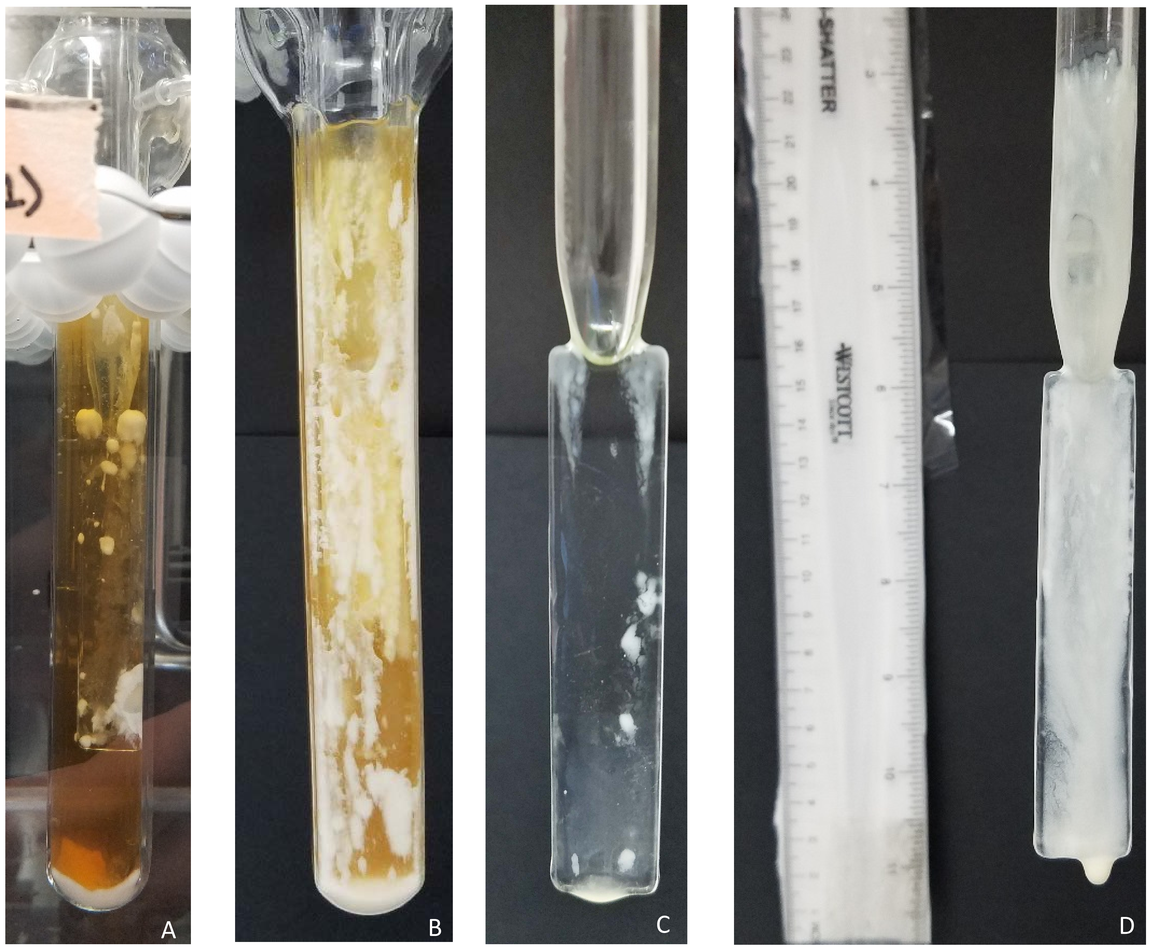


Fig.1. A) Microfermenter, 24 hr. growth in system; B) Microfermenter, 48 hr. growth; C) spatula, 24 hr. growth; D) spatula, 48 hr. growth

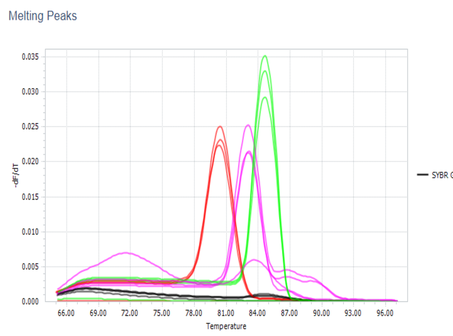
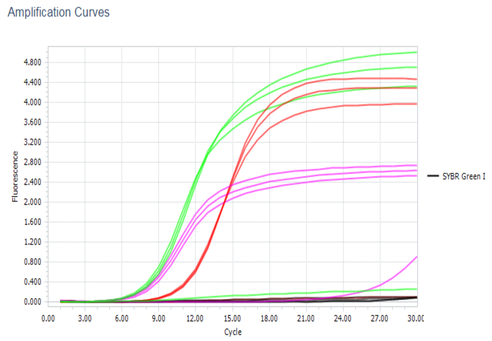


Fig. 2
L. plantarum primers
F-TTACATTTGAGTGAGTGGCGAACT
R-CCCAATGTGGCCGATTACC
Eubacteria Universal primers
F-ACTCCTACGGGAGGCAGCAGT
R-ATTACCGCGGCTGCTGGC
E. coli specific primers
F-GTGCCAGCGCCGCGGTAA
R-GCCTCAAGGGCACAACCTCCAAG
RecA primers
F-GGCAGAACAGATCAAGGAAGG
R- TATCCACTTCGGCACGCTTA



RESULTS

We were successful in visualizing and documenting the real-time growth and development of *L. plantarum*. The mean final weight of the biofilm was 0.61 ± 0.12g for 24 hours, 2.86± 0.30g for 48 hours, the weight of the attached phase was 0.41g (n=1), and the weight of the detached phase was 2.75g (n=1), (data are mean ± SEM). We were also able to quantify the dynamic growth phases of the biofilm formation during the 24 hour and 48 hour periods. Documentation via time-lapse video was compiled for the first 2 hours, second 24 hours, and full 48 hours for the dynamic phases of the *L. plantarum* biofilm. DNA isolation and Q-PCR were performed on the final biofilm growth utilizing house-designed specific primers for *L. plantarum* (Fig.1 and Fig.2).

CONCLUSION

The dynamic phases of the *L. plantarum* biofilm growth and development were documented via time-lapse video recording to be able to better visualize with the naked eye the various phases of biofilm growth, formation, and detachment. This will allow for future investigation of the dynamic phases of bacterial biofilms.

REFERENCE

- Barman, M., Unold, D., Shifley, K., Amir, E., Hung, K., Bos, N., and Salzman, N. (2008). Enteric Salmonellosis Disrupts the Microbial Ecology of the Murine Gastrointestinal Tract. Infection and Immunity, Mar. 2008, 907-915.
- Beloin, C., Fernandez-Hidalgo, N., Lebeaux, D. (2016). Understanding Biofilm Formation in Intravascular Device-Related Infections. Intensive Care Medicine, Mar. 2016, 443-446.

ACKNOWLEDGEMENTS

❖Dr. Kyle Beran, UTPB, for institutional support
❖Dr. Andrey Bednov, TTUHSC, for equipment design assistance
❖Eli Arazate, TTUHSC, for help with the time-lapse video compilation
❖TTUHSC laboratory staff for their assistance throughout project