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Purpose: To determine if *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 inhibits the growth of *Helicobacter pylori*

Test Materials:

- 1) Lyophilized *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 (powder CFU – 1×10^9 /g)
- 2) Brucella broth with 5% Fetal bovine serum (B-FBS)
- 3) Brucella agar plates with hemin, vitamin K and 5% sheep blood (BMB)
- 4) *Helicobacter pylori* #3, #5 and #8 (clinical strains)

Bacterial Inoculum

H. pylori

Previously prepared frozen stock cultures of *H. pylori* were used for these experiments. The bacterial concentrations were 1.58×10^7 CFU/mL for *H. pylori* #3, 1.11×10^7 CFU/mL for *H. pylori* #5 and 1.44×10^7 CFU/mL for *H. pylori* #8.

Control and test samples containing 5 ml of B-FBS were inoculated with each strain to achieve a bacterial concentration of approximately 1×10^5 CFU/mL.

Lactobacillus delbrueckii subsp. *bulgaricus* G-LB-44

1 gram of *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 was added to 9 mL of sterile water at room temperature. The solution was mixed using a vortex for 5 minutes to ensure the homogeneity of the mixture. 50ul (10^6) or 500ul (10^7) was added to each of the test samples. For 10^8 G-LB-44, 500 mg was added to 4.5 ml of B-FBS and vortexed for 5 minutes before inoculating with the *H. pylori*.

Test Procedure:

The following test samples were prepared for the *H. pylori* #3 and *H. pylori* #5.

Control Samples:

3 tubes containing 5 mL each B-FBS

Test Samples:

3 tubes containing 5 mL each B-FBS with 10^6 *Lactobacillus delbrueckii* subsp. *bulgaricus*

H. pylori was added to the control and test samples as described above. The bacterial concentration for each sample was determined at time 0. Serial 10 fold dilutions were made in phosphate buffered saline and plated onto BMB. The agar plates were placed in Gas-Pak jars containing microaerophilic gas generating sachets and incubated at 37°C for a minimum of 120 hours. Colonies were enumerated and all counts were recorded as

CFU/mL. All control and test samples were then placed in Gas-Pak jars containing microaerophilic gas generating sachets and incubated at 37° C for a 48 hours. Following incubation, the bacterial concentration for each sample was determined as previously described.

The following test samples were prepared for the *H. pylori* #8.

Control Samples:

3 tubes containing 5 mL B-FBS

Test Samples:

3 tubes containing 5 mL B-FBS with 10⁶, 10⁷ and 10⁸ *Lactobacillus delbrueckii* subsp. *bulgaricus*

H. pylori was added to the control and test samples as described above. The bacterial concentration for each sample at time 0 and at 48h was determined as described above.

Results:

H pylori #3 alone		H pylori #3 + 10 ⁶ Lb	%reduction
T=48	6.70E+06	3.90E+05	
	1.75E+07	3.10E+05	
	1.64E+07	6.00E+04	
Average	1.35E+07	2.53E+05	98.13%

H pylori #5 alone		H pylori #5 + 10 ⁶ Lb	
T=48	2.30E+07	3.20E+06	
	2.90E+07	4.40E+06	
	3.10E+07	3.70E+06	
Average	2.77E+07	3.77E+06	86.39%

H pylori #8 alone		H pylori #8 + 10 ⁶ Lb	
T=48	1.39E+07	3.90E+06	
	2.90E+06	3.60E+06	
	1.03E+07	2.60E+06	
Average	9.03E+06	3.37E+06	62.73%

H pylori #8 alone		H pylori #8 + 10 ⁷ Lb	
T=48	1.10E+04	0.00E+00	
	2.70E+06	0.00E+00	
	7.90E+06	0.00E+00	
Average	3.54E+06	0.00E+00	100.00%

H pylori #8 alone		H pylori #8 + 10 ⁸ Lb	
T=48	1.10E+04	0.00E+00	
	2.70E+06	0.00E+00	
	7.90E+06	0.00E+00	
Average	3.54E+06	0.00E+00	100.00%

Sincerely,

A handwritten signature in black ink, reading "Andrew Onderdonk". The script is fluid and cursive, with the first letter of each word being capitalized and larger than the others.

Andrew B. Onderdonk