

Clinical Laboratories
75 Francis Street
Boston, Massachusetts 02115
Tel: 617 732-7372, Lab: 617 525-2506
Fax: 617 277-1762
E-mail: aonderdonk@partners.org

Andrew B. Onderdonk, Ph.D.
Director
Clinical Microbiology Laboratory
Professor of Pathology
Harvard Medical School

Pathogen Inhibition Test

Purpose: To determine if *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 inhibits the growth of 1×10^5 /ml *Escherichia coli* in carrot juice.

Bacterial Inoculum

E. coli

Previously prepared frozen stock culture of *E. coli* was used for these experiments. The bacterial concentration was 2.29×10^9 /ml.

E. coli 1×10^5 /ml control and test samples:

The frozen tube containing *E. coli* was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:100) in sterile phosphate buffered saline to achieve a concentration of approximately 2.29×10^7 /ml. 20ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^5 /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. 0.05 ml of the prepared solution was added to 5 ml of test sample.

Test Procedure:

The following samples were prepared for the *E. coli*

Control Samples:

3 tubes containing 5 ml each of carrot juice

Test Samples:

3 tubes containing 5 ml each of carrot juice plus 0.05 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

E. coli was added to the control and test samples as described above. All of the samples were stored in a refrigerator (4°C) for 24 hours. Following refrigeration, the bacterial concentration for each sample was determined. Serial 10 fold dilutions were made in phosphate buffered saline. A 0.1mL aliquot of each dilution was plated onto TSA. The agar plates were incubated at 37°C for 24 hours before enumeration; all counts were

recorded as CFU/ml. All treatment and control samples were then placed at 37° C for 48 hours. Following incubation, the bacterial concentration for each sample was determined as previously described.

Results:

Incubation for 48 hours at 37° C		
Carrot Juice	Control - E. coli (1x10 ⁵ /ml)	0.05 ml L. bulgaricus + E. coli (1x10 ⁵ /ml)
1	530,000,000	0
2	710,000,000	0
3	210,000,000	0
Sample Average	483,333,333	0
% Inhibition of 48h Pathogenic Levels by L. bulgaricus		100%

Sincerely,



Andrew B. Onderdonk

September 12, 2014

Clinical Laboratories
75 Francis Street
Boston, Massachusetts 02115
Tel: 617 732-7372, Lab: 617 525-2506
Fax: 617 277-1762
E-mail: aonderdonk@partners.org

Andrew B. Onderdonk, Ph.D.
Director
Clinical Microbiology Laboratory
Professor of Pathology
Harvard Medical School

Pathogen Inhibition Test

Purpose: To determine if *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 inhibits the growth of *Escherichia coli* in vegetable juice.

Test Materials:

- 1) Lyophilized *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 (powder CFU – 1×10^9 /g)
- 2) Vegetable juice (3 bottles of Juice Press Organic Complete Source, 17 fluid ounces each)
- 3) Tryptic Soy Agar Plates (TSA)
- 4) *Escherichia coli* - ATCC 25922

Bacterial Inoculum

E.coli

Previously prepared frozen stock culture of *E. coli* was used for these experiments. The bacterial concentration was 1.32×10^9 /mL.

For the *E. coli* control and test samples:

The frozen tube containing *E. coli* was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:10000) in sterile phosphate buffered saline to achieve a concentration of approximately 1×10^5 /mL. 50ul of this dilution was added to each of the control and test samples to achieve a concentration of approximately 1×10^2 /mL.

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

2 grams of *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 was added to 18 mL of sterile water at room temperature. The solution was mixed using a vortex for 5 minutes to ensure the homogeneity of the mixture. 0.5 ml of the prepared solution was added to each of the test samples.

Test Procedure:

The following test samples were prepared for the *E. coli*.

Control Samples:

5 bottles containing 50 mL each of vegetable juice

Test Samples:

5 bottles containing 50 mL each of boiled vegetable juice plus *Lactobacillus delbrueckii* subsp. *bulgaricus*

E. coli was added to the control and test samples as described above. All of the samples were stored in a refrigerator (4°C) for 24 hours. Following refrigeration, the bacterial concentration for each sample was determined. Serial 10 fold dilutions were made in phosphate buffered saline. A 0.1mL aliquot of each dilution was plated onto TSA. The agar plates were incubated at 37° C for 24 hours before enumeration; all counts were recorded as CFU/mL. All treatment and control samples were then place at 37° C for 48 hours. Following incubation, the bacterial concentration for each sample was determined as previously described.

Results:

Vegetable Juice	Bacterial Incubation for 48h	
	Control - <i>E. coli</i> (CFU)	with <i>L. bulgaricus</i> - <i>E. coli</i> (CFU)
1	87,096	0
2	64,565	0
3	58,884	0
4	30,903	0
5	60,256	0
Sample Average	60,341	0
% Inhibition of 48h Pathogenic Levels by <i>L. bulgaricus</i>		100.00%

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Andrew B. Onderdonk

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Director
Clinical Microbiology Laboratory
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Harvard Medical School

Pathogen Inhibition Test

Purpose: To determine if *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 inhibits the growth of *Escherichia coli* in carrot juice.

Test Materials:

- 1) Lyophilized *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 (powder CFU – 1×10^9 /g, received 06/05/14 from K Petkov)
- 2) Carrot juice (received 06/05/14 from K Petkov)
- 3) Tryptic Soy Agar Plates with 5% sheep blood (TSA)
- 4) *Escherichia coli* - ATCC 35150 (O157:H7)

Bacterial Inoculum

E. coli

Previously prepared frozen stock culture of *E. coli* was used for these experiments. The bacterial concentration was 2.29×10^9 /ml.

For the *E. coli* control and test samples:

The frozen tube containing *E. coli* was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:100000) in sterile phosphate buffered saline to achieve a concentration of approximately 1×10^4 /ml. 50ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /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. Either 0.05 ml or 0.5 ml of the prepared solution was added to 5 ml of test sample.

Test Procedure:

The following samples were prepared for the *E. coli*

Control Samples:

3 tubes containing 5 ml each of carrot juice

Test Samples:

3 tubes containing 5 ml each of carrot juice plus 0.05 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*
 3 tubes containing 5 ml each of carrot juice plus 0.5 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

E. coli was added to the control and test samples as described above. All of the samples were stored in a refrigerator (4°C) for 24 hours. Following refrigeration, the bacterial concentration for each sample was determined. Serial 10 fold dilutions were made in phosphate buffered saline. A 0.1mL aliquot of each dilution was plated onto TSA. The agar plates were incubated at 37° C for 24 hours before enumeration; all counts were recorded as CFU/ml. All treatment and control samples were then place at 37° C for 48 hours. Following incubation, the bacterial concentration for each sample was determined as previously described.

Results:

Carrot Juice	Control - <i>E. coli</i> (CFU)	Incubation for 48 hours at 37° C	
		0.05 ml <i>L. bulgaricus</i> + <i>E. coli</i> (CFU)	0.5 ml <i>L. bulgaricus</i> + <i>E. coli</i> (CFU)
1	285,000,000	10	0
2	137,000,000	10	0
3	198,000,000	0	100
Sample Average	207,000,000	6.7	33.3
% Inhibition of 48h Pathogenic Levels by <i>L. bulgaricus</i>		>99%	>99%

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Director
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Harvard Medical School

Pathogen Inhibition Test

Purpose: To determine if *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 inhibits the growth of *Listeria monocytogenes* in carrot juice.

Test Materials:

- 1) Lyophilized *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 (powder CFU – 1×10^9 /g, received 06/05/14 from K Petkov)
- 2) Carrot juice (received 06/05/14 from K Petkov)
- 3) Tryptic Soy Agar Plates with 5% sheep blood (TSA)
- 4) *Listeria monocytogenes* - ATCC BAA-751
- 5) *Listeria monocytogenes* - BWH 432

Bacterial Inoculum

***L. monocytogenes* ATCC BAA-751**

Previously prepared frozen stock culture of *L. monocytogenes* BAA-751 was used for these experiments. The bacterial concentration was 9.9×10^8 /ml.

For the *L. monocytogenes* BAA-751 control and test samples:

The frozen tube containing *L. monocytogenes* BAA-751 was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:10000) in sterile phosphate buffered saline to achieve a concentration of approximately 9.9×10^4 /ml. 5ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /ml.

***L. monocytogenes* BWH 432**

Previously prepared frozen stock culture of *L. monocytogenes* BWH 432 was used for these experiments. The bacterial concentration was 1.68×10^9 /ml.

For the *L. monocytogenes* BWH 432 control and test samples:

The frozen tube containing *L. monocytogenes* BWH 432 was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:100000) in sterile phosphate buffered saline to achieve a concentration of approximately 1.68×10^4 /ml. 30ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /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. Either 0.05 ml or 0.5 ml of the prepared solution was added to 5 ml of test sample.

Test Procedure:

The following samples were prepared for each *L. monocytogenes* strain

Control Samples:

3 tubes containing 5 ml each of carrot juice

Test Samples:

3 tubes containing 5 ml each of carrot juice plus 0.05 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

3 tubes containing 5 ml each of carrot juice plus 0.5 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

L. monocytogenes BAA-751 or BWH 432 was added to the control and test samples as described above. All of the samples were stored in a refrigerator (4°C) for 24 hours. Following refrigeration, the bacterial concentration for each sample was determined. Serial 10 fold dilutions were made in phosphate buffered saline. A 0.1mL aliquot of each dilution was plated onto TSA. The agar plates were incubated at 37° C for 24 hours before enumeration; all counts were recorded as CFU/ml. All treatment and control samples were then place at 37° C for 48 hours. Following incubation, the bacterial concentration for each sample was determined as previously described.

Results:

Incubation for 48 hours at 37° C

Carrot Juice	Control – L monocyt BAA-751 (CFU)	0.05 ml L. bulgaricus + Lm BAA-751(CFU)	0.5 ml L. bulgaricus + Lm BAA-751 (CFU)
1	3200000	0	0
2	790000	0	0
3	0	0	0
Sample Average	1330000	0	0
% Inhibition of 48h Pathogenic Levels by L. bulgaricus		100%	100%

Incubation for 48 hours at 37° C

Carrot Juice	Control – L monocyt BWH 432 (CFU)	0.05 ml L. bulgaricus + Lm BWH 432 (CFU)	0.5 ml L. bulgaricus + Lm BWH 432 (CFU)
1	420000000	0	0
2	45000000	0	0
3	0	0	0
Sample Average	155000000	0	0
% Inhibition of 48h Pathogenic Levels by L. bulgaricus		100%	100%

Sincerely,



Andrew B. Onderdonk

August 1, 2014

Clinical Laboratories
75 Francis Street
Boston, Massachusetts 02115
Tel: 617 732-7372, Lab: 617 525-2506
Fax: 617 277-1762
E-mail: aonderdonk@partners.org

Andrew B. Onderdonk, Ph.D.
Director
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Harvard Medical School

Pathogen Inhibition Test

Purpose: To determine if *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 inhibits the growth of *Staphylococcus aureus* in carrot juice.

Test Materials:

- 1) Lyophilized *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 (powder CFU – 1×10^9 /g, received 06/05/14 from K Petkov)
- 2) Carrot juice (received 06/05/14 from K Petkov)
- 3) Tryptic Soy Agar Plates with 5% sheep blood (TSA)
- 4) *Staphylococcus aureus* – ATCC 12600
- 5) *Staphylococcus aureus* - ATCC 29213

Bacterial Inoculum

S. aureus 12600

Previously prepared frozen stock culture of *S. aureus* 12600 was used for these experiments. The bacterial concentration was 4.2×10^8 /ml.

For the *S. aureus* 12600 control and test samples:

The frozen tube containing *S. aureus* 12600 was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:10000) in sterile phosphate buffered saline to achieve a concentration of approximately 4.2×10^4 /ml. 12ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /ml.

S. aureus 29213

Previously prepared frozen stock culture of *S. aureus* 29213 was used for these experiments. The bacterial concentration was 1.8×10^8 /ml.

For the *S. aureus* 29213 control and test samples:

The frozen tube containing *S. aureus* 29213 was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:10000) in sterile phosphate buffered saline to achieve a concentration of approximately 1.8×10^4 /ml. 28ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /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. Either 0.05 ml or 0.5 ml of the prepared solution was added to 5 ml of test sample.

Test Procedure:

The following samples were prepared for each *S. aureus* strain

Control Samples:

3 tubes containing 5 ml each of carrot juice

Test Samples:

3 tubes containing 5 ml each of carrot juice plus 0.05 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

3 tubes containing 5 ml each of carrot juice plus 0.5 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

S. aureus 12600 or 29213 was added to the control and test samples as described above. All of the samples were stored in a refrigerator (4°C) for 24 hours. Following refrigeration, the bacterial concentration for each sample was determined. Serial 10 fold dilutions were made in phosphate buffered saline. A 0.1mL aliquot of each dilution was plated onto TSA. The agar plates were incubated at 37° C for 24 hours before enumeration; all counts were recorded as CFU/ml. All treatment and control samples were then place at 37° C for 48 hours. Following incubation, the bacterial concentration for each sample was determined as previously described.

Results:

Incubation for 48 hours at 37° C			
Carrot Juice	Control – <i>S. aureus</i> 12600 (CFU)	0.05 ml <i>L. bulgaricus</i> + <i>S. aureus</i> 12600 (CFU)	0.5 ml <i>L. bulgaricus</i> + <i>S. aureus</i> 12600 (CFU)
1	5,800,000	41,000	0
2	5,600,000	0	0
3	6,200,000	0	0
Sample Average	5,866,667	13,667	0
% Inhibition of 48h Pathogenic Levels by <i>L. bulgaricus</i>		>99%	100%

Incubation for 48 hours at 37° C

Carrot Juice	Control – S. aureus 29213 (CFU)	0.05 ml L. bulgaricus + S. aureus 29213 (CFU)	0.5 ml L. bulgaricus + S. aureus 29213 (CFU)
1	9,200,000	85,000	140
2	9,300,000	105,000	0
3	15,600,000	155,000	960
Sample Average	11,366,667	115,000	550
% Inhibition of 48h Pathogenic Levels by L. bulgaricus		>98%	>99%

Sincerely,



Andrew B. Onderdonk

July 21, 2014

Clinical Laboratories
75 Francis Street
Boston, Massachusetts 02115
Tel: 617 732-7372, Lab: 617 525-2506
Fax: 617 277-1762
E-mail: aonderdonk@partners.org

Andrew B. Onderdonk, Ph.D.
Director
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Pathogen Inhibition Test

Purpose: To determine if *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 inhibits the growth of *Acinetobacter baumannii*, *Enterococcus faecalis* or *Pseudomonas aeruginosa* in carrot juice.

Test Materials:

- 1) Lyophilized *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 (powder CFU – 1×10^9 /g, received 06/05/14 from K Petkov)
- 2) Carrot juice (received 06/05/14 from K Petkov)
- 3) Tryptic Soy Agar Plates with 5% sheep blood (TSA)
- 4) *Acinetobacter baumannii* - ATCC 19606
- 5) *Enterococcus faecalis* - ATCC 29212
- 6) *Pseudomonas aeruginosa* - ATCC 27853

Bacterial Inoculum

Acinetobacter baumannii

Previously prepared frozen stock culture of *A. baumannii* was used for these experiments. The bacterial concentration was 1.5×10^8 /ml.

For the *A. baumannii* control and test samples:

The frozen tube containing *A. baumannii* was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:10000) in sterile phosphate buffered saline to achieve a concentration of approximately 1.5×10^4 /ml. 33ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /ml.

Enterococcus faecalis

Previously prepared frozen stock culture of *E. faecalis* was used for these experiments. The bacterial concentration was 6.0×10^8 /ml.

For the *E. faecalis* control and test samples:

The frozen tube containing *E. faecalis* was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:10000) in sterile phosphate buffered saline to achieve a concentration of approximately 6.0×10^4 /ml. 8ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /ml.

P. aeruginosa

Previously prepared frozen stock culture of *P. aeruginosa* was used for these experiments. The bacterial concentration was 7.9×10^7 /ml.

For the *P. aeruginosa* control and test samples:

The frozen tube containing *P. aeruginosa* was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:1000) in sterile phosphate buffered saline to achieve a concentration of approximately 7.9×10^4 /ml. 6ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /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. Either 0.05 ml or 0.5 ml of the prepared solution was added to 5 ml of test sample.

Test Procedure: The following samples were prepared for *A. baumannii*, *E. faecalis* or *P. aeruginosa*

Control Samples:

3 tubes containing 5 ml each of carrot juice

Test Samples:

3 tubes containing 5 ml each of carrot juice plus 0.05 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

3 tubes containing 5 ml each of carrot juice plus 0.5 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

A. baumannii, *E. faecalis* or *P. aeruginosa* was added to the control and test samples as described above. All of the samples were stored in a refrigerator (4°C) for 24 hours. Following refrigeration, the bacterial concentration for each sample was determined. Serial 10 fold dilutions were made in phosphate buffered saline. A 0.1mL aliquot of each dilution was plated onto TSA. The agar plates were incubated at 37°C for 24 hours before enumeration; all counts were recorded as CFU/ml. All treatment and control samples were then place at 37°C for 48 hours. Following incubation, the bacterial concentration for each sample was determined as previously described.

Results:

Incubation for 48 hours at 37° C

Carrot Juice	Control – <i>A. baumannii</i> (CFU)	0.05 ml <i>L. bulgaricus</i> + <i>A. baumannii</i> (CFU)	0.5 ml <i>L. bulgaricus</i> + <i>A. baumannii</i> (CFU)
1	166,000,000	570,000	0
2	173,000,000	0	0
3	164,000,000	1,270,000	0
Sample Average	168,000,000	613,333	0
% Inhibition of 48h Pathogenic Levels by <i>L. bulgaricus</i>		>99%	100%

Incubation for 48 hours at 37° C

Carrot Juice	Control – E. faecalis (CFU)	0.05 ml L. bulgaricus + E. faecalis (CFU)	0.5 ml L. bulgaricus + E. faecalis (CFU)
1	540,000,000	22,300	3,200,000
2	650,000,000	360,000	4,100,000
3	330,000,000	1,790,000	3,500,000
Sample Average	510,000,000	724,100	3,600,000
% Inhibition of 48h Pathogenic Levels by L. bulgaricus		>99%	>99%

Incubation for 48 hours at 37° C

Carrot Juice	Control – P. aeruginosa (CFU)	0.05 ml L. bulgaricus + P. aeruginosa (CFU)	0.5 ml L. bulgaricus + P. aeruginosa (CFU)
1	1,470,000	0	0
2	35,000,000	0	0
3	10,700,000	0	0
Sample Average	15,723,333	0	0
% Inhibition of 48h Pathogenic Levels by L. bulgaricus		100%	100%

Sincerely,



Andrew B. Onderdonk

July 21, 2014

Clinical Laboratories
75 Francis Street
Boston, Massachusetts 02115
Tel: 617 732-7372, Lab: 617 525-2506
Fax: 617 277-1762
E-mail: aonderdonk@partners.org

Andrew B. Onderdonk, Ph.D.
Director
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Pathogen Inhibition Test

Purpose: To determine if *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 inhibits the growth of *Enterococcus faecalis*, *Enterococcus faecium* or *Staphylococcus aureus* in carrot juice.

Test Materials:

- 1) Lyophilized *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 (powder CFU – 1×10^9 /g, received 06/05/14 from K Petkov)
- 2) Carrot juice (received 06/05/14 from K Petkov)
- 3) Tryptic Soy Agar Plates with 5% sheep blood (TSA)
- 4) *Enterococcus faecalis* - ATCC 29200
- 5) *Enterococcus faecium* - ATCC 51559
- 6) *Staphylococcus aureus* - ATCC 25923

Bacterial Inoculum

Enterococcus faecalis

Previously prepared frozen stock culture of *E. faecalis* was used for these experiments. The bacterial concentration was 9.1×10^8 /ml.

For the *E. faecalis* control and test samples:

The frozen tube containing *E. faecalis* was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:10000) in sterile phosphate buffered saline to achieve a concentration of approximately 9.1×10^4 /ml. 6ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /ml.

Enterococcus faecium

Previously prepared frozen stock culture of *E. faecium* was used for these experiments. The bacterial concentration was 8.1×10^8 /ml.

For the *E. faecium* control and test samples:

The frozen tube containing *E. faecium* was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:10000) in sterile phosphate buffered saline to achieve a concentration of approximately 8.1×10^4 /ml. 6ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /ml.

S. aureus 25923

Previously prepared frozen stock culture of *S. aureus* was used for these experiments. The bacterial concentration was 9.4×10^7 /ml.

For the *S. aureus* control and test samples:

The frozen tube containing *S. aureus* was removed from the freezer and allowed to thaw at room temperature. The bacteria were diluted (1:1000) in sterile phosphate buffered saline to achieve a concentration of approximately 9.4×10^4 /ml. 5ul of this dilution was added to 5 ml each of the control and test samples to achieve a concentration of approximately 1×10^2 /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. Either 0.05 ml or 0.5 ml of the prepared solution was added to 5 ml of test sample.

Test Procedure: The following samples were prepared for *E. faecalis*, *E. faecium* or *S. aureus*

Control Samples:

3 tubes containing 5 ml each of carrot juice

Test Samples:

3 tubes containing 5 ml each of carrot juice plus 0.05 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

3 tubes containing 5 ml each of carrot juice plus 0.5 ml *Lactobacillus delbrueckii* subsp. *bulgaricus*

E. faecalis, *E. faecium* or *S. aureus* was added to the control and test samples as described above. All of the samples were stored in a refrigerator (4°C) for 24 hours. Following refrigeration, the bacterial concentration for each sample was determined. Serial 10 fold dilutions were made in phosphate buffered saline. A 0.1mL aliquot of each dilution was plated onto TSA. The agar plates were incubated at 37° C for 24 hours before enumeration; all counts were recorded as CFU/ml. All treatment and control samples were then place at 37° C for 48 hours. Following incubation, the bacterial concentration for each sample was determined as previously described.

Results:

Incubation for 48 hours at 37° C

Carrot Juice	Control – <i>E. faecalis</i> (CFU)	0.05 ml <i>L. bulgaricus</i> + <i>E. faecalis</i> (CFU)	0.5 ml <i>L. bulgaricus</i> + <i>E. faecalis</i> (CFU)
1	700,000,000	2,080,000	5,200,000
2	690,000,000	640,000	9,200,000
3	720,000,000	1,090,000	6,400,000
Sample Average	703,333,333	1,270,000	6,933,333
% Inhibition of 48h Pathogenic Levels by <i>L. bulgaricus</i>		>99%	98.6%

Incubation for 48 hours at 37° C

Carrot Juice	Control – <i>E. faecium</i>	0.05 ml <i>L. bulgaricus</i> +	0.5 ml <i>L. bulgaricus</i> +
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	(CFU)	E. faecium (CFU)	E. faecium (CFU)
1	1,060,000,000	45,000,000	3,600,000
2	1,070,000,000	45,000,000	2,500,000
3	1,190,000,000	64,000,000	4,400,000
Sample Average	1,106,666,667	51,333,333	3,500,000
% Inhibition of 48h Pathogenic Levels by L. bulgaricus		>99%	>99%

Incubation for 48 hours at 37° C

Carrot Juice	Control – S. aureus (CFU)	0.05 ml L. bulgaricus + S. aureus (CFU)	0.5 ml L. bulgaricus + S. aureus (CFU)
1	0	0	0
2	65,000,000	100	0
3	90,000,000	1,820	0
Sample Average	51,700,000	640	0
% Inhibition of 48h Pathogenic Levels by L. bulgaricus		>99%	100%

Sincerely,



Andrew B. Onderdonk

August 15, 2014