DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Tyler Homer	Chief Operations Officer

Organization *(if applicable)*: OmniLytics, Inc.

Mailing Address *(number and street)*: 9075 South Sandy Parkway

City	State or Province	Zip Code/Postal Code	Country
Sandy	Utah	84070	United States of America

Telephone Number	Fax Number	E-Mail Address
801-746-3600	801-746-3461	thomer@omnilytics.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Tyler Homer	Chief Operations Officer

Organization *(if applicable)*: OmniLytics, Inc.

Mailing Address *(number and street)*: 9075 South Sandy Parkway

City	State or Province	Zip Code/Postal Code	Country
Sandy	Utah	84070	United States of America

Telephone Number	Fax Number	E-Mail Address
801-746-3600	801-746-3461	thomer@omnilytics.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

ECLYPSE-STEC (E. coli-specific bacteriophage cocktail)

2. Submission Format: *(Check appropriate box(es))*

☒ Electronic Submission Gateway ☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

ECLYPSE-STEC™ is intended for use as an antimicrobial processing aid to control *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145 on food, when applied to food surfaces up to $1x10^8$ PFU (Plaque Forming Units) per

Food categories include: poultry, red meat, fruits, vegetables, eggs, fish, and shellfish.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☒ Yes ☐ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☒ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Tyler Homer
(name of notifier)

has concluded that the intended use*(s)* of ECLYPSE-STEC (E. coli-specific bacteriophage cocktail)
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Tyler Homer *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

9075 South Sandy Parkway, Sandy, UT 84070
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
	Tyler Homer, Chief Operations Officer	10/04/2018

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	ECLYPSE-STEC - GRAS Notice.pdf	Submission

OmniLytics, Inc.

GRAS Notification:

ECLYPSE-STEC™

OmniLytics, Inc.
9075 South Sandy Parkway, Sandy, UT 84070
Tel: 801-746-3600; Fax: 801-746-3461

October 4, 2018

Lane A. Highbarger, Ph.D.
Division of Biotechnology and GRAS Notice Review
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Reference: OmniLytics, Inc. GRAS Notification for ECLYPSE-STEC™

Dear Dr. Highbarger:

In accordance with 21 CFR Part 170 Subpart E, OmniLytics, Inc. is submitting a GRAS notification for the bacteriophage cocktail ECLYPSE-STEC™ for bio-control of O157:H7 and the top non-O157 Shiga toxin-producing *Escherichia coli* (STEC) in food: O26, O45, O103, O111, O121, and O145. OmniLytics has determined, through scientific procedures, that ECLYPSE-STEC™ is GRAS and therefore not subject to the pre-market approval requirements.

We also request that a copy of this notification be shared with the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) for determining the efficacy and suitability of ECLYPSE-STEC™ for use in meat, poultry, and egg products.

ECLYPSE-STEC™ is to be used as a processing aid and is substantially similar to many other GRAS notifications of bacteriophage products.

Please let me know if you have any questions.

Best regards

Dr. Ryan Bringhurst
Senior Research Scientist
OmniLytics, Inc.

Table of Contents

Part 1 Signed Statements and Certification

1.1 Compliance with 21 CFR 170 Subpart E

OmniLytics, Inc. is hereby submitting a GRAS notice in accordance with 21 CFR 170 Part E.

1.2 Name and Address of Notifier

OmniLytics, Inc.
9075 South Sandy Parkway
Sandy, UT 84070
Phone: 801-746-3600
Fax: 801-746-3461

1.3 Common or Usual Name

OmniLytics, Inc. produces an *Escherichia coli* specific bacteriophage cocktail under the trade name ECLYPSE-STEC™.

1.4 Intended Conditions of Use

ECLYPSE-STEC™ is intended for use as an antimicrobial processing aid to control *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145 on food, when applied to food surfaces up to $1x10^8$ PFU (Plaque Forming Units) per gram of food.

Food categories include:

- Poultry
- Red meat
- Fresh and processed fruits
- Fresh and processed vegetables
- Eggs
- Fish and shellfish

1.5 Basis for GRAS Determination

Pursuant to 21 CFR 170.30 (a) and (b), OmniLytics, Inc. has determined that ECLYPSE-STEC™ is GRAS through scientific procedures.

1.6 Exemption from Premarket Approval

ECLYPSE-STEC™ was determined by OmniLytics to be GRAS and is therefore exempt from premarket approval requirements when used under the intended use conditions described within this notification.

1.7 Availability of Information

The data and information that are the basis for OmniLytics' determination of GRAS for ECLYPSE-STEC™ are available for review and copying by FDA during customary business hours at the location below or will be send to FDA upon request, made to:

Tyler Homer
OmniLytics, Inc.
9075 South Sandy Parkway
Sandy, UT 84070
Email: thomer@omnilytics.com
Phone: 801-746-3600

A complete copy of data and information will be provided in an electronic format that is accessible for evaluation or on paper.

1.8 Freedom of Information Act

The information contained in parts 2 through 7 of this notification is not exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

1.9 Certification

The undersigned certifies that to the best of their knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to OmniLytics, Inc. and pertinent to the evaluation of the safety and GRAS status of the use of ECLYPSE-STEC™.

1.10 Signature

Ryan Bringhurst

Digitally signed by Ryan Bringhurst
DN: cn=Ryan Bringhurst, o=OmniLytics, ou, email=rbringhurst@omnilytics.com, c=US
Date: 2018.10.04 08:47:21 -06'00'

10/4/2018

Dr. Ryan Bringhurst
Senior Research Scientist
OmniLytics, Inc.

Date

1.11 FSIS Authorization

We request that a complete copy, including trade secrets, of this notification be shared with the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) for determining the efficacy and suitability of ECLYPSE-STEC™ for use in meat, poultry, and egg products as a processing aid.

Part 2 Identity and Specifications of ECLYPSE-STEC™

2.1 Identity

ECLYPSE-STEC™ consists of a mixture of equal concentrations of three *E. coli* specific lytic bacteriophages (hereinafter referred to as "monophage(s)". These monophages are specifically effective against Shiga toxin-producing *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145. These phages were isolated by OmniLytics scientists from city waterways in the US.

ECLYPSE-STEC™ is a liquid concentrate made up of equal parts of three monophages (MLF4, OLB35, and OLB145), which are produced and purified separately and mixed in equal phage concentrations. The commercial product ECLYPSE-STEC™ has a minimal titer of $1x10^{10}$ PFU/mL. This solution is concentrated and will be diluted with water at application sites to ensure application rate at a maximum of $1x10^{8}$ PFU/g of food.

2.1.1 Phage Identity

The monophages were isolated by OmniLytics scientists from city waterways in the US. The phages were isolated from the natural environment and have not been genetically modified. Each phage was fully characterized by a variety of methods, including polymerase chain reaction (PCR), full-genome analysis, lytic activity against a large number of *E. coli* O157:H7 strains, and lytic activity against non-*E. coli* O157:H7-related bacteria strains.

Name: MLF4
Order: Caudovirales
Family: *Myoviridae*
Properties: Double-stranded DNA, lytic

Name: OLB35
Order: Caudovirales
Family: *Myoviridae*
Properties: Double-stranded DNA, lytic

Name: OLB145
Order: Caudovirales
Family: *Podoviridae*
Properties: Double-stranded DNA, lytic

The DNA genome of phages MLF4, OLB35, and OLB145 were sequenced and deposited in GenBank. Accession numbers: MH992121 for MLF4, MH992122 for OLB35, and MH992123 for OLB145.

2.1.2 Host Identity

Each monophage is produced in non-pathogenic hosts; 11-1178D for MLF4, 43888™ for OLB35, and 12-799F for OLB145. 43888™ is an ATCC™ *Escherichia coli* strain that has been widely studied because of its lack of pathogenicity, and according to www.atcc.org the strain "does not produce either Shiga-like toxin I or II and does not possess the genes for these toxins". 11-1178D and 12-799F are from OmniLytics' library and have been PCR verified that they do not contain Shiga toxins.

The production hosts were also tested for antibiotic resistance against tetracycline, chloramphenicol, kanamycin, nalidixic acid, nitrofurantoin, and penicillin. The production hosts were sensitive to all antibiotics, except penicillin. Resistance to penicillin is not an issue because the production host is completely removed during the down-stream processing and each batch goes through rigorous QC testing (Tables 2 & 3). In addition, the bacteria inoculum for fermentation is always derived from the original master stock, which prevents the development of resistance to the other antibiotics.

The one undesirable host-derived components including host DNA and Lipopolysaccharides (LPS or endotoxins) are removed by clarification and purification and will be described in sections 2.1.6, 3.6, and 6.4.1.

2.1.3 Host Range

Host range studies were conducted by OmniLytics, Inc. scientists on 113 *E. coli* isolates.

- O157:H7 – 45 strains
- O26 – 15 strains
- O45 – 11 strains
- O103 – 12 strains
- O111 – 18 strains
- O121 – 7 strains
- O145 – 5 strains

ECLYPSE-STEC™ was shown to be *E. coli* specific and has a broad host range. Lytic activity was demonstrated on over 96% of the tested *E. coli* strains.

ECLYPSE-STEC™ was also tested 17 non-*E.coli* strains and did not show any lytic activity against the panel. Most importantly, MLF4, OLB35, and OLB145 are non-transducing phages and cannot integrate or transfer genes.

The tested non-*E. coli* strains included, *Salmonella, Bacillus subtilis, Enterobacter aerogenes, Pantoea agglomerans, Enterococcus faecalis, Listeria monocytogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus epidermidis, Listeria innocua,* and *Staphylococcus aureus*.

2.1.4 ECLYPSE-STEC™ Characteristics

ECLYPSE-STEC™ is a clear to opalescent, odorless liquid with an average phage weight of $8.97x10^{7}$ Dalton or $1.49x10^{-16}$ grams.

TABLE 1

MONOPHAGE WEIGHT

	Number of Base Pairs	Weight/Phage #bp x 660 (Dalton)	Weight/Phage (grams)	Weight in 1 mL of ECLYPSE-STEC™ $1x10^{10}$ PFU/mL (grams)
MLF4	167,379	110,470,140	$1.83x10^{-16}$	$6.10x10^{-7}$
OLB35	169,140	111,632,400	$1.85x10^{-16}$	$6.18x10^{-7}$
OLB145	70,173	46,314,180	$7.69x10^{-17}$	$2.56x10^{-7}$
Average	135,564	89,472,240	$1.49x10^{-16}$	$1.49x10^{-6}$
Total	406,692	268,416,720	$4.46x10^{-16}$	

*1 bp DNA = 660 Dalton

1 mL of ECLYPSE-STEC™ has a total phage weight of $1.49x10^{-6}$ g, with the remainder of the weight being attributed to the buffer consisting of 1.0 mM dipotassium phosphate, 10 mM magnesium sulfate, and 10 mM sodium chloride (0.174 mg/mL K_2HPO_4, 2.46 mg/mL $MgSO_4 \cdot 7H_2O$, and 0.584 mg/mL NaCl). The monophages are estimated to be 0.00015% of the total weight of the concentrated liquid.

2.1.5 ECLYPSE-STEC™ Specifications

Quality control consists of 2 steps: each monophage batch needs to pass the specification tests from Table 2, and each batch of the final cocktail of ECLYPSE-STEC™ needs to pass the specification tests from Table 3. The Quality Control tests consist of analyzing:

a) **The Potency:** Standard phage titration protocols are used to confirm lytic activity. Batched that are < $1x10^{10}$ PFU/mL may be concentrated and retested.

b) **The Identity:** Identity is determined by specific PCR with predetermined reference profiles or a bacteria reference panel.

c) **The Bacterial sterility:** Sterility is tested by plating 100 µL aliquots of the monophage or final cocktail onto 3 non-selective LB plates and incubating them at 30°C for 7 days. If any bacterial colonies appear after 7 days, the product must be re-filtered or discarded.

d) **The Endotoxin Content:** Endotoxin content is tested by using a commercially available quantitative LAL-based assay. If the batch fails the quality standard, the batch can be washed again with buffer and then be retested (potency and bacterial sterility must also be retested).

TABLE 2

QUALITY CONTROL OF INDIVIDUAL MONOPHAGE BATCHES

PARAMETER	SPECIFICATIONS
Potency	$\geq 1x10^{10}$ PFU/mL
Identity	PCR: Matches reference bands or bacteria panel
Bacterial sterility	No growth after 7 days

TABLE 3

QUALITY CONTROL OF ECLYPSE-STEC™

PARAMETER	SPECIFICATIONS
Potency	$\geq 1x10^{10}$ PFU/mL
Identity	PCR: Matches reference bands or bacteria panel
Endotoxin Content	< 250,000 EU/mL for concentrated product containing $1x10^{10}$ PFU/mL
Bacterial sterility	No growth after 7 days

TABLE 4

PHYSICAL PROPERTIES

Physical properties	**ECLYPSE-STEC™ Lot # 93-048002**	**ECLYPSE-STEC™ Lot # 93-048003**	**ECLYPSE-STEC™ Lot # 93-048004**
Odor	Odorless	Odorless	Odorless
Color	Opalescent	Opalescent	Opalescent
Physical State & Appearance	Liquid	Liquid	Liquid
pH	7.40	7.46	7.38
Endotoxin (EU/mL)	12,363	11,115	9,623
Solubility	Soluble in water	Soluble in water	Soluble in water

* All tests were conducted by OmniLytics, Inc.

TABLE 5

CHEMICAL COMPOSITION

	Units	Detection Limit	ECLYPSE-STEC™ Lot# 93-048002	ECLYPSE-STEC™ Lot# 93-048003	ECLYPSE-STEC™ Lot# 93-048004	Average	Standard Deviation
Specific Gravity	g/mL	0.001	**0.996**	**0.997**	**1.003**	0.9987	0
Nitrate + Nitrite, Total	mg/L	0.1	**1.1**	**1.8**	**ND**	1.45	0.35
Total Kjeldahl Nitrogen	mg/L	1.0	**85.2**	**81.9**	**82.3**	83.13	1.47
Total Nitrogen	mg/L	1.0	**86.3**	**83.7**	**82.3**	84.10	1.66
Total Organic Carbon	mg/L	5.0	**104**	**111**	**164**	126.3	26.79
Arsenic, Total	mg/L	0.0005	**ND**	**ND**	**ND**	N/A	N/A
Calcium, Total	mg/L	0.2	**0.6**	**0.5**	**0.4**	0.50	0.082
Copper, Total	mg/L	0.0010	**0.0112**	**0.0100**	**0.0046**	0.00860	0.0029
Iron, Total	mg/L	0.02	**0.04**	**0.04**	**0.04**	N/A	N/A
Lead, Total	mg/L	0.0005	**ND**	**ND**	**ND**	N/A	N/A
Magnesium, Total	mg/L	0.2	**256**	**256**	**247**	253.0	4.24
Manganese, Total	mg/L	0.0005	**0.0148**	**0.0116**	**0.0097**	0.01203	0.00210
Mercury, Total	mg/L	0.0002	**ND**	**ND**	**ND**	N/A	N/A
Phosphorus, Total as P	mg/L	0.2	**77.1**	**76.9**	**69.1**	74.4	3.72
Potassium, Total	mg/L	0.5	**87.4**	**86.8**	**83.7**	86.0	1.62
Sodium, Total	mg/L	0.5	**245**	**245**	**252**	247.33	3.30

ND = not detected

* All tests were conducted by Chemtech-Ford Laboratories.

2.1.6 Know Toxins

Endotoxin is the only known human toxin present in ECLYPSE-STEC™ commercial product. The non-pathogenic *E. coli* strains used for manufacturing are Gram-negative bacteria. As with all Gram-negative bacteria, they produce bacterial endotoxins or lipopolysaccharide (LPS). Each batch of ECLYPSE-STEC™ is tested for LPS content to ensure it meets the release criteria. Endotoxins are further discussed below in sections 3.6 and 6.4.1. As tested, the selected non-pathogenic *E. coli* strains used for monophage production do not contain any virulence genes.

2.1.7 Stability

The proposed shelf-life of ECLYPSE-STEC™ is one year when stored at 2-6°C in a dark, UV-protected area.

2.2 Method of Manufacture

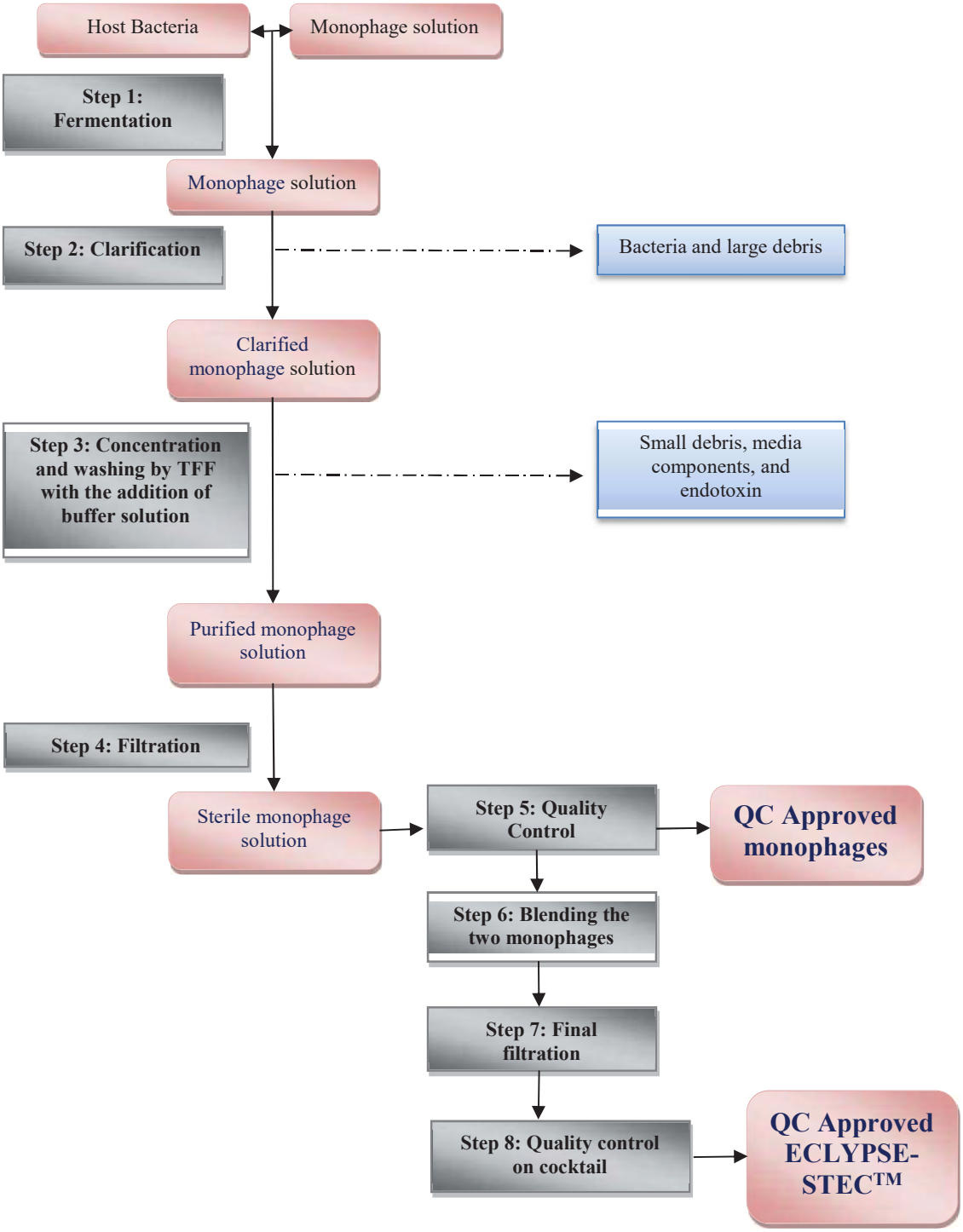
Batches of the three monophages are produced separately by aerobic fermentation using a broth media which is animal-product free. Initially, the host bacteria (non-pathogenic *E. coli*) is grown from a working bank sample (itself derived from the master bank) to a pre-determined optical density (OD) in an animal-free medium. Each monophage (from a working bank sample) is then added at a pre-determined multiplicity of infection (MOI; phage to bacteria ratio). The culture is incubated under specific aeration and agitation conditions.

After a determined time of incubation, the culture is clarified by filtration to remove bacteria cell debris. The filtrate is washed with a buffer (1.0 mM dipotassium phosphate, 10 mM magnesium sulfate, and 10 mM sodium chloride) and concentrated by tangential flow filtration (TFF). Most endotoxins are expected to be removed during clarification and washing. The monophage solution is then filtered with a sterile-grade 0.2 μm filter.

Finally, after each monophage solution has passed the Quality Control (QC) specification steps (Table 2), they are blended and diluted to form ECLYPSE-STEC™ for commercialization, with each phage representing 1/3 of the minimum final product titer of $1x10^{10}$ PFU/mL. ECLYPSE-STEC™ is filtered with a sterile-grade 0.2 μm filter, packaged into sterile packaging components, and placed in refrigerated storage (2-6°C). Quality Control (QC) is performed on each final batch (Table 3). Only after passing QC testing is the batch released for sale.

ECLYPSE-STEC™ is diluted with water at the application site to form the "working solution" with a maximum lytic activity of $1x10^{9}$ PFU/mL. Figure 1 is an overview of the manufacturing process.

Figure 1: Overview of ECLYPSE-STEC™ Method of Manufacturing



2.3 Food-Grade Material

All components used in the manufacturing of ECLYPSE-STEC™ are animal-free and food grade. The final ECLYPSE-STEC™ product contains no preservatives, known allergenic substances, or additives.

Part 3 Dietary Exposure

3.1 Application Rates

For the dietary exposure estimation, the assumption is that ECLYPSE-STEC™ will be diluted and applied at the maximum rate of $1x10^8$ PFU/g of food.

3.2 Dietary Intakes

ECLYPSE-STEC™ is expected to be used on the following foods:

- Poultry
- Red meat
- Fruit
- Vegetables
- Eggs
- Fish and shellfish

The estimated daily dietary intake of each food was determined by data collected from USDA's Food Availability (Per Capita) Data System. The Loss-Adjusted Food Availability database, updated on 7/26/2017, was used to obtain the estimated average daily food consumption. It is also assumed that all foods on the market have been treated with ECLYPSE-STEC™ and 100% of the available food will be consumed without waste. Thus, this estimated dietary exposure is much higher than expected consumption.

TABLE 6

AVERAGE AMERICAN FOOD CONSUMPTION

HTTPS://WWW.ERS.USDA.GOV/DATA-PRODUCTS/FOOD-AVAILABILITY-PER-CAPITA-DATA-SYSTEM/

	Average Annual Per Capita Consumption (lbs)	Average Daily Per Capita Consumption (g)
Poultry	59.0	73.3
Red Meat	71.4	88.7
Fruit	115.4	335.1
Vegetables	156.3	194.3
Eggs	19.5	24.3
Fish & Shellfish	9.4	11.6
Total	**431.0**	**727.3**

3.3 Estimated Dietary Exposure to ECLYPSE-STEC™ Bacteriophages

The following calculation estimates the consumption of ECLYPSE-STEC™ when using a working solution of $1x10^9$ PFU/mL and applied at $1x10^8$ PFU/g of food:

Number of ECLYPSE-STEC™ phage per gram multiplied by the average daily per capita consumption in grams equals the total number of phages consumed per day.

$$\frac{1x10^8 \text{ P U}}{\text{g}} \times 727.3 \text{ g} = \mathbf{7.27x10^{10} \text{ PFU per day}}$$

The total amount of phages consumed per day multiplied by the average phage weight (see section 2.1.4), then divided by the daily average diet weight equals the daily concentration of phage consumption.

Assuming an average diet is 3 kg per day, the dietary concentration of phages is:

$$\frac{7.27x10^{10} \text{ P U} \times 1.49x10^{-16} \text{ g}}{3000} = \mathbf{3.58 \text{ pp}}$$

3.4 Estimated Dietary Exposure to ECLYPSE-STEC™

The following calculation estimates the consumption of ECLYPSE-STEC™:

ECLYPSE-STEC™ is diluted with water to a working concentration of $1x10^9$ PFU/mL and applied at a maximum rate of $1x10^8$ PFU/g of food. One gram of food is treated with 0.1 mL of ECLYPSE-STEC™.

The average American consumes a total of 727.3 g of food daily that has been treated with ECLYPSE-STEC™ and will consume 72.73 mL of ECLYPSE-STEC™ at a maximum concentration of $1x10^9$ PFU/mL.

$$727.3 \text{ g} \times 0.1 \text{ mL} = \mathbf{72.73 \text{ mL}}$$

3.5 Estimated Dietary Exposure to Dipotassium Phosphate, Magnesium Sulfate & Sodium Chloride

As indicated in section 2.1.4, only 0.00015% of ECLYPSE-STEC™ is bacteriophages and the remainder 99.99985% is 1.0 mM dipotassium phosphate, 10 mM magnesium sulfate, and 10 mM sodium chloride (0.174 mg/mL K_2HPO_4, 2.46 mg/mL $MgSO_4 \cdot 7H_2O$, and 0.584 mg/mL NaCl).

Diluted ECLYPSE-STEC™ contains 0.0174 mg/mL K_2HPO_4, 0.246 mg/mL $MgSO_4 \cdot 7H_2O$, and 0.0584 mg/mL NaCl.

The average American will consume an estimated maximum of 72.73 mL of ECLYPSE-STEC™ per day and each mL has 0.0174 mg/mL K_2HPO_4, 0.246 mg/mL $MgSO_4 \cdot 7H_2O$, and 0.0584 mg/mL NaCl.

$$72.73 \text{ mL} \times 0.0174 \text{ mg} = \mathbf{1.27 \text{ mg dipotassium phosph te}}$$

$$72.73 \text{ mL} \times 0.246 \text{ mg} = \mathbf{17.89 \text{ mg m gnesium sulfate}}$$

$$72.73 \text{ mL} \times 0.0584 \text{ mg} = \mathbf{4.25 \text{ mg sodium chloride}}$$

This amounts to 1.27 mg of dipotassium phosphate, 17.9 mg of magnesium sulfate, and 4.25 mg of sodium chloride per day.

3.6 Estimated Dietary Exposure to Endotoxins

Bacterial endotoxins, found in the outer membrane of Gram-negative bacteria are members of a class of phospholipids called lipopolysaccharides (LPS). As a consequence, endotoxins are found everywhere in the environment and consumed by humans on a daily basis. Also Gram-negative organisms releasing LPS are found in very high numbers in our intestines. In the bloodstream, endotoxins can lead to toxic shock syndrome and regulations exist for medicinal reparations that are injected.

No regulations exist for food; moreover foodstuffs could contain high levels of endotoxins. For example, Jay *et al.,* (1979) found endotoxin levels in ground beef in ranges of 500-75,000 EU/g. Townsend *et al.,* (2007) investigated the presence of endotoxins in infant formula in 75 samples collected from seven countries (representing 31 brands). The endotoxin levels ranged from 40 to 55,000 EU/g and did not correlate with the number of viable bacteria. Gehring *et al.,* (2008) measured endotoxin in approximately 400 farm milk and shop milk samples and found levels ranging from 100,000 to 1,000,000 EU/mL of milk samples in Switzerland and Germany.

Additionally, Gram-negative organisms living in the oral cavity also produce endotoxin and Leenstra *et al.* (1996) showed that saliva contains approximately 1 mg of endotoxin/mL. In a nationwide study, Thorne *et al.* (2009) assayed 2,552 house dust samples, the weighted geometric mean endotoxin concentration ranged from 18.7 to 80.5 EU/mg for 5 sampling locations in the houses, and endotoxin load ranged from 4,160 to 95,000 EU/m^2.

Complete removal of endotoxin during the production process of ECLYPSE-STEC™ is not feasible. However, following removal of cellular debris, endotoxin levels are extremely low, and will not significantly contribute to the daily dietary intake of endotoxins by consumers.

The Limulus Amoebocyte Lysate (LAL) method was used to detect and quantify Gram-negative bacteria endotoxins (aka: lipopolysaccharides [LPS], or endogenous pyrogens) that may be present in biotechnological product.

The LAL method was used to detect endotoxin levels in each lot of ECLYPSE-STEC™ purified lots produced. The level of Endotoxin in each of the 3 purified lots was less than *250,000 EU/mL.* Using the maximum allowed for product release, we can calculate the daily consumption of endotoxins:

$$\frac{72.73\ \text{mL}}{\text{day}} \times \frac{2.5\text{x}10^{5}\ \text{EU}}{\text{mL}} = \frac{\mathbf{1.82\text{x}10^{7}\ EU}}{\mathbf{day}}$$

Human saliva contains approximately 1 mg of endotoxins/mL (Leenstra et al. 1996) which is equivalent to $1\text{x}10^{6}$ EU/mL. Saliva is produced at levels exceeding 500 mL/day, which amounts to $5\text{x}10^{8}$ EU/day. The maximum amount of ECLYPSE-STEC™ only constitutes 3.6% of the daily endotoxin load from saliva and is thus considered safe.

Part 4 Self-limiting Levels of Use

The proposed use of ECLYPSE-STEC™ is as an antibacterial processing aid for foods that are at high risk to be contaminated with Shiga toxin-producing *E. coli*. The purpose of ECLYPSE-STEC™ is to significantly reduce or eliminate toxic *E. coli* in the finished product.

The use of the product and potential intake would be self-limiting levels by several factors:

- Due to the cost of the product, the manufacturer would use the minimum dose required to achieve the desired reduction levels of *E. coli*.

- After the *E. coli* host is depleted, the phage will stop replicating and will degrade; virions consist of only proteins and DNA.

- The bacteriophages of cannot replicate or survive in the natural environment without a viable bacterial host. Thus no long-term technical effect is expected with ECLYPSE-STEC™.

- Phages are susceptible to a variety of environmental factors, including sunlight (K. Eric Wommack et al. 1996), heat (Quiberoni, Guglielmotti, and Reinheimer 2003), and UV light (Rigvava et al. 2013). Exposure to these environmental factors will cause the number of phages to decrease.

Part 5 Experience Based on Common Use in Food Before 1958

This part is not applicable to this GRAS notification.

Part 6 Narrative

OmniLytics' determination of ECLYPSE-STEC™ as GRAS is based on scientific procedures and will be shown in the following sections.

6.1 Background on *E. coli*

Shiga toxin-producing *E. coli* (STEC) is a common cause of severe food-borne diseases worldwide, causing severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. According to the CDC, "Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons." (https://www.cdc.gov/ecoli/general/index.html). *E. coli* is considered to be one of the principal causes of zoonotic disease reported worldwide. The top 7 STECs (O157, O26, O45, O103, O111, O121, and O145) are responsible for the majority of the outbreaks, and most events are reported to be due to consumption of foods contaminated with Shiga toxin-producing *E. coli*. "Cattle are a major reservoir of O157 and non-O157 STEC, which harbor the organisms in the hindgut and shed in the feces. Consumption of water, beef and fresh produce contaminated with cattle feces leads to human illnesses"(Shridhar et al. 2017).

> Shiga toxin (Stx) is one of the most potent bacterial toxins known. Stx is found in *Shigella dysenteriae* 1 and in some serogroups of *Escherichia coli* (called Stx1 in *E. coli*). In addition to or instead of Stx1, some *E. coli* strains produce a second type of Stx, Stx2, that has the same mode of action as Stx/Stx1 but that is antigenically distinct.
>
> The Stx(s) act as ribotoxins that halt protein synthesis within the cell and induce apoptosis, but can also prompt altered gene/protein expression in epithelial cells, endothelial cells, monocytes, and mesangial cells. (Melton-Celsa 2014).

Each year in the United States, *E. coli* causes approximately 99,392 illnesses, 2,625 hospitalizations, and 115 deaths (https://wwwn.cdc.gov/norsdashboard/). Total estimated costs of STEC infections in the US are over $300 million annually. (https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx)

> *E. coli* O157:H7 is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Faecal contamination of water and other foods, as well as cross-contamination during food preparation (with beef and other meat products, contaminated surfaces and kitchen utensils), will also lead to infection.
>
> An increasing number of outbreaks are associated with the consumption of fruits and vegetables (including sprouts, spinach, lettuce, coleslaw, and salad) whereby contamination may be due to contact with faeces from domestic or wild animals at some stage during cultivation or handling. STEC has also been isolated from bodies of water

(such as ponds and streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments. Waterborne transmission has been reported, both from contaminated drinking-water and from recreational waters. (http://www.who.int/mediacentre/factsheets/fs125/en/)

Antibiotic-resistance will inevitably increase worldwide as the bacteria they are meant to kill mutate and multiply. "Multidrug resistant strains of *E. coli* are a matter of concern as resistance genes are easily transferable to other strains" (Rasheed et al. 2014). Antibiotic-resistant pathogens constitute a worsening global health problem exacerbated by interconnected travel, antibiotic overuse, horizontal gene transfer, and bacterial evolution. New classes of antimicrobials are needed to treat these pathogens but the drug development pipeline is dry (Boucher et al. 2009; Freire-Moran et al. 2011). As a result, regulatory agencies worldwide have shown a renewed interest in novel biocontrol measures; and phages, are considered as the single most promising processing aid (Nilsson 2014). Multi-phage cocktails have been shown to protect against bacteria developing a resistance to phages (Sulakvelidze 2001).

6.2 Lytic Phages are GRAS

6.2.1 Lytic versus Lysogenic: All Lytic Phages are GRAS

Phages can be classified into two broad categories: lytic (virulent) and lysogenic (temperate).

- Lytic phages are viruses that attack and kill specific bacteria, adhering to specific cell-surface proteins. Once attached to the bacterial host, phages inject their genetic material into the cytoplasm of the host cell, hijacking the bacterium's replication machinery via the expression of specific enzymes encoded by the phage genome, which redirects the bacterial synthesis machinery to reproduction of the new phage particles. The production of phage's enzymes in the later stage, such as lysins and holins, induce destruction of the cell membrane, enabling the newly formed virions to burst out from the lysed bacterial host cell into the extracellular environment. The lytic cycle of the virulent phages fit the class of 'natural antimicrobial controlling agents'.

- Temperate phages, in addition to being capable to enter the lytic cycle, possess the ability to persist as a Prophage in the genome of their bacterial host in the lysogenic cycle. The phage genome remains in a repressed state in the host genome and is replicated as part of the bacterial chromosome until lytic cycle is induced. Hence, temperate phages are not suitable for direct therapeutic use as they may mediate transduction by transferring genetic material of one bacterium to the other.

The biology of lytic phages has been exhaustively studied, demonstrating their safety. Development of recent techniques and the power of comparative genomics are moving us towards more satisfying answers about bacteriophages' biology and understanding the bacteria-phage interaction (Koskella and Meaden 2013). These studies have clearly shown that phages

are obligate intracellular parasites of bacteria and are not infectious or toxic to humans or other mammals.

The host range of a bacteriophage, defined by which bacteria strains can be infected, depends on the host cell surface receptor (proteins, lipopolysaccharide, or other surface components) recognized through functional receptors located on their tail extremity (Kutter and Sulakvelidze 2005). Many phages are known to be highly specific for their receptors and are therefore characterized by a narrow host range, limiting their infectivity to a single species or to specific bacterial strains within a species (Chan, Abedon, and Loc-Carrillo 2013). However, some phages show a broader host range allowing them to infect a large number of strains within a bacterial species, the application of such phages may help prevent an incidence of foodborne diseases caused by pathogens like *Escherichia, Salmonella, Campylobacter, Listeria,* and others. For example Micreos' Listex™, a phage preparation containing a single *Listeria monocytogenes* lytic phage, P100, is used for biocontrol of all *Listeria* strains in susceptible foodstuffs.

Bacteriophages serve as the natural counterbalance to bacteria and herewith have become the most abundant and diverse biological entities on Earth (10^{30}-10^{32}). They are approximately 10 times more abundant than bacteria and archaea. Bacteriophages are probably the most diverse micro-organisms identified on Earth, and in theory, all bacteria are susceptible to viral infection, often by several types of phages (Ackermann and DuBow 1987).

6.2.2 The Major Advantages of Lytic Phages

- Lytic phages replicate exponentially and eradicate the bacteria rapidly regardless of their antibiotic-resistance profile.
- Most lytic phages display very limited host range even among specific bacteria and bacteria strains;
- Phages are **self-replicating and self-limiting**: *In situ* activity increases numbers (though only given favorable bacterial densities).
- Lytic phages have a reduced potential for bacterial development of resistance. They constantly evolve as do the bacteria and overcome mutating resistant bacteria strains.
- Antibiotic-resistant bacteria tend to retain phage sensitivity
- Phages are natural products: Potential appeal to natural medicinal market; Public perception of use of phages as antibacterials seemingly is positive
- Phages have low inherent toxicity; virions consist of only proteins and DNA
- Phages eliminate pathogens more rapidly and effectively than standard antibiotics
- Phages can be grouped in cocktails and can be used with other agents: Versatility in formulation development and combination with other drugs including antibiotics.
- Certain phages, unlike most chemical antibiotics, can be relatively good at biofilm clearance

Phages present a viable alternative and, potentially, the last resort for the treatment of antibiotic-resistant pathogens.

6.2.3 Phages as Biocontrol Agents of *E. coli*

Ensuring food safety is a complex process that depends on the implementation of a wide range of coordinated control measures at all levels of the food production chain (based on the farm-to-fork principle). Among the various approaches of food safety currently under exploration, bacteriophages have emerged as a novel tool for the biocontrol of bacterial contamination in foods. In the following sections, we will focus on the biocontrol of *E. coli.* (Kazi and Annapure 2016; Sillankorva, Oliveira, and Azeredo 2012)

Studies on the Pre-harvest Control of *E. coli*:

"It is widely believed that phage therapy may have potential in the reduction of harmful bacteria in animals. Research involving the application of phage therapies in animal models has produced very promising results." (O'Flynn et al. 2004)

Several researchers demonstrated the use of bacteriophage as a pre-harvest intervention to decrease *E. coli* concentration in poultry (W. Huff et al. 2003; W. E. Huff et al. 2002; A. Oliveira, Sereno, and Azeredo 2010).

> "Recent phage therapy to decrease *E. coli* levels on farm animals has focused mainly on poultry and ruminants.
>
> Application of phages to poultry has been successful to prevent fatal respiratory infections in broiler chickens. Several different approaches have been used; however, aerosol spraying and intramuscular (i.m.) injection have given the best results and reduced significantly the mortality of broiler chicken. Despite these results, phage administration via addition to bird drinking water proved to be inefficient in protecting the birds from fatal *E. coli* respiratory infections." (Sillankorva, Oliveira, and Azeredo 2012)

Even at refrigerated temperatures, phages can attach and effectively reduce *E. coli* contamination (Sulakvelidze 2010).

> "Previous studies demonstrating phage-mediated biocontrol of pathogenic *E. coli* in animals have generated very promising results. For example, calves and piglets with diarrhea due to experimentally administered pathogenic *E. coli* were cured within 8 h following phage administration. Further studies found that phage could act very successfully as a prophylactic. Experimentally induced diarrhea could be prevented by spraying the litter in the calf rooms with aqueous phage suspensions or by keeping calves in uncleaned rooms previously occupied by calves whose *E. coli* infection had been treated by phage administration. Recent results of phage therapy against other bacterial pathogens have shown considerable potential. For example, it has been shown that intraperitoneal injections of bacteria (*Staphlococcus aureus* or vancomycin-resistant *Enterococcus faeicum*) eventually cause death in mice whereas the administration of an

intraperitoneal injection of phage following the initial injection significantly reduces the lethality of the bacteria." (O'Flynn et al. 2004)

Studies on the Post-harvest Control of *E. coli*:

In the post-harvest control of *E. coli*, promising results were obtained when bacteriophages were used to control the growth of *E. coli* on foods, such as milk, spinach, lettuce, broccoli, tomato, cantaloupe, and meat (Sharma et al. 2009; Viazis et al. 2011; Patel et al. 2011; Tomat et al. 2013; Abuladze et al. 2008; Sulakvelidze 2010).

Abuladze et al. (2008) demonstrated at least a 99% *E. coli* reduction in broccoli, tomatoes, and spinach. "The data suggest that naturally occurring bacteriophages may be useful for reducing contamination of various hard surfaces, fruits, vegetables, and ground beef by *E. coli* O157:H7".

Magnone *et al*. (2013) found that combined treatment of fresh vegetables (phage application before storage at 10°C and levulinic acid produce wash after storage at 10°C) was more successful in reduction in bacterial count (*E. coli O157:H7, Shigella* spp. *and Salmonella*) in cases where one-step treatment did not bring satisfactory results.

In addition to these examples, many bacteriophage GRAS processing aid products have been reviewed by USDA for efficacy. It is very clear that the use of lytic bacteriophages is an effective control for a variety of targeted bacteria.

"Phages are highly active and specific against their host with no adverse effects on the intestinal microbiota. Bacteriophages are auto-replicative, hence when bacterial contamination is high, low concentrations of phage can get the desired pathogen reduction. Phage production is relatively simple and has high storage stability under different environ- mental conditions." (Kazi and Annapure 2016)

In conclusion, many studies and regulatory submissions have shown that bacteriophages can be used to successfully control the prevalence of targeted bacterial organisms. ECLYPSE-STEC™ is no different and will effectively reduce the amount of Shiga toxin-producing *E. coli* in food processing.

6.3 GRAS Status of Starting Material

All ingredients used in the manufacturing process are animal-product free, GRAS substances or food ingredients.

Soytone: Peptones are GRAS affirmed in 21 CFR § 184.1553.

Yeast Extract: Baker's yeast extract is a GRAS affirmed direct food substance, 21 CFR § 184.1983.

K_2HPO_4: According to 21 CFR § 182.6285, dipotassium phosphate is generally recognized as safe when used in accordance with good manufacturing practice.

$MgSO_4 \cdot 7H_2O$: Magnesium Sulfate is a GRAS substance according to 21 CFR § 184.1443.

NaCl: Sodium Chloride is a GRAS substance according 21 CFR § 182.70

Polypropylene Glycol 2000: This antifoam emulsion is approved for many food additive uses and is used in several GRAS products (GRAS# 435, 528, and 672).

Host strains: The *E. coli* production host strains are nonpathogenic and safe, see section 2.1.2 for details. In addition, the bacteria used for phage production is completely removed via filtration and goes through two QC checks before commercialization (tables 2 & 3).

Monophages: Lytic phages are generally recognized as being safe and numerous phage solutions are already approved either as GRAS product or by other regulatory authorities (see section 6.5 for details). In particular, ECLYPSE-STEC™ was determined to be generally recognized as safe by OmniLytics through scientific procedures.

6.4 Safety

ECLYPSE-STEC™ is a mixture of three monophages (active ingredients), added salts and residual fermentation by-products. Sections below are presenting the safety of these ingredients or residuals.

6.4.1 LPS (only toxic by-products known)

Within the manufacturing process, the only known toxic ingredient is the Lipopolysaccharides which are released from the non-pathogenic *E. coli* host bacteria (LPS is a component of the outer membrane of Gram-negative bacteria). The non-pathogenic *E.coli* hosts were tested for absence of undesirable genes.

During the manufacturing process, the clarification and washing ensures a final concentration of less than 250,000 EU/mL in a $1x10^{10}$ PFU/mL phage preparation, as assessed by QC procedure for each lot of ECLYPSE-STEC™ (Table 3).

6.4.2 Phages are Non-toxic

All available data indicate that the oral consumption of lytic phages (even at high levels) is entirely harmless to humans. Safety studies have been performed for example with the Listeria-phage P100, in which rats were fed high doses of phages with no measurable effects compared to the control group (Carlton et al. 2005). A study with *E. coli* phages, both in mice and in human volunteers, also showed no significant effects on the test subjects (Chibani-chennou et al. 2004; Bruttin, Brüssow, and Bru 2005). In our hands (Mandeville et al. 2003), pre-treatment of piglets with bacteriophages three hours prior to bacterial challenge, or treatment at the onset of diarrhea, demonstrated a statistically significant reduction in the severity of diarrhea in phage-treated animals. No adverse effects such as fever or any other adverse reactions were observed with these treatments. In these studies, and in contrast to antibiotics, phages seemed to have little effect on the *E. coli* occurring in the animals' intestinal flora.

6.4.3 Phages are Ubiquitous in the Environment

Whether found in the soil (Gómez and Buckling 2011; Griffiths et al. 2011), the ocean (Marston et al. 2012) or the human body (Smillie et al. 2011), bacteriophages play a key role in shaping bacterial population dynamics, serving as the natural counterbalance to bacteria. Phages have been or can be isolated from virtually any aquatic or terrestrial habitat where bacteria exist. A single drop of seawater can hold literally millions of phages (K. E. Wommack and Colwell 2000). The abundance of phages in the environment and the continuous exposure of humans to them, explains the extremely good tolerance of the human organism to phages.

The human gut contains approximately 10^5 bacteriophages (the phageome) (Dalmasso, Hill, and Ross 2014) having been consumed by humans via various foods. In this context, bacteriophages have been commonly isolated from a wide variety of foods and food products; including carrots (Endley et al. 2003); cheese (Michel Gautier, Sommer, and Briandet 1995), meat (Atterbury et al. 2003), with fermented foods like wine (Poblet-Icart, Bordons, and Lonvaud-Funel 1998), yogurt (Kiliç et al. 1996) and Sauerkraut (Lu et al. 2003) having especially high number of these phages. In one study (Lu et al. 2003) 26 different phages were isolated from the product of 4 different Sauerkraut fermentation plants. Phages infecting *Propionibacterium freudenreichii* have been isolated from Swiss cheese at levels of up to $7x10^5$ PFU/g (M Gautier et al. 1995). In Argentina, phages infecting thermophilic lactic acid bacteria have been isolated from dairy plant samples at numbers up to 10^9 PFU/mL (Suárez et al. 2007). Also Campylobacter phages have been isolated at levels of $4x10^6$ PFU/g from chickens (Atterbury et al. 2003) and *Brochothrix thermosphacia* phages from beef (Greer 1983).

In humans, phages have been isolated from dental plaques (Delisle and Donkersloot 1995), feces (Grabow et al. 1995; Gantzer, Henny, and Schwartzbrod 2002), saliva (Bachrach et al. 2002),

and vagina (Kiliç et al. 2001). Phages were shown to be present in municipal water supplies of large cities in Spain and Israel, indicating resistance to physico-chemical methods of purification of drinking water (Armon et al. 1997). This example clearly shows the continuous direct contact of humans with phages. Such widespread and frequent consumption of phages every day supports the view that phages can safely be consumed and therefore deserve the GRAS status.

6.4.4 Circumventing Phage Resistance Mechanisms

The prospect of using phages to combat bacterial infection in food has rendered the understating of the interactions between phages and their hosts crucial. Effectively controlling bacterial populations in bio-industries implicates a better understanding of phage resistance barriers and the evolutionary strategies that phages employ to circumvent them. Many bacterial antiviral mechanisms have been reported in the literature reviewed by (Labrie, Samson, and Moineau 2010), and can be classified in 4 categories depending on which step is targeted in the phage replication cycle. Interestingly, for every antiviral mechanism reported, a counter-mechanism has been uncovered, allowing the phages to overcome and persist. Table 7 summarizes the co-evolutionary host-phage mechanisms.

Bacteria can alter their cell surface to limit phage propagation by blocking phage receptors. In the case of *Salmonella,* phages can use a number of cell surface moieties as receptors, including glycolipids (O-and Vi-antigens), integral membrane proteins (e.g. *OmpF, BtuB, and TolC*), and flagella proteins (FliC, FljB, and FliK) (Chaturongakul and Ounjai 2014; Ho and Slauch 2001). This variety in host receptors leads to wider possibilities in successful host-phage adsorption when using a cocktail of different phages. Moreover, phages have been shown to evolve to target new receptors by acquiring mutations in the genes encoding the receptor binding proteins or tail fibers. For example, *OmpC* porin is used as a receptor by *Salmonella Gifsy* and T4-like phages (Ho and Slauch 2001), while vitamin B_{12} uptake protein BtuB is used by T5-like phages (Kim and Ryu 2011). Although resistance to BtuB-targeting phages have been shown to develop in *Salmonella*, the trait is not heritable and progeny bacteria can revert and become susceptible to these phages again.

Bacteria can prevent phage adsorption by producing an extracellular matrix; the expression of surface molecules at the receptor site can limit or prevent phage access. However, many phages have been shown to possess a depolymerase which degrades secreted substances and unmask the receptors. In *Salmonella*, tail spike proteins of *Siphophages* and *Podophages* recognize and hydrolyze the O-antigen of LPS. *Siphophage* SSU5 can also use core oligosaccharides of LPS as receptors (Kim et al. 2014) making it a beneficial part of a cocktail against insensitive *Salmonella* populations capable of O-antigen glycosylation. It is thus important to note that phage-host interactions are not exclusive to single types of protein-receptor recognition and that bacterial hosts resistant to flagellatropic phages are sensitive to phages targeting BtuB and LPS. **Cross-infection by different types of phages naturally limits the development and abundance of resistant strains.**

Preventing phage DNA entry is another tactic used by both bacteria and phages to ensure their environmental fitness. Superinfection exclusion systems are used by prophages to confer immunity to their host again secondary infection by other incoming phages. In lysogenic *S. enterica*, expression of SieA and SieB proteins encoded by lysogenic Podophage P22, induces lysis of superinfected host cells and degradation of superinfecting phage genome.

When a phage manages to inject its DNA in its host, a restriction endonuclease can cut the invading foreign DNA at specific recognition sites. Moreover, **restriction modification (RM) systems** cluster with other antivirus defense systems (toxin-antitoxin, abortive infection) and operate synergistically in order to increase the overall resistance to phage infection (P. H. Oliveira, Touchon, and Rocha 2014). It has recently been shown that a majority of novel motifs observed in *Salmonella enterica* serovars were modified by Type I RM systems (Pirone-Davies et al. 2015). Phages employ diverse strategies to escape these systems: (a) Some phage have few restriction sites in their genomes, or these sites are too far apart to be recognized by the restriction endonuclease; (b) the phage can be modified by the host metlytransferase (MTase) or acquire its own MTase, and thus be protected during replication of its DNA; (c) the phage can co-inject proteins that directly bind to the DNA and mask the restriction sites; (d) a phage protein can mimic the target DNA and sequester the restriction enzyme, or (e) a phage protein can activate the activity of the MTase or inhibit it by perturbing the REase-MTase complex (Samson et al. 2013).

Targeting and cleaving foreign DNA: CRISPR–Cas can target and cleave invading foreign phage DNA. Phages can circumvent this system by acquiring mutations in the phage protospacers or in the protospacer-adjacent motif (PAM). Some phages, such as *Pseudomonas aeruginosa* lysogens, encode an anti-CRISPR protein that prevents the formation or blocks the action of the CRISPR–Cas complexes (Samson et al. 2013). Interestingly, new research shows that in *Salmonella*, the CRISPR-Cas locus has ceased undergoing adaptive events suggesting that the *Salmonella* CRISPR-Cas systems are no longer immunogenic (Shariat et al. 2015).

Abortive infection systems consist of two proteins, a toxin and an antitoxin. During phage infection, an imbalance in the toxin–antitoxin ratio or inactivation of the antitoxin results in liberation of the toxin, which is free to act on its target and inhibits bacterial growth, thus aborting phage infection. Phages can by-pass abortive-infection (Abi) systems, by acquiring certain mutations of genes involved in nucleotide metabolism or by encoding a molecule that replaces the bacterial antitoxin, thereby counteracting toxin activity and avoiding host death.

TABLE 7

CIRCUMVENTING PHAGE RESISTANCE MECHANISMS

Antiviral mechanisms		Phage evasion tactics
Preventing phage adsorption	Blocking phage receptors	Diversity generating retroelement systems
	Production of extracellular matrix	Extracellular polymer degradation mechanisms (i.e. lyases, hydrolases, and hasluronidases)
	Production of competitive inhibitors	Recognition of multiple receptors
Preventing phage DNA entry	Superinfection exclusion systems	
Cutting phage nucleic acid	Restriction-modification systems	Anti-restriction strategies (e.g. absence of endonuclease recognition sites by point mutations, acquisition of the cognate methylase gene, acquisition of a gene encoding internal proteins, acquisition of restriction alleviation mechanism encoded by *ral*, etc.)
	CRISPR-Cas systems	Acquisition of simple point mutation (or deletion) in the targeted proto-spacer, or mutation in the conserved PAM of the phage genome.
Abortive infection systems		Acquisition of point mutations (e.g. mutation in gene 1.2 and/or 10 in T7 to bypass PifA resistance mechanisms).

6.4.5 Immune Interactions

Treatment with phages can give rise to immunological reactions, depending on where the location of the infection is, and how the phages are administered. It is important to mention that each phage is unique; phage surfaces are covered with peptides that the body does not recognize. Moreover, phage titers fall rapidly after intravenous administration, mainly due to innate immunity and phagocytosis in the blood and liver, and less due to the adaptive immune system (Sokoloff et al. 2000).

A number of studies reported that consumption of large amounts of phages did not lead to any immunological complications (McCallin et al. 2013; Sarker et al. 2012), and topical application has not shown any adverse effects (Wright et al. 2009; Merabishvili et al. 2009).

Phages may inhibit interleukin (IL-2), tumor necrosis factor and, to some extent, Interferon-gamma (Górski et al. 2012; Dbrowska et al. 2014). Phages were also shown to increase non-neutralizing antibodies, IgM and later IgG, and enhance the immune response (Biswas et al. 2002). Previous clinical and animal trials have, however, not resulted in serious immunologic reactions (Skurnik, Pajunen, and Kiljunen 2007), but the risk after intravenous phage therapy cannot be completely ruled out since all phages are different. It is therefore very important to test the immunological response of every single phage, particularly if intravenous therapy is being considered.

Despite these intriguing findings, virtually nothing is known about whether phages can influence innate and adaptive immunity during natural associations with mammals. Although there have been no reports of adverse effects or incidents resulting from the direct exposure to naturally occurring bacteriophage, in treating patients with phage there is reason for caution regarding potential immunological reactions perhaps associated with the lack of formulation purification. Phage preparations for therapy must, however, be purified and free from any toxic or allergenic substances emanating from the bacteria used for the propagation of the phage.

6.4.6 Determination of Absence of Undesirable Genes from Sequence

The DNA genome of phages MLF4, OLB35, and OLB145 was sequenced and deposited in the GenBank. Accession number: MH992121 for MLF4, MH992122 for OLB35, and MH992123 for OLB145.

The size of the DNA and comparative studies of the DNA sequences demonstrates the uniqueness of these phages. Bioinformatic analysis of data generated on the genomic analysis of MLF4, OLB35, and OLB145 sequences demonstrated the lack of harmful or undesired genes against a panel of virulence or transduction genes identified in GenBank.

6.4.7 Worker Safety

ECLYPSE-STEC™ will not harm or jeopardize the safety of workers or Federal Inspection Program Personnel (IPP). Lytic bacteriophages have been scientifically evaluated as GRAS over and over again. Phages cannot infect mammalian cells. Our phage preparation is any not different.

The inert buffer makes up about 99.99985% of ECLYPSE-STEC™, consisting of 1.0 mM dipotassium phosphate, 10 mM magnesium sulfate, and 10 mM sodium chloride which are also GRAS substances (working solution rates are: 0.174 mg/mL K_2HPO_4, 2.46 mg/mL $MgSO_4 \cdot 7H_2O$, and 0.584 mg/mL NaCl).

See Attachment II: ECLYPSE-STEC™ Safety Data Sheet (SDS) for additional information regarding worker safety.

6.5 Substantial Equivalence to Approved Products

Many lytic phage products targeting various bacterial pathogens have already been designated GRAS and/or cleared for food safety usage by a number of regulatory agencies:

Listex™

- Listex™ a phage preparation containing a single *Listeria monocytogenes* lytic phage, P100, used for biocontrol of Listeria in susceptible foodstuffs, is GRAS (GRAS Notice No.000218.)

- Listex™ is also listed by the USDA FSIS for use as processing aid for use on RTE meat products (FSIS Directive 7120.1).

- Listex™ is also approved as a processing aid for susceptible foodstuffs in many countries, including approval in Canada by Health Canada and FSANZ in Australia and New Zealand. The Dutch Ministry of Health has issued a formal statement confirming that Listex™ can be used as a processing aid. Additionally, Listex™ has been approved for use in Switzerland in cheese-making and also as processing aids in keeping with European legislation on food safety

- Listex™ is listed by the Organic Materials Review Institute (OMRI). This means that Listex™ may be used in the certified organic production of food processing and handling according to the USDA National Organic Program Rule

ListShield™

- ListShield™ (formerly known as LMP-102), a phage preparation containing six lytic *Listeria monocytogenes*-specific phages, is FDA-cleared as food additive (21 CFRF§172.785);

- ListShield™ is also listed by the USDA FSIS for use as processing aid with no labeling requirements when applied to various RTE meats and poultry products (FSIS Directive 7120.1).

- ListShield™, is GRAS for direct application to fish and shellfish (including smoked varieties; e.g., smoked salmon), fresh and processed fruits, fresh and processed vegetables, and dairy products (including cheese) (GRN No. 528).

- ListShield™ is also EPA-registered for use on non-food surfaces in food processing plants to prevent or significantly reduce contamination of *Listeria monocytogenes* (EPA registration #74234-1.)

- ListShield™ is Health Canada approved for use on ready-to-eat meat and poultry, smoked salmon, fresh-cut apples, and long leaf lettuce (iLONO).

- ListShield™ is National Food Service of Israel approved as a food processing aid for the treatment of ready-to-eat meat and poultry products (Ref: 70275202).

EcoShield™

- EcoShield™ (formerly ECP-100™), a phage preparation containing three lytic phages *E. coli* 0157:H7-specific phages, is FDA-cleared, through a "Food Contact Notification" or FCN, for use on red meat parts and trim intended to be ground (FCN No. 1018).for use as a food contact substance (FCN No. 1018).

- EcoShield™ is also listed by the USDA FSIS as safe and suitable for use in the production of red meat parts and trim prior to grinding as processing aid with no labeling requirements (FSIS Directive 7120.1).

- EcoShield™ is Health Canada approved for use on red meat parts and trim prior to grinding (iLONO).

- EcoShield™ is National Food Service of Israel approved as food processing aid for the treatment of meat immediately before grinding (Ref: 70275202).

AgriPhage™

- AgriPhage™, a phage preparation targeting *Xanthomonas campestris* pv. *vesicatoria* and

  symptomatic plants or preventively prior to visual signs of damage. (EPA Reg. No.67986-1)

- AgriPhage has been amended to now include organic usage on tomato and pepper plants as governed by the USDA National Organic Program (NOP).

AgriPhage-CMM™

- AgriPhage-CMM™, a phage preparation targeting *Clavibacter michiganensis* pv. *michiganensis*, is EPA-registered for use on tomatoes. AgriPhage-CMM can be applied directly as a foliar spray and can be used as a curative on symptomatic plants or preventively prior to visual signs of damage (EPA Reg. No.67986-6).

- The Canadian Pest Management Regulatory Agency (PMRA) has approved biopesticide AgriPhage-CMM for bacterial stem canker in tomato caused by *Clavibacter michiganensis* pv. *michiganensis* (30301).

AgriPhage-Fire Blight™

- AgriPhage-Fire Blight, a phage preparation targeting *Erwinia amylovora*, is EPA-registered for use on apple and pear trees. AgriPhage-Fire Blight can be applied directly as a foliar spray and can be used as a curative on symptomatic plants or preventively prior to visual signs of damage (EPA Reg. No.67986-8).

AgriPhage-Citrus Canker™

- AgriPhage-Citrus Canker, a phage preparation targeting *Xanthomonas citri* subsp. *citri*, is EPA-registered for use on citrus. AgriPhage-Citrus Canker can be applied directly as a foliar spray and can be used as a curative on symptomatic plants or preventively prior to visual signs of damage (EPA Reg. No.67986-9).

Finalyse™

- Finalyse™, a phage preparation targeting *E.coli* O157:H7, received USDA's Food Safety and Inspection Services approval for commercialization and application as a spray mist or wash on live animals prior to slaughter to decrease pathogen transfer to meat.

Armament™

- Armament™, a phage preparation targeting *Salmonella,* received USDA's Food Safety and Inspection Services approval for commercialization and application as a spray mist or wash on the feathers of live poultry prior to slaughter to decrease pathogen transfer to meat.

Salmonelex™

- Salmonelex™, a phage preparation containing two specific phages, S16 and FO1a, for use as antimicrobial to control *Salmonella* serovars in certain pork and poultry products at levels up to 10^8 PFU/g of food was designated as GRAS (GRAS Notice No. GRN 000468).

SalmoFresh™

- SalmoFresh™, a phage preparation for controlling the foodborne bacterial pathogen *Salmonella enterica,* is GRAS for direct application onto poultry, fish and shellfish, and fresh and processed fruits and vegetables (GRN No. 435).

- SalmoFresh™, is also FSIS-listed as safe and suitable antimicrobial for use in the production of poultry products as a processing aid with no labeling requirements (FSIS Directive 7120.1).

- SalmoFresh™, is Health Canada approved as a processing aid for use on fish, shellfish, and fresh and process fruits and vegetables or on ready-to-eat poultry products prior to slicing and on raw poultry prior to grinding or after grinding (iLONO).

- SalmoFresh™, is National Food Service of Israel approved as a as a food processing aid for the treatment of fish, shellfish, fresh and processed fruits and vegetables and poultry immediately before or after grinding, and on ready to eat products before slicing (ref: 70275202).

ShigaShield™

- ShigaShield™, a phage preparation for controlling the foodborne bacterial pathogen *Shigella,* is GRAS for direct application onto Ready-to-eat meats, fish and shellfish, and fresh and processed fruits and vegetables, and dairy products (GRN No. 672).

Biotector™

- BIOTECTOR™ S1 phage product from CheilJedang Corporation is developed to replace antibiotics in animal feed. It is particularly efficient to control *Salmonella Galliinarum* (SG) and *S. Pullorum* (SP) responsible for fowl typhoid and pullorum disease, respectively. While BIOTECTOR™ S4 is a phage product (additives in swine feed) which could specifically control *S. typhimurium* (ST).

SalmoPro™

- SalmoPro™ is a phage preparation for use as an antimicrobial agent to control *Salmonella* in food (including meat, poultry, and egg products) (GRN No. 752).

6.6 Efficacy Data at the Intended Levels of Use

The literature reports on multiple studies concerning the application of bacteriophages on food for the reduction of *E. coli* (Abuladze et al. 2008; Carter et al. 2012; O'Flynn et al. 2004; Patel et al. 2011; Sharma et al. 2009; Hudson et al. 2013).

O'Flynn et al. (2004) had very compelling results which showed his cocktail of three virulent phages resulted in a 5-log-unit reduction of pathogen numbers in 1 h at 37°C. Abduladze et al. (2008) showed significant *E. coli* reductions on hard surfaces, broccoli, tomato slices, spinach, and ground beef. Sharma et al. (2009) showed similar results on lettuce and cantaloupe. Patel et

al. (2011) demonstrated their cocktail "of bacteriophages reduced E. coli O157:H7 populations by 4.5 log CFU on blades after 2h of phage treatment".

EcoShield™ "significantly ($p < 0.05$) reduced the levels of the bacterium in experimentally contaminated beef by ≥ 94% and in lettuce by 87% after a 5 min contact time. The reduced levels of bacteria were maintained for at least one week at refrigerated temperatures. However, the one-time application of EcoShield™ did not protect the foods from recontamination with E. coli O157:H7. Our results demonstrate that EcoShield™ is effective in significantly reducing contamination of beef and lettuce with E. coli O157:H7, but does not protect against potential later contamination due to, for example, unsanitary handling of the foods post processing"(Carter et al. 2012). This clearly demonstrates that after the initial treatment and initial reduction of bacterial load, any remaining bacteria will grow out at similar growth rates as the untreated controls, thus having no lasting technical effect.

Based on the above results, we designed multiple comprehensive challenge studies to determine whether ECLYPSE-STEC™ would significantly reduce the population of *E. coli*. We show that the application of ECLYPSE-STEC™ at a maximum rate of $1x10^8$ PFU/g of food is effective in reducing the prevalence of Shiga toxin-producing *E. coli* (see Appendix I).

6.7 Summary and Basis for GRAS

ECLYPSE-STEC™ is an *E. coli* specific cocktail of three naturally occurring monophages (MLF4, OLB35, and OLB145). A number of bacteriophage products for the biocontrol of pathogens have previously been scientifically accepted as GRAS. The current ECLYPSE-STEC™ phage product is equivalent to other phage preparations that have been accepted as GRAS.

Based on genetic and biologic/chemical analysis as well as experimental challenges, scientific data are showing that the individual phages contained in ECLYPSE-STEC™ are safe:

- By nature: strict lytic phage devoid of harmful genes
- By manufacturing process controls: QC analysis of each batch ensures that ECLYPSE-STEC™ is effective, devoid of live contaminants (bacterial sterility testing) and has a minimal safe amount of residual LPS.

ECLYPSE-STEC™ is also shown to be effective in reducing *E. coli* on many types of food (Appendix I).

OmniLytics has reviewed the available data and information and is not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

Based on these findings and significant equivalence with the other accepted GRAS phage products, ECLYPSE-STEC™ should also be considered GRAS.

Part 7 List of Supporting Data and Information

7.1 Appendices (Not Generally Available)

Appendix I: Efficacy Studies of ECLYPSE-STEC™ on Foods

Appendix II: ECLYPSE-STEC™ SDS

7.2 References (Generally Available)

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APPENDIX I: EFFICACY STUDIES OF ECLYPSE-STEC™ ON FOOD

Test Substance: ECLYPSE-STEC™ bacteriophages

Products Tested:

- Raw chicken breast
- Spinach
- Crab
- Pre-cut apples
- Deli sliced honey baked ham
- Eggs
- Salmon
- Ground beef

Treatment Amounts: ECLYPSE-STEC™ was applied to the surfaces of the products tested at the concentration of $1x10^8$ PFU (Plaque Forming Units) per gram.

Labeling Requirements: None under the accepted conditions of use

ECLYPSE-STEC™ consists of a mixture of equal concentrations of three *E. coli* O157:H7-specific lytic bacteriophages. ECLYPSE-STEC™ is intended for use as an antimicrobial processing aid to control *E. coli* O157:H7 on food, when applied to food surfaces up to $1x10^8$ PFU (Plaque Forming Units) per gram of food.

Efficacy: ECLYPSE-STEC™ has been shown to be effective in significantly reducing *E. coli* O157:H7 on food.

Products Tested	Study	*Salmonella* Reduction	Log Reduction	Significant
Raw chicken breast	50-RP-00016 A	98%	1.75	Yes
Spinach	50-RP-00017 A	95%	1.32	Yes
Crab	50-RP-00018 A	99%	2.12	Yes
Pre-cut apples	50-RP-00019 A	97%	1.47	Yes
Deli sliced ham	50-RP-00020 A	93%	1.15	Yes
Eggs	50-RP-00021 A	99%	2.11	Yes
Salmon	50-RP-00022 A	96%	1.41	Yes
Ground beef	50-RP-00023 A	95%	1.26	Yes

Project Summary

Title of Report:

Evaluation of the ability of ECLYPSE-STEC™ to reduce top Shiga toxin-producing E. coli strains (O26, O111, O103, O121, O45, O145, O157) contamination in experimentally contaminated chicken breast

Document # 50-RP-00016 A

Report Approval

The following personnel of OmniLytics are responsible for the accuracy and completeness of the information reported herein:

Analyst/Lead: Amanda Mulia, Laboratory Manager

[redacted] 8-31-18

Signature Date

Supervisor: Ryan Bringhurst, Senior Scientist

[redacted] 8/31/18

Signature Date

Project Summary

OMNILYTICS™ THE PHAGE COMPANY

ECLYPSE-STEC™ reduction on chicken breast

Scientist Name(s): *Amanda Mulia, Ryan Bringhurst*
Date(s) of Testing: 5/8/18 thru 5/9/18
Relevant Notebook Page(s): AM-023-003 and AM-023-004

Purpose / Abstract

The purpose of this study is to test the efficacy of reducing Shiga toxin-producing E. coli on chicken breast using ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g.

Results Summary and Conclusions

Results of Applying ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g on experimentally contaminated chicken breast. One way ANOVA (α= 0.05) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
ECLYPSE-STEC™	3	4.47E+04	98%	Yes	0.0003
PBS	3	2.51E+06			

ECLYPSE-STEC™ can significantly reduce viable Shiga toxin-producing E. Coli levels on experimentally contaminated chicken breast by 98% in 30 min at room temperature when used at $1x10^8$ PFU/g.

Materials and Methods

Challenge Organism:

- 93-111 (*E. coli* O157:H7 strain isolated in Washington State)

Procedure:

1) Grow culture of bacteria in LB to an OD_{600} of 0.5-1.
2) Obtain about a 10 g piece of test material. Bring to room temperature.
3) Coat surface of sample with 0.1 mL of bacterial culture.
4) Allow bacteria to attach to sample at room temperature.
5) Dilute ECLYPSE-STEC™ in PBS to a titer of 10^9. ECLYPSE-STEC™ (93-048004) has a titer of $1.9x10^{11}$, so this lot was diluted 1:100 to prepare the test solution.
6) Apply 1 mL of diluted ECLYPSE-STEC™ to sample.
7) Cover sample and allow to sit for 30 min.
8) Add 10 mL of peptone water.
9) Vortex or mix for at least 30 seconds.
10) Plate 100 µL of 10^{-2} and 10^{-3} dilutions for samples and 10^{-3} and 10^{-4} for controls on MacConkey Sorbitol Agar.
11) Incubate at 37°C overnight.
12) Perform above test in triplicate.
13) Also, perform above without inoculating or applying ECLYPSE-STEC™ to assess background colony load.

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

Data

Raw data from colony counts.

Sample	Replicate	Count	Plate portion	Plate dilution	Titer (CFU/mL)	Average Titer	LOG Drop
ECLYPSE-STEC™	1	20	1	3	2.00E+04	4.47E+04	1.75
	2	67	1	3	6.70E+04		
	3	47	1	3	4.70E+04		
Control	1	73	0.25	4	2.92E+06	2.51E+06	
	2	60	0.25	4	2.40E+06		
	3	55	0.25	4	2.20E+06		
none		0	1	2	0.00E+00		

Project Summary

Title of Report:

Evaluation of the ability of ECLYPSE-STEC™ to reduce top Shiga toxin-producing E. coli strains (O26, O111, O103, O121, O45, O145, O157) contamination in experimentally contaminated spinach

Document # 50-RP-00017 A

Report Approval

The following personnel of OmniLytics are responsible for the accuracy and completeness of the information reported herein:

Analyst/Lead: Amanda Mulia, Laboratory Manager

[redacted] 8-31-18

Signature Date

Supervisor: Ryan Bringhurst, Senior Scientist

[redacted] 8/31/18

Signature Date

Project Summary

OMNILYTICS™ THE PHAGE COMPANY

ECLYPSE-STEC™ reduction on spinach

Scientist Name(s): *Amanda Mulia, Ryan Bringhurst*
Date(s) of Testing: 5/8/18 thru 5/9/18
Relevant Notebook Page(s): AM-023-003 and AM-023-004

Purpose / Abstract

The purpose of this study is to test the efficacy of reducing Shiga toxin-producing E. coli on spinach using ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g.

Results Summary and Conclusions

Results of Applying ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g on experimentally contaminated spinach. One way ANOVA (α= 0.05) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
ECLYPSE-STEC™	3	9.23E+04	95%	Yes	0.0005
PBS	3	2.36E+06			

ECLYPSE-STEC™ can significantly reduce viable Shiga toxin-producing E. Coli levels on experimentally contaminated spinach by 95% in 30 min at room temperature when used at $1x10^8$ PFU/g.

Materials and Methods

Challenge Organism:

- 93-111 (*E. coli* O157:H7 strain isolated in Washington State)

Procedure:

1) Grow culture of bacteria in LB to an OD_{600} of 0.5-1.
2) Obtain about a 10 g piece of test material. Bring to room temperature.
3) Coat surface of sample with 0.1 mL of bacterial culture.
4) Allow bacteria to attach to sample at room temperature.
5) Dilute ECLYPSE-STEC™ in PBS to a titer of 10^9. ECLYPSE-STEC™ (93-048004) has a titer of $1.9x10^{11}$, so this lot was diluted 1:100 to prepare the test solution.
6) Apply 1 mL of diluted ECLYPSE-STEC™ to sample.
7) Cover sample and allow to sit for 30 min.
8) Add 10 mL of peptone water.
9) Vortex or mix for at least 30 seconds.
10) Plate 100 µL of 10^{-2} and 10^{-3} dilutions for samples and 10^{-3} and 10^{-4} for controls on MacConkey Sorbitol Agar.
11) Incubate at 37°C overnight.
12) Perform above test in triplicate.
13) Also, perform above without inoculating or applying ECLYPSE-STEC™ to assess background colony load.

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

Data

Raw data from colony counts.

Sample	Replicate	Count	Plate portion	Plate dilution	Titer (CFU/mL)	Average Titer	LOG Drop
ECLYPSE-STEC™	1	15	1	3	1.50E+04	1.74E+05	1.32
	2	40	1	4	4.00E+05		
	3	54	0.5	3	1.08E+05		
Control	1	89	0.25	4	3.56E+06	3.63E+06	
	2	78	0.25	4	3.12E+06		
	3	105	0.25	4	4.20E+06		
none		0	1	2	0.00E+00		

Project Summary

Title of Report:

Evaluation of the ability of ECLYPSE-STEC™ to reduce top Shiga toxin-producing E. coli strains (O26, O111, O103, O121, O45, O145, O157) contamination in experimentally contaminated crab

Document # 50-RP-00018 A

Report Approval

The following personnel of OmniLytics are responsible for the accuracy and completeness of the information reported herein:

Analyst/Lead: Amanda Mulia, Laboratory Manager

[redacted] 8-31-18

Signature Date

Supervisor: Ryan Bringhurst, Senior Scientist

[redacted] 8/31/18

Signature Date

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

ECLYPSE-STEC™ reduction on crab

Scientist Name(s): *Amanda Mulia, Ryan Bringhurst*
Date(s) of Testing: 5/8/18 thru 5/9/18
Relevant Notebook Page(s): AM-023-003 and AM-023-004

Purpose / Abstract

The purpose of this study is to test the efficacy of reducing Shiga toxin-producing E. coli on crab using ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g.

Results Summary and Conclusions

Results of Applying ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g on experimentally contaminated crab. One way ANOVA (α= 0.05) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
ECLYPSE-STEC™	3	1.90E+04	99%	Yes	<0.00001
PBS	3	2.52E+06			

ECLYPSE-STEC™ can significantly reduce viable Shiga toxin-producing E. Coli levels on experimentally contaminated crab by 99% in 30 min at room temperature when used at $1x10^8$ PFU/g.

Materials and Methods

Challenge Organism:

- 93-111 (*E. coli* O157:H7 strain isolated in Washington State)

Procedure:

1) Grow culture of bacteria in LB to an OD_{600} of 0.5-1.
2) Obtain about a 10 g piece of test material. Bring to room temperature.
3) Coat surface of sample with 0.1 mL of bacterial culture.
4) Allow bacteria to attach to sample at room temperature.
5) Dilute ECLYPSE-STEC™ in PBS to a titer of 10^9. ECLYPSE-STEC™ (93-048004) has a titer of $1.9x10^{11}$, so this lot was diluted 1:100 to prepare the test solution.
6) Apply 1 mL of diluted ECLYPSE-STEC™ to sample.
7) Cover sample and allow to sit for 30 min.
8) Add 10 mL of peptone water.
9) Vortex or mix for at least 30 seconds.
10) Plate 100 µL of 10^{-2} and 10^{-3} dilutions for samples and 10^{-3} and 10^{-4} for controls on MacConkey Sorbitol Agar.
11) Incubate at 37°C overnight.
12) Perform above test in triplicate.
13) Also, perform above without inoculating or applying ECLYPSE-STEC™ to assess background colony load.

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

Data

Raw data from colony counts.

Sample	Replicate	Count	Plate portion	Plate dilution	Titer (CFU/mL)	Average Titer	LOG Drop
ECLYPSE-STEC™	1	24	1	3	2.40E+04	1.90E+04	2.12
	2	19	1	3	1.90E+04		
	3	14	1	3	1.40E+04		
Control	1	65	0.25	4	2.60E+06	2.52E+06	
	2	65	0.25	4	2.60E+06		
	3	59	0.25	4	2.36E+06		
none		0	1	2	0.00E+00		

Project Summary

Title of Report:

Evaluation of the ability of ECLYPSE-STEC™ to reduce top Shiga toxin-producing E. coli strains (O26, O111, O103, O121, O45, O145, O157) contamination in experimentally contaminated pre-cut apples

Document # 50-RP-00019 A

Report Approval

The following personnel of OmniLytics are responsible for the accuracy and completeness of the information reported herein:

Analyst/Lead: Amanda Mulia, Laboratory Manager

[redacted] 8-31-18

Signature Date

Supervisor: Ryan Bringhurst, Senior Scientist

[redacted] 8/31/18

Signature Date

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

ECLYPSE-STEC™ reduction on pre-cut apples

Scientist Name(s): *Amanda Mulia, Ryan Bringhurst*
Date(s) of Testing: 5/8/18 thru 5/9/18
Relevant Notebook Page(s): AM-023-003 and AM-023-004

Purpose / Abstract

The purpose of this study is to test the efficacy of reducing Shiga toxin-producing E. coli on pre-cut apples using ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g.

Results Summary and Conclusions

Results of Applying ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g on experimentally contaminated apples. One way ANOVA ($\alpha = 0.05$) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
ECLYPSE-STEC™	3	8.07E+04	97%	Yes	0.0008
PBS	3	2.39E+06			

ECLYPSE-STEC™ can significantly reduce viable Shiga toxin-producing E. Coli levels on experimentally contaminated apples by 97% in 30 min at room temperature when used at $1x10^8$ PFU/g.

Materials and Methods

Challenge Organism:

- 93-111 (*E. coli* O157:H7 strain isolated in Washington State)

Procedure:

1) Grow culture of bacteria in LB to an OD_{600} of 0.5-1.
2) Obtain about a 10 g piece of test material. Bring to room temperature.
3) Coat surface of sample with 0.1 mL of bacterial culture.
4) Allow bacteria to attach to sample at room temperature.
5) Dilute ECLYPSE-STEC™ in PBS to a titer of 10^9. ECLYPSE-STEC™ (93-048004) has a titer of $1.9x10^{11}$, so this lot was diluted 1:100 to prepare the test solution.
6) Apply 1 mL of diluted ECLYPSE-STEC™ to sample.
7) Cover sample and allow to sit for 30 min.
8) Add 10 mL of peptone water.
9) Vortex or mix for at least 30 seconds.
10) Plate 100 µL of 10^{-2} and 10^{-3} dilutions for samples and 10^{-3} and 10^{-4} for controls on MacConkey Sorbitol Agar.
11) Incubate at 37°C overnight.
12) Perform above test in triplicate.
13) Also, perform above without inoculating or applying ECLYPSE-STEC™ to assess background colony load.

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

Data

Raw data from colony counts.

Sample	Replicate	Count	Plate portion	Plate dilution	Titer (CFU/mL)	Average Titer	LOG Drop
ECLYPSE-STEC™	1	43	0.5	3	8.60E+04	8.07E+04	1.47
	2	37	0.5	3	7.40E+04		
	3	82	1	3	8.20E+04		
Control	1	48	0.25	4	1.92E+06	2.39E+06	
	2	61	0.25	4	2.44E+06		
	3	70	0.25	4	2.80E+06		
none		0	1	2	0.00E+00		

Project Summary

Title of Report:

Evaluation of the ability of ECLYPSE-STEC™ to reduce top Shiga toxin-producing E. coli strains (O26, O111, O103, O121, O45, O145, O157) contamination in experimentally contaminated deli sliced ham

Document # 50-RP-00020 A

Report Approval

The following personnel of OmniLytics are responsible for the accuracy and completeness of the information reported herein:

Analyst/Lead: Amanda Mulia, Laboratory Manager

[redacted] 8-31-18

Signature Date

Supervisor: Ryan Bringhurst, Senior Scientist

[redacted] 8/31/18

Signature Date

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

ECLYPSE-STEC™ reduction on deli sliced ham

Scientist Name(s): *Amanda Mulia, Ryan Bringhurst*
Date(s) of Testing: 5/8/18 thru 5/9/18
Relevant Notebook Page(s): AM-023-003 and AM-023-004

Purpose / Abstract

The purpose of this study is to test the efficacy of reducing Shiga toxin-producing E. coli on deli sliced ham using ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g.

Results Summary and Conclusions

Results of Applying ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g on experimentally contaminated deli sliced ham. One way ANOVA ($\alpha = 0.05$) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
ECLYPSE-STEC™	3	1.73E+05	93%	Yes	0.0047
PBS	3	2.43E+06			

ECLYPSE-STEC™ can significantly reduce viable Shiga toxin-producing E. Coli levels on experimentally contaminated deli sliced ham by 93% in 30 min at room temperature when used at $1x10^8$ PFU/g.

Materials and Methods

Challenge Organism:

- 93-111 (*E. coli* O157:H7 strain isolated in Washington State)

Procedure:

1) Grow culture of bacteria in LB to an OD_{600} of 0.5-1.
2) Obtain about a 10 g piece of test material. Bring to room temperature.
3) Coat surface of sample with 0.1 mL of bacterial culture.
4) Allow bacteria to attach to sample at room temperature.
5) Dilute ECLYPSE-STEC™ in PBS to a titer of 10^9. ECLYPSE-STEC™ (93-048004) has a titer of $1.9x10^{11}$, so this lot was diluted 1:100 to prepare the test solution.
6) Apply 1 mL of diluted ECLYPSE-STEC™ to sample.
7) Cover sample and allow to sit for 30 min.
8) Add 10 mL of peptone water.
9) Vortex or mix for at least 30 seconds.
10) Plate 100 µL of 10^{-2} and 10^{-3} dilutions for samples and 10^{-3} and 10^{-4} for controls on MacConkey Sorbitol Agar.
11) Incubate at 37°C overnight.
12) Perform above test in triplicate.
13) Also, perform above without inoculating or applying ECLYPSE-STEC™ to assess background colony load.

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

Data

Raw data from colony counts.

Sample	Replicate	Count	Plate portion	Plate dilution	Titer (CFU/mL)	Average Titer	LOG Drop
ECLYPSE-STEC™	1	47	0.25	3	1.88E+05	1.73E+05	1.15
	2	43	0.25	3	1.72E+05		
	3	40	0.25	3	1.60E+05		
Control	1	58	0.25	4	2.32E+06	2.43E+06	
	2	79	0.25	4	3.16E+06		
	3	45	0.25	4	1.80E+06		
none		0	1	2	0.00E+00		

Project Summary

Title of Report:

Evaluation of the ability of ECLYPSE-STEC™ to reduce top Shiga toxin-producing E. coli strains (O26, O111, O103, O121, O45, O145, O157) contamination in experimentally contaminated eggs

Document # 50-RP-00021 A

Report Approval

The following personnel of OmniLytics are responsible for the accuracy and completeness of the information reported herein:

Analyst/Lead: Amanda Mulia, Laboratory Manager

[redacted]
Signature

8-31-18
Date

Supervisor: Ryan Bringhurst, Senior Scientist

[redacted]
Signature

8/31/18
Date

Project Summary

OMNILYTICS™ THE PHAGE COMPANY

ECLYPSE-STEC™ reduction on eggs

Scientist Name(s): *Amanda Mulia, Ryan Bringhurst*
Date(s) of Testing: 5/8/18 thru 5/9/18
Relevant Notebook Page(s): AM-023-003 and AM-023-004

Purpose / Abstract

The purpose of this study is to test the efficacy of reducing Shiga toxin-producing E. coli on eggs using ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g.

Results Summary and Conclusions

Results of Applying ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g on experimentally contaminated eggs. One way ANOVA (α= 0.05) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
ECLYPSE-STEC™	3	3.10E+04	99%	Yes	0.0002
PBS	3	4.00E+06			

ECLYPSE-STEC™ can significantly reduce viable Shiga toxin-producing E. Coli levels on experimentally contaminated eggs by 99% in 30 min at room temperature when used at $1x10^8$ PFU/g.

Materials and Methods

Challenge Organism:

- 93-111 (*E. coli* O157:H7 strain isolated in Washington State)

Procedure:

1) Grow culture of bacteria in LB to an OD_{600} of 0.5-1.
2) Obtain about a 10 g piece of test material. Bring to room temperature.
3) Coat surface of sample with 0.1 mL of bacterial culture.
4) Allow bacteria to attach to sample at room temperature.
5) Dilute ECLYPSE-STEC™ in PBS to a titer of 10^9. ECLYPSE-STEC™ (93-048004) has a titer of $1.9x10^{11}$, so this lot was diluted 1:100 to prepare the test solution.
6) Apply 1 mL of diluted ECLYPSE-STEC™ to sample.
7) Cover sample and allow to sit for 30 min.
8) Add 10 mL of peptone water.
9) Vortex or mix for at least 30 seconds.
10) Plate 100 µL of 10^{-2} and 10^{-3} dilutions for samples and 10^{-3} and 10^{-4} for controls on MacConkey Sorbitol Agar.
11) Incubate at 37°C overnight.
12) Perform above test in triplicate.
13) Also, perform above without inoculating or applying ECLYPSE-STEC™ to assess background colony load.

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

Data

Raw data from colony counts.

Sample	Replicate	Count	Plate portion	Plate dilution	Titer (CFU/mL)	Average Titer	LOG Drop
ECLYPSE-STEC™	1	28	1	3	2.80E+04	3.10E+04	2.11
	2	44	1	3	4.40E+04		
	3	21	1	3	2.10E+04		
Control	1	85	0.25	4	3.40E+06	4.00E+06	
	2	43	1	5	4.30E+06		
	3	43	1	5	4.30E+06		
none		0	1	2	0.00E+00		

Project Summary

Title of Report:

Evaluation of the ability of ECLYPSE-STEC™ to reduce top Shiga toxin-producing *E. coli* strains (O26, O111, O103, O121, O45, O145, O157) contamination in experimentally contaminated salmon

Document # 50-RP-00022 A

Report Approval

The following personnel of OmniLytics are responsible for the accuracy and completeness of the information reported herein:

Analyst/Lead: Amanda Mulia, Laboratory Manager

[redacted] 8-31-18

Signature Date

Supervisor: Ryan Bringhurst, Senior Scientist

[redacted] 8/31/18

Signature Date

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

ECLYPSE-STEC™ reduction on salmon

Scientist Name(s): *Amanda Mulia, Ryan Bringhurst*
Date(s) of Testing: 5/8/18 thru 5/9/18
Relevant Notebook Page(s): AM-023-003 and AM-023-004

Purpose / Abstract

The purpose of this study is to test the efficacy of reducing Shiga toxin-producing E. coli on salmon using ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g.

Results Summary and Conclusions

Results of Applying ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g on experimentally contaminated salmon. One way ANOVA (α= 0.05) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
ECLYPSE-STEC™	3	9.23E+04	96%	Yes	0.0023
PBS	3	2.36E+06			

ECLYPSE-STEC™ can significantly reduce viable Shiga toxin-producing E. Coli levels on experimentally contaminated salmon by 96% in 30 min at room temperature when used at $1x10^8$ PFU/g.

Materials and Methods

Challenge Organism:

- 93-111 (*E. coli* O157:H7 strain isolated in Washington State)

Procedure:

1) Grow culture of bacteria in LB to an OD_{600} of 0.5-1.
2) Obtain about a 10 g piece of test material. Bring to room temperature.
3) Coat surface of sample with 0.1 mL of bacterial culture.
4) Allow bacteria to attach to sample at room temperature.
5) Dilute ECLYPSE-STEC™ in PBS to a titer of 10^9. ECLYPSE-STEC™ (93-048004) has a titer of $1.9x10^{11}$, so this lot was diluted 1:100 to prepare the test solution.
6) Apply 1 mL of diluted ECLYPSE-STEC™ to sample.
7) Cover sample and allow to sit for 30 min.
8) Add 10 mL of peptone water.
9) Vortex or mix for at least 30 seconds.
10) Plate 100 µL of 10^{-2} and 10^{-3} dilutions for samples and 10^{-3} and 10^{-4} for controls on MacConkey Sorbitol Agar.
11) Incubate at 37°C overnight.
12) Perform above test in triplicate.
13) Also, perform above without inoculating or applying ECLYPSE-STEC™ to assess background colony load.

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

Data

Raw data from colony counts.

Sample	Replicate	Count	Plate portion	Plate dilution	Titer (CFU/mL)	Average Titer	LOG Drop
ECLYPSE-STEC™	1	47	0.5	3	9.40E+04	9.23E+04	1.41
	2	59	0.5	3	1.18E+05		
	3	65	1	3	6.50E+04		
Control	1	54	0.25	4	2.16E+06	2.36E+06	
	2	68	0.25	4	2.72E+06		
	3	55	0.25	4	2.20E+06		
none		7	1	2	7.00E+02		

Project Summary

Title of Report:

Evaluation of the ability of ECLYPSE-STEC™ to reduce top Shiga toxin-producing E. coli strains (O26, O111, O103, O121, O45, O145, O157) contamination in experimentally contaminated ground beef

Document # 50-RP-00023 A

Report Approval

The following personnel of OmniLytics are responsible for the accuracy and completeness of the information reported herein:

Analyst/Lead: Amanda Mulia, Laboratory Manager

[redacted] 8-31-18

Signature Date

Supervisor: Ryan Bringhurst, Senior Scientist

[redacted] 8/31/18

Signature Date

Project Summary

OMNILYTICS™ THE PHAGE COMPANY

ECLYPSE-STEC™ reduction on ground beef

Scientist Name(s): *Amanda Mulia, Ryan Bringhurst*
Date(s) of Testing: 5/8/18 thru 5/9/18
Relevant Notebook Page(s): AM-023-003 and AM-023-004

Purpose / Abstract

The purpose of this study is to test the efficacy of reducing Shiga toxin-producing E. coli on ground beef using ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g.

Results Summary and Conclusions

Results of Applying ECLYPSE-STEC™ at a concentration of $1x10^8$ PFU/g on experimentally contaminated ground beef. One way ANOVA (α= 0.05) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
ECLYPSE-STEC™	3	6.07E+04	95%	Yes	0.0010
PBS	3	1.11E+06			

ECLYPSE-STEC™ can significantly reduce viable Shiga toxin-producing E. Coli levels on experimentally contaminated ground beef by 95% in 30 min at room temperature when used at $1x10^8$ PFU/g.

Materials and Methods

Challenge Organism:

- 93-111 (*E. coli* O157:H7 strain isolated in Washington State)

Procedure:

1) Grow culture of bacteria in LB to an OD_{600} of 0.5-1.
2) Obtain about a 10 g piece of test material. Bring to room temperature.
3) Coat surface of sample with 0.1 mL of bacterial culture.
4) Allow bacteria to attach to sample at room temperature.
5) Dilute ECLYPSE-STEC™ in PBS to a titer of 10^9. ECLYPSE-STEC™ (93-048004) has a titer of $1.9x10^{11}$, so this lot was diluted 1:100 to prepare the test solution.
6) Apply 1 mL of diluted ECLYPSE-STEC™ to sample.
7) Cover sample and allow to sit for 30 min.
8) Add 10 mL of peptone water.
9) Vortex or mix for at least 30 seconds.
10) Plate 100 µL of 10^{-2} and 10^{-3} dilutions for samples and 10^{-3} and 10^{-4} for controls on MacConkey Sorbitol Agar.
11) Incubate at 37°C overnight.
12) Perform above test in triplicate.
13) Also, perform above without inoculating or applying ECLYPSE-STEC™ to assess background colony load.

Project Summary

OMNILYTICS™
THE PHAGE COMPANY

Data

Raw data from colony counts.

Sample	Replicate	Count	Plate portion	Plate dilution	Titer (CFU/mL)	Average Titer	LOG Drop
ECLYPSE-STEC™	1	49	1	3	4.90E+04	6.07E+04	1.26
	2	53	1	3	5.30E+04		
	3	40	0.5	3	8.00E+04		
Control	1	44	0.5	4	8.80E+05	1.11E+06	
	2	57	0.5	4	1.14E+06		
	3	65	0.5	4	1.30E+06		
none		0	1	2	0.00E+00		

APPENDIX II: ECLYPSE-STEC™ Safety Data Sheet (SDS)

SAFETY DATA SHEET

SDS Revision Date: October 4, 2018

ECLYPSE-STEC ™

SECTION 1: Identification

PRODUCT NAME: **ECLYPSE-STEC™**
FDA GRAS #: **Undetermined**
MANUFACTURER: **OmniLytics, Inc.**
ADDRESS: **9075 South Sandy Parkway, Sandy, Utah 84070**
PHONE: **801.746.3600**
TOLL FREE: **866.285.2644**
FAX: **801.746.3461**

PRODUCT USE: ECLYPSE-STEC ™ is intended for use as an antimicrobial processing aid to control *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145 on food, when applied to food surfaces up to $1x10^8$ PFU (Plaque Forming Units) per gram of food.

SECTION 2: Hazard(s) Identification

As bacteriophages are not hazardous, toxicology information is based on the non-hazardous inert buffer solution (1.0 mM dipotassium phosphate, 10 mM magnesium sulfate, and 10 mM sodium chloride) that makes up 99.99985% of ECLYPSE-STEC™.

Classification according to the Hazard Communication Standard (HCS):

ECLYPSE-STEC™ is not a hazardous substance or mixture

Hazard Statement:

ECLYPSE-STEC™ is not a hazardous substance or mixture

Health Hazards:

Skin Contact:	Contact is unlikely to cause injury; excessive amounts may cause mild irritation
Eye Contact:	Contact is unlikely to cause injury; excessive amounts may cause mild irritation
Inhalation:	Inhalation is unlikely to cause injury, excessive amounts may cause mild irritation
Ingestion:	Ingestion is unlikely to cause injury, excessive amounts may cause mild irritation

Environmental Hazard:
No known environmental hazards.

Routes of Entry: Dermal, Eyes, Inhalation, Ingestion

Occupational Exposure Limits:
Threshold Limit Values: None listed
Permissible Exposure Limits: None listed

SECTION 3: Composition/Information on Ingredients

Chemical Name: Bacteriophages active against *E. coli* species
Common Name: ECLYPSE-STEC ™
CAS #: Not applicable
FDA GRAS #: Undetermined
Active Ingredient: 0.00015% (Bacteriophages)
Inert Ingredients: 99.99985% (1.0 mM dipotassium phosphate, 10 mM magnesium sulfate, and 10 mM sodium chloride)

SECTION 4: First-Aid Measures

General Information: Immediately remove any clothing soiled by the product.

Symptoms: Possible symptoms may be eye, skin, or throat irritation.

Dermal: Rinse skin immediately with plenty of water for 2-5 minutes.
If skin irritation continues, consult a doctor.

Eyes: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.
If eye irritation continues, consult a doctor.

Inhalation: If inhaled, suppy fresh air.
Consult doctor is symptoms persist.

Ingestion: Drink 2-3 glasses of water.
Consult doctor is symptoms persist.

SECTION 5: Fire-Fighting Measures

ECLYPSE-STEC ™ is Non-Flammable.

Suitable Extiguishing Media:	Not applicable
Specific Hazards:	None known
Advice for Firefighters:	No special advice

SECTION 6: Accidental Release Measures

If material is spilled or released, recover free product. Use absorbent material to minimize runoff of spilled product, clean up with absorbant cloth and mild cleanser as normal. ECLYPSE-STEC™ is not a hazard.

SECTION 7: Handling and Storage

Container Handling: Non-refillable container. Do not reuse or refill this container. Triple rinse container (or equivalent) promptly after emptying. Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container ¼ full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later

use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. Then offer for recycling if available or reconditioning if appropriate, or puncture and dispose of in a sanitary landfill, or by incineration, or if allowed by state and local authorities, by burning. If burned, stay out of smoke.

Method of Storage: Store at 4°C. Product in packaging should be stored in a secure, protected area. Shaded or darkened space is recommended. Moisture and humidity should be kept to a minimum to maintain integrity of corregated paper packaging.

SECTION 8: Exposure Controls/Personal Protection

Engineering Controls:	No specialized engineering controls required.
Ventilation:	No specialized ventilation required.
Personal Protective Equipement:	Use a lab coat, Long Sleeved Shirt, Long Pants, Waterproof Gloves, Waterproof Shoes plus socks.
Eye Protection:	Avoid contact with eyes. Use eye protection.
Hygenic Practices:	Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.

SECTION 9: Physical and Chemical Properties

General Information

Appearance:	
Physical State:	Liquid
Color:	Opalescent
Odor:	None to Slight (pure solution & working solution)
Odor Threshold:	Not Determined
pH:	7.0-7.5 (pure solution & working solution)
Freezing Point:	0°C (32°F)
Boiling Point:	100°C (212°F)
Flash Point:	Not Applicable
Evaporation Rate:	Not Determined
Flamability:	Not Applicable
Dangers of Explosion:	Product does not present an explosion hazard.
Vapor Pressure:	Not Determined
Vapor Density:	Not Determined
Relative Density to water:	0.996 – 1.003 g/ml
Solubility:	Fully Miscible
Partition Coefficient:	Not Determined
Autoignition Temperature:	Product is not selfigniting
Decomposition Temperature:	Not Determined
Viscocity:	Not Determined

SECTION 10: Stability and Reactivity

Reactivity:	Stable
Chemical Stability:	Stable
Hazardous Reactions:	None known
Conditions to Avoid:	No known conditions to avoid.
Incompatible Materials:	None known

Hazardous Decompostion Products: No dangerous decomposition products known.

SECTION 11: Toxicology Information

As bacteriophages are not hazardous, toxicology information is based on the non-hazardous inert buffer solution (1.0 mM dipotassium phosphate, 10 mM magnesium sulfate, and 10 mM sodium chloride) that makes up 99.99985% of ECLYPSE-STEC™.

Likely Routes of Exposure:	Dermal, Eyes, Inhalation, Ingestion
Acute Toxicity:	No data available
Inhalation:	No data available
Dermal:	No data available
Skin Corrosion/Irritation:	No data available
Serious Eye Damage/Irritation:	No data available
Respiratory or Skin Sensitization:	No data available
Mutagenic Effects:	No known effects
Carcinogenicity:	No known effects
Reproductive Toxicity:	No known effects

ECLYPSE-STEC™ does not contain any known hazards or have any toxic effects.

SECTION 12: Ecological Information

ECLYPSE-STEC ™ has no known hazards to any ecological systems.

SECTION 13: Disposal Considerations

WASTE DISPOSAL METHOD: Waste resulting from the use of this product may be disposed of on site or at an approved waste disposal facility. Triple rinse empty containers and offer for recycling, or puncture and dispose of in an approved sanitary landfill.

SECTION 14: Transport Information

UN/NA #:	Not Classified
Proper Shipping Name:	None
Transport Hazard Class:	Not Hazardous
Packaging Group:	Not Applicable
Environmental Hazards:	None

SECTION 15: Regulatory Information

ECLYPSE-STEC ™ is generally recognized as safe (GRAS) and has been reviewed by FDA and FSIS.

SECTION 16: Other Information

To the best of our knowledge, the information contained herein is accurate. All material may present unknown health hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards which exist. OmniLytics, Inc. and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product

OMNILYTICS™
THE PHAGE COMPANY

September 10, 2019

RE: GRAS Notice # 827 ECLYPSE-STEC: USDA Regulated Uses

Dear Mr. Wafula,

On July 23, 2019, OmniLytics requested that the GRAS Notice #827 be amended to "Remove all USDA regulated uses in the notice" because USDA couldn't make a suitability determination in the allotted timeframe. Therefore, USDA regulated areas were removed from the GRAS notice #827.

FDA issued a response letter on August 12, 2019 stating the FDA has ''no questions at this time regarding OmniLytics's conclusion that *E. coli* phage preparation is GRAS under its intended conditions of use."

On September 6, 2019 USDA issued a letter to OmniLytics stating:

"Specifically, you requested the use of GRN 827 as described below:

- An aqueous solution containing up to 1 x 10^8 PFU per gram applied using a surface spray, dip, or wash on red meat carcasses, parts, and trim (prior to grinding)."

"FSIS completed its review and has no objection to the use of GRN 827, as described above. Food ingredients intended for use in the production of FSIS-regulated meat, poultry, and egg products are subject to the Food and Drug Administration (FDA) regulations. GRN 827 became effective with FDA on August 12, 2019."

OmniLytics would like to request that FDA considers USDA regulated uses for GRAS Notice #827 based on the recent opinion letter from the USDA. Since these recent developments have occurred within one month of FDA's response letter, we would like to request that this review avoid the amendment process in order to expedite the changes.

We greatly appreciate your consideration.

Sincerely,

Digitally signed by Tyler Homer
DN: cn=Tyler Homer, o=OmniLytics, Inc., ou, email=thomer@omnilytics.com, c=US
Date: 2019.09.10 12:48:05 -06'00'
Adobe Acrobat version: 11.0.0

Tyler Homer, Chief Operations Officer

9075 South Sandy Parkway, Sandy, UT 84070 (801) 746-3600 (Fax) 801-746-3461

United States Department of Agriculture

Food Safety and Inspection Service

Office of Policy and Program Development

Risk Management and Innovations Staff

Patriot's Plaza III
1400 Independence Avenue, SW,
Washington, D.C.
20250-3700

September 6, 2019

Ryan Bringhurst
OmniLytics, Inc.
9075 South Sandy Parkway
Sandy, UT 84070

Mr. Ryan Bringhurst:

This letter is being issued in response to your submission, Log No. 2019-46-ING. The submission requested an acceptability determination for food notification (GRN) 827. GRN 827 (ECLYPSE-STEC) is an aqueous mixture containing three bacterial monophages (MLF4, OLB35, and OLB145) to be used in red meat carcasses, parts, and trim (prior to grinding), using a surface spray, dip, or wash as an antimicrobial specific to shiga toxin-producing *Escherichia coli* (STEC) including serogroups O26, O45, O103, O111, O121 and O145.

Specifically, you requested the use of GRN 827 as described below:

- An aqueous solution containing up to 1 x 10^8 PFU per gram applied using a surface spray, dip, or wash on red meat carcasses, parts, and trim (prior to grinding).

Under the Federal Meat Inspection Act (FMIA), Poultry Products Inspection Act (PPIA), and the Egg Products Inspection Act (EPIA), the Food Safety and Inspection Service (FSIS) is responsible for determining the efficacy and suitability of food ingredients in meat, poultry, and egg products. Suitability relates to the effectiveness of the ingredient in performing the intended purpose of use and the assurance that the conditions of use will not result in an adulterated product, or one that misleads the consumer.

FSIS completed its review and has no objection to the use of GRN 827, as described above. Food ingredients intended for use in the production of FSIS-regulated meat, poultry, and egg products are subject to the Food and Drug Administration (FDA) regulations. GRN 827 became effective with FDA on August 12, 2019.

The use of this antimicrobial bacteriophage solution (GRN 827), as described in your notification, will need to be addressed in an establishment's hazard

analysis and, as appropriate, incorporated into a Hazard Analysis and Critical Control Point (HACCP) plan, Sanitation Standard Operating Procedures (SSOPs), or other prerequisite program, validated for its application, and verified on an "ongoing" basis for its effectiveness. If the establishment does not address the effects of using this ingredient application in its hazard analysis, FSIS would be unable to determine that product processed using this ingredient is not adulterated. Therefore, the product would not be eligible to bear the mark of inspection.

All manufacturers, suppliers, and establishments are to comply with the Occupational Safety and Health Administration (OSHA) regulations to ensure that the use of this substance will not jeopardize the safety of FSIS inspection program personnel (IPP). This includes compliance with Safety Data Sheets (SDS), hazard communication, employee safety information and training, hazard assessments for personal protective equipment (PPE) selection, and air sampling data for substances with Permissible Exposure Limits (PELs). The use of this substance will need to be addressed in the establishment's hazard communication program to ensure adequate controls are in place to protect IPP from the associated safety and health hazards. For questions regarding OSHA standards, visit www.osha.gov or call OSHA at 1-800-321-OSHA (6742), TTY 1-877-889-5627.

As described in the October 19, 2005 *Federal Register* Notice, Vol. 70, No. 201, pages 60784-60786, a summary description on your new technology will be posted on the FSIS New Technology Information Table. If you do not object within five (5) business days from the date that you receive this letter, the Agency will post the included alternative description of the technology on the website. If you do object to the description, you should state in writing that you object to the description, explain the basis for your objection (*e.g.*, proprietary agreement, confidential commercial information), and provide an alternative description. FSIS will post the alternative description, unless the Agency concludes that the description does not fairly describe the technology. In such case, FSIS will post the description that it prepared and will notify the company of its decision. FSIS will post the following summary description of your technology:

Case Number	**Company Name**	**Summary of the Notification/Protocol**
2019-46-ING	OmniLytics, Inc.	An aqueous preparation containing three bacterial monophages (MLF4, OLB35and OLB145) to be used in red meat

The substance will become effective with this letter. The next scheduled revision of FSIS Directive 7120.1, "Safe and Suitable Ingredients in Meat, Poultry, and Egg Products" will be amended to reflect the changes as shown below:

Substance	Intended use of Product	Amount	Reference	Labeling Requirements
Antimicrobials				
An aqueous preparation containing three bacterial monophages (MLF4, OLB35, and OLB145) as an antimicrobial specific to shiga toxin-producing *Escherichia coli* (STEC), including serogroups O26, O45, O103, O111, O121, and O145	red meat carcasses, parts, and trim (prior to grinding)	Up to 1 x 10^8 PFU/g	GRN 827	None under the accepted conditions

If you have any questions, please contact Scott Updike by e-mail at Michael.Updike@usda.gov or by phone at 301-504-0896.

Sincerely,

MELVIN CARTER Digitally signed by MELVIN CARTER Date: 2019.09.06 15:35:19 -04'00'

Melvin Carter, Ph.D.
Director
Risk Management and Innovations Staff
Office of Policy and Program Development

ECP-100 treatment significantly reduces the concentration of *Escherichia coli* O157:H7 in experimentally-contaminated beef, and it does not exhibit a residual anti-*E. coli* effect after initially reducing the bacterium's concentration in ground beef

Running title: "Residual Effect Study"

Study #051908

INTRALYTIX, INC.
The Columbus Center
701 E. Pratt Street
Baltimore, MD 21202
www.intralytix.com

TABLE OF CONTENTS

1. STUDY TITLE

"ECP-100 significantly reduces the concentration of *Escherichia coli* O157:H7 in experimentally-contaminated beef, and it does not exhibit a residual anti-*E. coli* effect after initially reducing the bacterium's concentration in ground beef"

Running title: "Residual Effect Study"

2. STUDY DURATION

The study was performed during May 2008.

3. STUDY DIRECTOR

Alexander Sulakvelidze, Ph.D.

4. STUDY PERSONNEL

The following personnel of Intralytix Inc. contributed to the conduct and reporting of the studies reported herein:

	Name	Title	Role
-	Alexander Sulakvelidze	Chief Scientist	Study Director
-	Manrong Li	Research Scientist	Hands-on research

5. PERFORMING LABORATORY

Intralytix, Inc.
Research and Development
The Columbus Center
701 E. Pratt Street
Baltimore, MD 21202

6. STUDY OBJECTIVES

The study had the following two objectives:

1. Determine whether application of ECP-100 significantly reduces the concentration of *E. coli* 0157:H7 in experimentally-contaminated ground beef stored refrigerated (at 4 to 7^{o}C) and frozen (at -20^{o}C).

2. Determine whether ECP-100 has a residual effect in ground beef stored at 4 to 7^{o}C and at -20^{o}C, after initially reducing the *E. coli* O157:H7 concentration. A "residual effect" was defined, for the purpose of this study, as the ability of ECP-100's phages to elicit a statistically significant further (after initially reducing the bacterium's concentration) reduction in the concentration of viable *E. coli* O157:H7 in ground beef, compared to the ground beef samples contaminated with the same residual levels of bacterial cells but not treated with phage.

7. STUDY DESIGN AND RATIONALE

1. Objective of the "efficacy study"

The study's experimental protocol was designed to mimic conditions likely to be encountered in a real-life meat processing facility where ECP-100 would be used. Specifically, (i) beef was treated with ECP-100 shortly before it was ground, and (ii) the anti-*E. coli* O157:H7 effect of ECP-100 was examined during the contaminated ground beef's storage for 7 days at 4 to 7^{o}C and at -20^{o}C.

2. Objective of the "residual effect study"

The study was designed to determine whether ECP-100's phages are able to reduce the concentration of *E. coli* 0157:H7 remaining in contaminated ground beef after the initial reduction resulting from treatment with ECP-100. The study was conducted utilizing two sequential protocols. First, we determined the number of surviving *E. coli* in the contaminated, ECP-100-treated ground beef samples (Group A). Second, (i) we prepared Group C samples by contaminating ground beef with the same number of *E. coli* 0157:H7 found to survive during the first protocol (Group A), but we did not treat the samples in Group C with ECP-100, and (ii) we quantitated the Group A, B, and C samples' *E. coli* concentrations during a 7-days-long storage period at 4 to 7^{o}C and at -20^{o}C. The study's rationale was that if the phages continued to have technical effect on the ECP-100-treated samples (Group A), we would see a statistically significant difference between the *E. coli* concentrations present in the Group A and Group C samples during their storage. However, if the concentrations of *E. coli* 0157:H7 in the Group A and Group C samples were not significantly different, it would indicate that after the initial reduction due to ECP-100 treatment, ECP-100 did not have a residual effect on *E. coli* in the ground beef.

8. TEST MATRIX

Beef slices were purchased from a local grocery store (Giant) in Baltimore, MD, and they were not washed or pre-treated prior to our studies.

9. ECP-100 LOT AND APPLICATION METHOD AND RATE

- ECP-100, lot #0708C120181
- Titer: ca. 2 x 10^9 PFU/ml
- ECP-100 was applied with a Basic Spray Gun, model #250-2 (Badger Air-Brush Co., Franklin Park, IL).
- The application rate was ca. 2 ml of ECP-100/500 cm^2 of unground/sliced beef.

10. BACTERIAL STRAINS USED TO EXPERIMENTALLY CONTAMINATE THE BEEF

The beef slices were experimentally contaminated with a 1:1:1 mixture of three *E. coli* 0157:H7 strains:

- *Ec* 229: A nalidixic acid-resistant mutant developed from *Ec* 133 (also known as EHEC 5/strain 2886-75). EHEC 5 caused the first known case of foodborne disease produced by *E. coli* O157:H7 in the U.S.A.
- *Ec* 230: A nalidixic acid-resistant mutant developed from *Ec* 136 (also known as EHEC 8/strain G5101). EHEC 8 is a human isolate.
- *Ec* 231: A nalidixic acid-resistant mutant developed from *Ec* 130 (also known as EHEC 2/strain 93-111). EHEC 2 was responsible for an outbreak of foodborne disease in Washington State during 1993.

The strains were selected for nalidixic acid resistance by serially passaging the original isolates on LB agar plates supplemented with increasing concentrations of nalidixic acid. Each strain underwent ≤8 serial passages before it was determined to be nalidixic acid-resistant at a concentration of 25 µg/ml. After the passaging, the above-noted Intralytix strain designations were assigned (i.e., *Ec* 229, *Ec* 230, and *Ec* 231). The strains were stored at -80°C, at Intralytix, in 70% LB broth/30% glycerol supplemented with nalidixic acid (25 µg /ml).

Shortly before performing the study, samples of the three strains were thawed and grown (37 ± 2°C, O/N) in LB broth (3 ml) supplemented with nalidixic acid (25 µg/ml).

Each of the bacterial cultures was diluted 100-fold, and equal volumes of the dilutions were mixed together just prior to performing the study.

Approximate bacterial challenge dose: 3,000 CFU/g food.

11. MEDIA AND REAGENTS

- Sorbitol MacConkey agar supplemented with Cefixime and rhamnose (CR-SMAC agar; purchased [catalog #611052] from Remel Inc., Lenexa, KS)
- Cefixime supplement (Remel, Inc.; Lenexa, KS [catalog #66391]) #66391])
- Peptone water (Becton, Dickinson and Company; Sparks, MD [catalog #218105])
- Nalidixic acid (Tokyo Kasei Kogyo Co., Ltd. [catalog #AG])

12. GENERAL OUTLINE OF THE STUDY

1. Determined the weight and surface dimensions (one side only) of the three beef slices (Groups A, B and C) to be used during the experiments.

2. Applied the challenge dose of *E. coli* to one side of the Group A and Group B beef slices. Stored the uncontaminated Group C slice (covered with Saran Wrap) at 4 to 7^{o}C.

3. Allowed the bacteria to colonize the Group A and Group B beef slices at room temperature (RT) for 30 min.

4. Applied PBS (2 ml/500 cm^2) to the colonized side of the Group B beef slice (control group)

5. Applied ECP-100 (2 ml/500 cm^2) to the contaminated side of the Group A beef slice, as described in Section 9 (test group).

6. Covered the treated samples with Saran Wrap, stored them at 4 to 7^{o}C, and tested them as described below.

7. After 24 h of storage, ground the three beef slices (Groups A, B, and C) with a Meat Grinder (Northern Industrial Tools, Burnsville, MN [catalog #168610]).

8. Aliquots (ca. 400 g) of the ground beef prepared from each of the three beef slices were divided into two approximately equal portions (200 g each), for a total of 6 groups/portions designated:

A
A-1
B
B-1
C
C-1

9. Removed triplicate samples (ca. 25 g) of ground beef from Group A and from Group B. Did not remove anything from Group C.

10. Placed the three 25 g samples in three separate, bagged aliquots (100 ml) of sterile peptone water containing sodium nalidixate (25 mg/L).

11. Each sample was "hand-mushed" briefly and stomached for a minimum of 30 seconds on the medium setting.

12. Aliquots (0.3 ml and 0.5 ml) of the stomached samples were spread uniformly on CR-SMAC agar in Petri dishes, and the inoculated medium was incubated at 37 ± 2°C for 24 ± 2 h.

13. After incubation, we counted the *E. coli* colonies and calculated the CFU/g of ground beef, using the following formula:

    CFU/g = Total CFU/bag (actual CFU/0.5 ml x 2 x 100 ml peptone water) ÷ 25 g weight of sample analyzed

    Note: We used the counts obtained with the 0.5 ml aliquots in order to calculate the CFU/g because many of the 0.3 ml aliquots did not contain colonies (see
    TABLE 1).

14. Determined the phage titer (plaque-forming units[PFU]/g) of the ground beef, by passing an aliquot (5 ml) of each stomached sample through a 0.45 µm filter, and analyzing the supernatant fluids by a standard titering protocol. The following formula was used to calculate PFU/g:

    PFU/g = PFU/ml x 100 ml peptone water ÷ 25 g weight of sample analyzed

15. We contaminated ground beef samples from Group C and Group C-1 with the same number of residual *E. coli* 0157:H7 counted in the Group A samples.

16. We immediately analyzed the ground beef samples in Groups A, B, and C, in order to determine and compare their concentrations of *E. coli* 0157:H7

and anti-*E. coli* O157:H7 phage, as described in steps 9-14. Stored the remaining Group A, B and C samples at 4 to 7°C. Placed the Group A-1, B-1, and C-1 samples in a -20°C freezer.

17. Repeated step 16 for all samples (Groups A, A-1, B, B-1, C, and C-1) on day 5.

18. Repeated step 16 for all samples (Groups A, A-1, B, B-1, C, and C-1) on day 7.

13. RESULTS

13.1. Raw data

TABLE 1. Raw data

Group	Time & Sample		Colonies		CFU/g		Phage Titer		Group	Time & Sample		Colonies		CFU/g		Phage Titer	
	Time	#	0.5 ml	0.3 ml	CFU/g	Average	PFU/ml	PFU/g		Time	#	0.5 ml	0.3 ml	CFU/g	Average	PFU/ml	PFU/g
A	Day 0	A1	1	0	8	5	2.0E+05	8.0E+05									
		A2	0	0	0												
		A3	1	0	8												
B	Day 0	B1	16	11	128	91	0										
		B2	10	7	80												
		B3	8	5	64												
C	Day 0	C1	2	1	16	11	0										
		C2	1	1	8												
		C3	1	0	8												
A	Day 5	A1	1	0	8	5	3.0E+05	1.2E+06	A-1	Day 5	A1-1	1	0	8	11	2.0E+05	8.00E+05
		A2	0	0	0						A2-1	1	0	8			
		A3	1	0	8						A3-1	2	0	16			
B	Day 5	B1	20	8	160	171	0		B-1	Day 5	B1-1	25	15	200	197	0	0
		B2	26	12	208						B2-1	28	20	224			
		B3	18	10	144						B3-1	21	17	168			
C	Day 5	C1	2	0	16	8	0		C-1	Day 5	C1-1	3	0	24	16	0	0
		C2	0	0	0						C2-1	1	0	8			
		C3	1	0	8						C3-1	2	0	16			
A	Day 7	A1	1	0	8	3	2.0E+05	8.0E+05	A-1	Day 7	A1-1	1	0	8	3	3.0E+05	1.20E+06
		A2	0	0	0						A2-1	0	0	0			
		A3	0	0	0						A3-1	0	0	0			
B	Day 7	B1	19	12	152	149	0	0	B-1	Day 7	B1-1	6	4	48	75	0	0
		B2	20	10	160						B2-1	10	8	80			
		B3	17	7	136						B3-1	12	9	96			
C	Day 7	C1	1	0	8	8	0	0	C-1	Day 7	C1-1	1	0	8	7	0	0
		C2	2	0	16						C2-1	0	0	0			
		C3	0	0	0						C3-1	1	0	8			

13.2. Percent reduction

Day 0, storage at 4 to 7°C

Group A (ECP-100-treated) vs. Control (PBS-treated) 95% reduction

Day 5, storage at 4 to 7°C

Group A (ECP-100-treated) vs. Control (PBS-treated) 97% reduction

Day 7, storage at 4 to 7°C

Group A (ECP-100-treated) vs. Control (PBS-treated) 98% reduction

Day 5, storage at -20°C

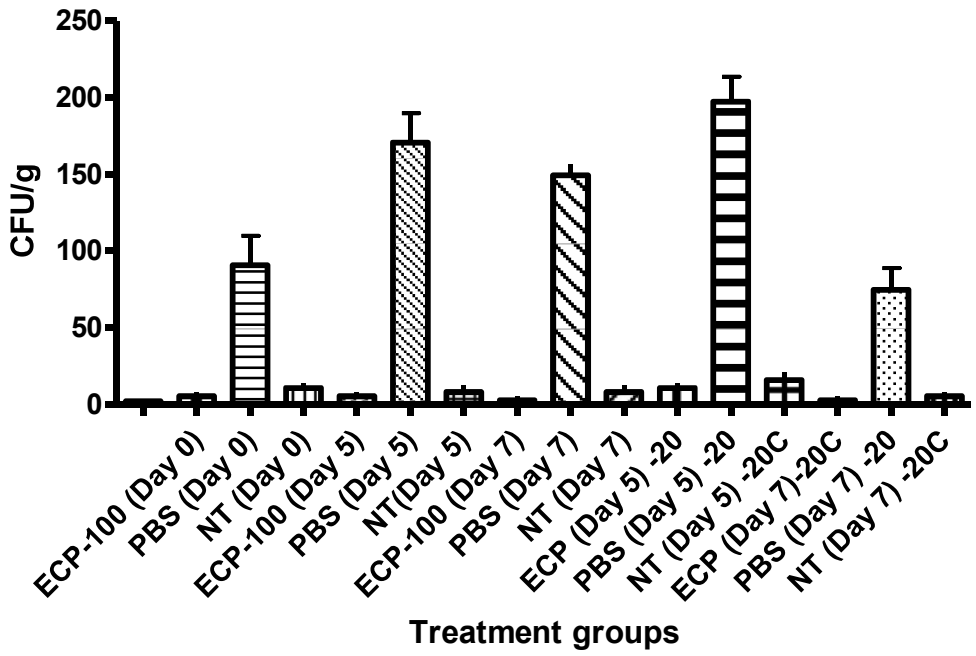
Group A (ECP-100-treated) vs. Control (PBS-treated) 94% reduction

Day 7, storage at -20°C

Group A (ECP-100-treated) vs. Control (PBS-treated) 96% reduction

13.3. Graphical presentation of the efficacy results

Figure 1. Efficacy of ECP-100 in reducing the number of viable *E. coli* O157:H7 in experimentally-contaminated ground beef (based on triplicate samples; bars = SEM).



13.4. Statistical analysis

The efficacy of the ECP-100 treatment in reducing the number of viable *E. coli* O157:H7 in experimentally-contaminated beef was determined by comparing the data obtained with the PBS-treated control samples and the ECP-100-treated samples.

Statistical analysis was performed, and a chart summarizing the data was constructed, with the GraphPad InStat (version 3.05) and GraphPad Prism (version 4.0) programs, respectively (GraphPad Software; San Diego, CA; www.graphpad.com). A *p* value of <0.05 indicated a statistically significant difference between the results.

Results of the "efficacy study" for the samples stored at 4 to 7°C

1. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples significantly less than that in the PBS-treated samples on Day 0?*

Unpaired t test: Do the means of ECP-100 (Day 0) and PBS (Day 0) differ significantly?

The one-tailed *p* value is 0.0059, which is considered to be very significant.

2. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples significantly less than that in the PBS-treated samples on Day 5?*

Unpaired t test: Do the means of ECP-100 (Day 5) and PBS (Day 5) differ significantly?

The one-tailed *p* value is 0.0005, which is considered to be extremely significant.

3. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples significantly less than that in the PBS-treated samples on Day 7?*

Unpaired t test: Do the means of ECP-100 (Day 7) and PBS (Day 7) differ significantly?

The one-tailed *p* value is <0.0001, which is considered to be extremely significant.

Results of the "efficacy study" for the samples stored at -20^{o}C

1. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples significantly less than that in the PBS-treated samples stored at -20^{o}C for 5 days?*

Unpaired t test: Do the means of ECP-100 (Day 5) and PBS (Day 5) differ significantly in the samples stored at -20^{o}C?

The one-tailed *p* value is 0.0002, which is considered to be extremely significant.

2. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples significantly less than that in the PBS-treated samples stored at -20^{o}C for 7 days?*

Unpaired t test: Do the means of ECP-100 (Day 7) and PBS (Day 7) differ significantly in the samples stored at -20^{o}C?

The one-tailed *p* value is 0.0037, which is considered to be very significant.

Results of the "residual effect" study for the samples stored at 4 to 7^{o}C

1. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples significantly different from the number of viable E. coli O157:H7 in the after-treatment samples (Group C) on Day 0?*

Unpaired t test: Do the means of ECP-100 (Day 0) and NT (Day 0) differ significantly?

The one-tailed *p* value is 0.1151, which is not considered to be significant.

2. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples significantly different from the number of viable E. coli O157:H7 in the after-treatment samples (Group C) on Day 5?*

Unpaired t test: Do the means of ECP-100 (Day 5) and NT(Day 5) differ significantly?

The one-tailed *p* value is 0.3217, which is not considered to be significant.

3. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples significantly different from the number of viable E. coli O157:H7 in the after-treatment samples (Group C) on Day 7?*

Unpaired t test: Do the means of ECP-100 (Day 7) and NT(Day 7) differ significantly?

The one-tailed *p* value is 0.1870, which is not considered to be significant.

Results of the "residual effect" study for the samples stored at -20°C

1. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples stored at -20°C significantly different from the number of viable E. coli O157:H7 in the after-treatment samples (Group C) stored at -20°C on Day 5?*

Unpaired t test: Do the means of ECP (Day 5) -20°C and NT (Day 5) -20°C differ significantly?

The one-tailed *p* value is 0.1870, which is not considered to be significant.

2. *Was the number of viable E. coli O157:H7 in the ECP-100-treated samples stored at -20°C significantly different from the number of viable E. coli O157:H7 in the after-treatment samples (Group C) stored at -20°C on Day 7?*

Unpaired t test: Do the means of ECP (Day 7) -20°C and NT (Day 7) -20°C differ significantly?

The one-tailed *p* value is 0.2593, which is not considered to be significant.

13.5. Brief discussion of the results and the study's conclusions

- Applying ECP-100 to beef before grinding reduced the number of viable *E. coli* O157:H7 in the ground beef by ca. 95% after 24 h of storage at 4 to 7°C. The observed reduction was statistically significant (p = <0.05).

- Applying ECP-100 to beef before grinding reduced the number of viable *E. coli* O157:H7 in the ground beef by ca. 97% after 5 days of storage at 4 to 7°C. The observed reduction was statistically significant (p = <0.05).

- Applying ECP-100 to beef before grinding reduced the number of viable *E. coli* O157:H7 in the ground beef by ca. 98% after 7 days of storage at 4 to 7^{o}C. The observed reduction was statistically significant (p = <0.05).

- Applying ECP-100 to beef before grinding reduced the number of viable *E. coli* O157:H7 in the ground beef by ca. 94% after 5 days of storage at -20^{o}C. The observed reduction was statistically significant (p = <0.05).

- Applying ECP-100 to beef before grinding reduced the number of viable *E. coli* O157:H7 in the ground beef by ca. 96% after 7 days of storage at -20^{o}C. The observed reduction was statistically significant (p = <0.05).

- The reduction in the concentration of viable *E. coli* 0157:H7 observed during the present study was similar to that observed during the previous studies designated #01232007 and #05242007. This observation supports the robustness and reproducibility of our phage treatment data.

- The concentration of viable *E. coli* O157:H7 in the ECP-100-treated samples was not significantly different from that in the Group C samples on Day 0. Therefore, the Group C samples were contaminated with essentially the same number of *E. coli* O157:H7 present in the Group A samples.

- The concentration of viable *E. coli* O157:H7 in the ECP-100-treated samples (Group A) was not significantly different from that in the Group C samples at any time during the testing and at any storage condition. Therefore, after initially reducing the concentration of *E. coli* 0157:H7, ECP-100 did not have an additional, residual effect in the recontaminated ground beef.

- The inability of ECP-100 to continue to reduce the concentration of *E. coli* 0157:H7 in ground beef after the initial reduction may have resulted from a combination of at least two interrelated factors. *First*, the residual phage concentration in the ground beef may have been insufficient to reduce further the number of viable *E. coli*. In that regard, the amount of ECP-100 applied to the beef slices before grinding was ca. 2 ml/500 cm^2 of surface area, a dose which significantly reduced the initial contamination with *E. coli* O157:H7. However, since grinding drastically increased the surface area of the beef, the amount of phages/cm^2 may have been reduced to a level that could not further reduce the bacterial counts. *Second*, direct contact (which is required for phage-mediated lysis of bacteria) between the phages and *E. coli* O157:H7 in the ground beef also may have been significantly reduced because many of the bacteria embedded in the ground meat may have been significantly less accessible to the phages.

14. SUMMARY CONCLUSION OF THE STUDY

ECP-100 significantly reduced the *E. coli* O157:H7 concentration in ground beef when it was applied to experimentally-contaminated meat before grinding. The reduction was apparent during the entire study's duration (7 days) for meat samples stored both under refrigerated conditions and frozen.

The concentration of *E. coli* in the ECP-100-treated beef after the initial reduction was not significantly different from that in untreated samples "spiked" to contain the same number of *E. coli* present in the ECP-100-treated samples after the initial reduction. Thus, ECP-100 treatment did not exhibit a residual anti-*E. coli* effect during our studies with experimentally-contaminated ground beef.

15. TEST SUBSTANCE / ECP-100 RETENTION

Remaining aliquots of ECP-100 lots used during the studies described in this report are stored (2-6^{0}C, in dark) at the Intralytix, Inc. Research and Development Facility, Baltimore, MD 21202. Lots will be stored until their expiration date, at which time they may be discarded.

16. SIGNATURES

Data analyzed by:

May 19, 2008

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Alexander Sulakvelidze, Ph.D. *Date*
Study Director