

**FDA USE ONLY**

GRN NUMBER 000752	DATE OF RECEIPT 01/03/2018
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Food and Drug Administration  
**GENERALLY RECOGNIZED AS SAFE  
(GRAS) NOTICE** (Subpart E of Part 170)

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    If yes, enter the date of communication (*yyyy/mm/dd*): \_\_\_\_\_  
 No

**SECTION B – INFORMATION ABOUT THE NOTIFIER**

<b>1a. Notifier</b>	Name of Contact Person Dr. Rosemond Mandeville	Position or Title Chief Scientific Officer	
	Organization ( <i>if applicable</i> ) Phagelux, Inc.		
	Mailing Address ( <i>number and street</i> ) 1600 Royalmount		
City Montreal	State or Province Quebec	Zip Code/Postal Code H4P 2R2	Country Canada
Telephone Number 514-246-5329	Fax Number 514-496-1521	E-Mail Address mandeville@phagelux.com	
<b>1b. Agent or Attorney (if applicable)</b>	Name of Contact Person Tyler Homer	Position or Title Director of Regulatory Affairs	
	Organization ( <i>if applicable</i> ) OmniLytics, Inc.		
	Mailing Address ( <i>number and street</i> ) 9100 South 500 West		
City Sandy	State or Province Utah	Zip Code/Postal Code 84070	Country United States of America
Telephone Number 801-746-3600	Fax Number 801-746-3461	E-Mail Address thomer@omnilytics.com	

## SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

SalmoPro (Salmonella-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 603 \_\_\_\_\_  
 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.

SalmoPro® is intended for use as an antimicrobial processing aid to control *Salmonella* on food, when applied to food surfaces up to  $1 \times 10^8$  PFU (Plaque Forming Units) per gram of food.

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 SalmoPro (Salmonella-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. Dr. Rosemonde Mandeville agrees to make the data and information that are the basis for the  
*(name of notifier)* 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.

1600 Royalmount, Montreal, Quebec, H4P 2R2, Canada  
*(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 of 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**

**Tyler Homer**

Digitally signed by Tyler Homer  
DN: cn=Tyler Homer, o=OmniLytics, Inc., ou=  
email=thomer@omnilytics.com, c=US  
Date: 2017.12.28 14:28:50 -07'00'

**Printed Name and Title**

Tyler Homer, Director of Regulatory Affairs

**Date (mm/dd/yyyy)**

12/28/2017

**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.

<b>Attachment Number</b>	<b>Attachment Name</b>	<b>Folder Location (select from menu)</b> (Page Number(s) for paper Copy Only)
	SalmoPro 2.2 - Final.pdf	Submission

**OMB Statement:** Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, [PRAStaff@fda.hhs.gov](mailto:PRAStaff@fda.hhs.gov). (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

**Phagelux (Canada), Inc.**

**GRAS Notification:**

**SalmoPro<sup>®</sup>**

**Phagelux (Canada), Inc.**

6100 Royalmount, Montreal, Quebec, H4P 2R2, Canada

Tel: 514-496-7722; Fax: 514-496-1521

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December 22, 2017

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: Phagelux GRAS Notification for SalmoPro<sup>®</sup>*

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Dear Dr. Highbarger:

In accordance with 21 CFR Part 170 Subpart E, Phagelux (Canada), Inc. is submitting a GRAS notification for the bacteriophage cocktail SalmoPro<sup>®</sup> for bio-control of *Salmonella enterica* in food. This GRAS submission contains several changes from GRN No. 603. Phagelux has again determined, through scientific procedures, that SalmoPro<sup>®</sup> 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 SalmoPro<sup>®</sup> for use in meat, poultry, and egg products.

SalmoPro<sup>®</sup> 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

(b) (6)



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Rosemonde Mandeville, M.B., Ch.B.; PhD  
Chief Scientific Officer  
Phagelux, Inc.

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## **Part 1 Signed Statements and Certification**

### **1.1 Compliance with 21 CFR 170 Subpart E**

Phagelux (Canada), Inc. is hereby submitting a GRAS notice in accordance with 21 CFR 170 Part E.

### **1.2 Name and Address of Notifier**

Phagelux (Canada), Inc.  
1600 Royalmount, Montreal,  
Quebec, H4P 2R2, Canada  
Phone: 514-246-5329  
Fax: 514-496-1521

### **1.3 Common or Usual Name**

Phagelux (Canada), Inc. produces a *Salmonella*-specific bacteriophage cocktail under the trade name SalmoPro<sup>®</sup>.

### **1.4 Intended Conditions of Use**

SalmoPro<sup>®</sup> is intended for use as an antimicrobial processing aid to control *Salmonella* on food, when applied to food surfaces up to  $1 \times 10^8$  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), Phagelux (Canada), Inc. has determined that SalmoPro<sup>®</sup> is GRAS through scientific procedures.

## 1.6 Exemption from Premarket Approval

SalmoPro<sup>®</sup> was determined by Phagelux 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 Phagelux's determination of GRAS for SalmoPro<sup>®</sup> 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.  
9100 South 500 West  
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 Phagelux (Canada), Inc. and pertinent to the evaluation of the safety and GRAS status of the use of SalmoPro<sup>®</sup>.

## 1.10 Signature

(b) (6)



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Rosemonde Mahdeville, M.B., Ch.B.; PhD  
Chief Scientific Officer

*Dec 22, 2017*

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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 SalmoPro<sup>®</sup> for use in meat, poultry, and egg products as a processing aid.

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## Part 2 Identity and Specifications of SalmoPro®

### 2.1 Identity

SalmoPro® consists of a mixture of equal concentrations of two *Salmonella*-specific lytic bacteriophages (hereinafter referred to as “monophage(s)”). Each of these monophages is specifically effective against a wide host range of *Salmonella enterica* serotypes. These phages were isolated by Phagelux and OmniLytics scientists from farms in the US and Canada.

SalmoPro® is a liquid made up of equal parts of two monophages (BP-63 and LVR16-A), which are produced and purified separately and mixed in equal concentrations. The commercial product SalmoPro® has a minimal titer of  $1 \times 10^{10}$  PFU/mL. This solution is concentrated and will be diluted with water at application sites to ensure application rate at a maximum of  $1 \times 10^8$  PFU/g of food.

#### 2.1.1 Phage Identity

The monophages were isolated by Phagelux and OmniLytics scientists from farms in the US and Canada. 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 *Salmonella* strains, and lytic activity against non-*Salmonella*-related bacteria strains.

Name: BP-63  
Order: Caudovirales  
Family: Myoviridae  
Properties: Double-stranded DNA, lytic

Name: LVR16A  
Order: Caudovirales  
Family: Siphoviridae  
Properties: Double-stranded DNA, lytic

The DNA genome of phages BP-63 and LVR16A was sequenced and deposited in the GenBank. Accession number: KM366099 for BP-63 and MF681663 for LVR16A.

#### 2.1.2 Host Identity

Both monophages are produced in non-pathogenic hosts; BP-63 in *E. coli* strain 12-869E and LVR16A in *Salmonella enterica* strain 17-37A. Identification and pathogenicity testing was performed by OmniLytics using standard PCR protocols for the respective tests. *E. coli* production strain 12-869E tests negative for Shiga toxins stx1 and stx2, and *Salmonella* production strain 17-37A lacks pathogenicity islands SPI-1 and SPI-2.

Both production hosts were tested for antibiotic resistance against tetracycline, chloramphenicol, kanamycin, nalidixic acid, nitrofurantoin, and penicillin. The production hosts were sensitive to all antibiotics, except *E. coli* strain 12-869E was resistant to penicillin.

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 65 strains of *Salmonella*. SalmoPro<sup>®</sup> was shown to be *Salmonella* specific and has a broad host range. Lytic activity was demonstrated on over 93% of the tested *Salmonella enterica* strains and non-serotyped strains. The tested *Salmonella enterica* strains included strains of Enteritidis, Hadar, Heidelberg, Kentucky, Montevideo, Typhimurium, Agona, Anatum, Braenderup, Bredeney, Infantis, Mbandaka, Minnesota, Muenchen, Newport, Panama, Paratyphi B, Saintpaul, and Schwarzengrund.

SalmoPro<sup>®</sup> was also tested 17 non-*Salmonella enterica* strains and did not show any lytic activity against the panel, except for two *E. coli* and one *Listeria monocytogenes* strains. *Salmonella* is known to be phylogenetically closely related to *E. coli* bacteria (Bern & Goldberg, 2005). More importantly, both BP-63 and LVR16A are non-transducing phages and cannot integrate or transfer any genes. The tested non-*Salmonella* strains included *E. coli*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Pantoea agglomerans*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Listeria innocua*, and *Staphylococcus aureus*.

### 2.1.4 SalmoPro<sup>®</sup> Characteristics

SalmoPro<sup>®</sup> is a clear to opalescent, odorless liquid with an average phage weight  $5.4 \times 10^7$  Dalton or  $9.02 \times 10^{-17}$  grams.

**TABLE 1**  
**MONOPHAGE WEIGHT**

	Number of Base Pairs	Weight/Phage #bp x 660 (Dalton)	Weight/Phage (grams)	Weight in 1 mL of SalmoPro <sup>®</sup> $1 \times 10^{10}$ PFU/mL (grams)
BP63	52,437	34,608,420	$5.75 \times 10^{-17}$	$2.87 \times 10^{-7}$
LVR16A	112,254	74,087,640	$1.23 \times 10^{-16}$	$6.15 \times 10^{-7}$
Average	82,346	54,348,030	$9.02 \times 10^{-17}$	$9.02 \times 10^{-7}$
Total	164,691	108,696,060	$1.80 \times 10^{-16}$	

\*1 bp DNA = 660 Dalton

1 mL of SalmoPro<sup>®</sup> has a total phage weight of  $9.02 \times 10^{-7}$  g, with the remainder of the weight being attributed to the buffer consisting of 0.01M dipotassium phosphate and 0.01M magnesium sulfate (0.00174 g/mL  $K_2HPO_4$  and 0.00246 g/mL  $MgSO_4 \cdot 7H_2O$ ). The monophage is estimated to be 0.00009% of the total weight of the concentrated liquid.

## 2.1.5 SalmoPro<sup>®</sup> 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 SalmoPro<sup>®</sup> 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  $< 1 \times 10^{10}$  PFU/mL may be concentrated and retested.
- b) **The Identity:** Identity is determined by specific PCR with predetermined reference profiles.
- c) **The Bacterial sterility:** Sterility is tested by plating 100  $\mu$ L 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**

<b>PARAMETER</b>	<b>SPECIFICATIONS</b>
<b>Potency</b>	$\geq 1 \times 10^{10}$ PFU/mL
<b>Identity</b>	PCR: Matches reference bands
<b>Bacterial sterility</b>	No growth after 7 days

**TABLE 3**

**QUALITY CONTROL OF SALMOPRO<sup>®</sup>**

<b>PARAMETER</b>	<b>SPECIFICATIONS</b>
<b>Potency</b>	$\geq 1 \times 10^{10}$ PFU/mL
<b>Identity</b>	PCR: Matches reference bands
<b>Endotoxin Content</b>	< 25,000 EU/mL for concentrated product containing $1 \times 10^9$ PFU/mL
<b>Bacterial sterility</b>	No growth after 7 days

**TABLE 4**

**PHYSICAL PROPERTIES**

<b>Physical properties</b>	<b>SalmoPro<sup>®</sup> Lot # 94-117002</b>	<b>SalmoPro<sup>®</sup> Lot # 94-117003</b>	<b>SalmoPro<sup>®</sup> Lot # 94-117004</b>
Odor	Odorless	Odorless	Odorless
Color	Opalescent	Opalescent	Opalescent
Physical State & Appearance	Liquid	Liquid	Liquid
pH	7.55	7.56	7.55
Endotoxin (EU/mL)	18,915	18,275	18,071
Solubility	Soluble in water	Soluble in water	Soluble in water

\* All tests were conducted by OmniLytics, Inc.



**TABLE 5**

**CHEMICAL COMPOSITION**

	Units	Detection Limit	SalmoPro® Lot# 94-117002	SalmoPro® Lot# 94-117003	SalmoPro® Lot# 94-117004	Average	Standard Deviation
Specific Gravity	g/mL	0.001	<b>0.995</b>	<b>0.997</b>	<b>0.998</b>	0.9967	0
Nitrate as N	mg/L	0.1	<b>ND</b>	<b>0.1</b>	<b>0.1</b>	0.10	0
Nitrite as N	mg/L	0.1	<b>ND</b>	<b>ND</b>	<b>ND</b>	N/A	N/A
Total Kjeldahl Nitrogen	mg/L	1.0	<b>7.8</b>	<b>22.1</b>	<b>22.8</b>	17.57	6.91
Total Nitrogen	mg/L	1.0	<b>7.8</b>	<b>22.2</b>	<b>22.9</b>	17.63	6.96
Total Organic Carbon	mg/L	5.0	<b>104</b>	<b>114</b>	<b>123</b>	113.7	7.76
Arsenic, Total	mg/L	0.0005	<b>0.0007</b>	<b>0.0008</b>	<b>0.0008</b>	0.00077	0.000047
Calcium, Total	mg/L	0.2	<b>0.4</b>	<b>0.3</b>	<b>0.3</b>	0.33	0.047
Copper, Total	mg/L	0.0010	<b>0.0090</b>	<b>0.0070</b>	<b>0.0060</b>	0.00733	0.0012
Iron, Total	mg/L	0.02	<b>ND</b>	<b>ND</b>	<b>ND</b>	N/A	N/A
Lead, Total	mg/L	0.0005	<b>ND</b>	<b>ND</b>	<b>ND</b>	N/A	N/A
Magnesium, Total	mg/L	0.2	<b>199</b>	<b>197</b>	<b>207</b>	201.0	4.32
Manganese, Total	mg/L	0.0005	<b>0.0075</b>	<b>0.0073</b>	<b>0.0067</b>	0.00717	0.00034
Mercury, Total	mg/L	0.0002	<b>ND</b>	<b>ND</b>	<b>ND</b>	N/A	N/A
Phosphorus, Total as P	mg/L	0.2	<b>281</b>	<b>281</b>	<b>296</b>	286.0	7.07
Potassium, Total	mg/L	0.5	<b>803</b>	<b>796</b>	<b>850</b>	816.3	23.98
Sodium, Total	mg/L	0.5	<b>9.8</b>	<b>9.4</b>	<b>9.8</b>	9.67	0.19

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 SalmoPro® commercial product. The non-pathogenic *Salmonella* and *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 SalmoPro® 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 *Salmonella* 17-37A and *E. coli* 12-869E used for monophage production do not contain any virulence genes.

### 2.1.7 Stability

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

## 2.2 Method of Manufacture

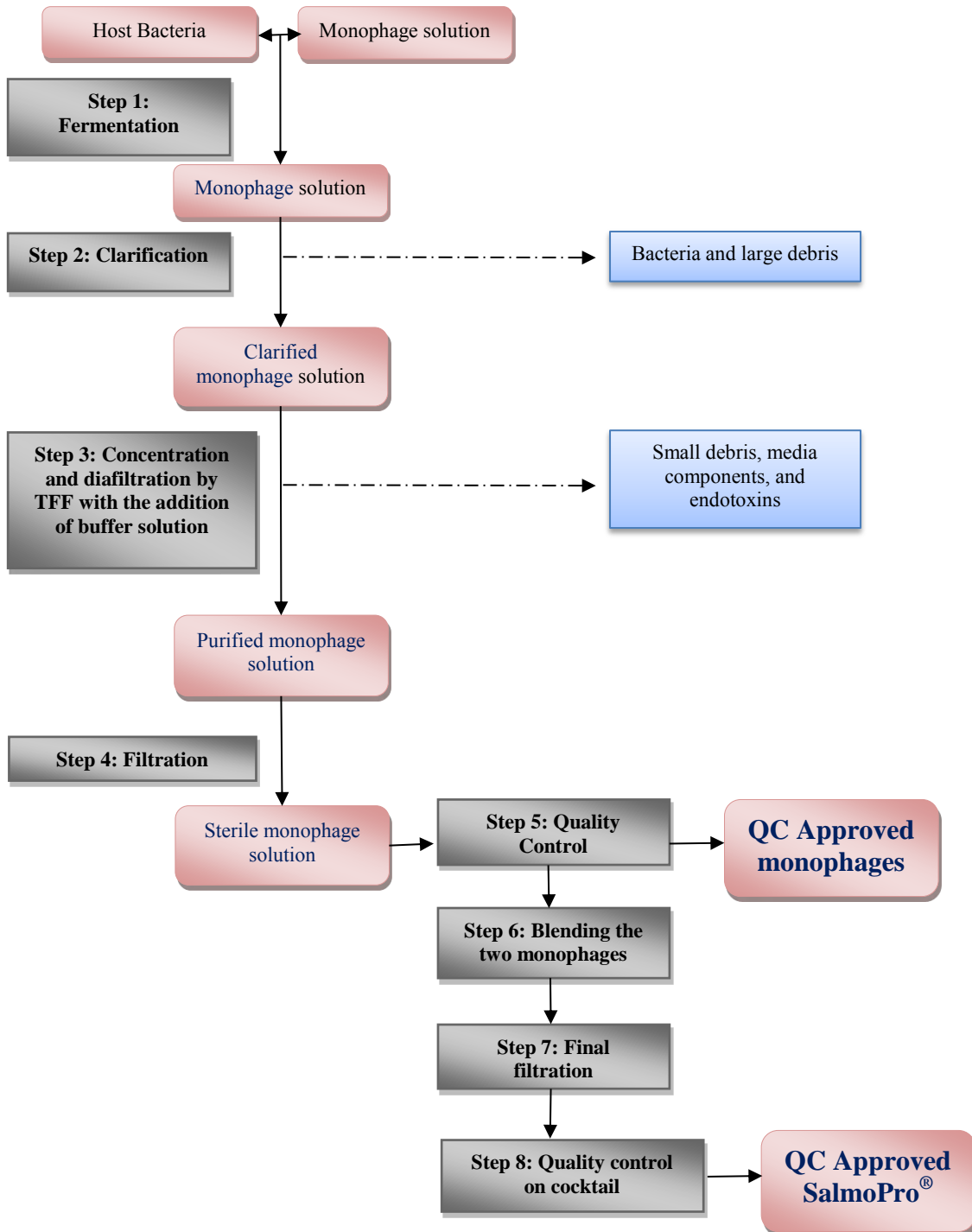
Batches of the two monophages are produced separately by aerobic fermentation using a broth media which is animal-product free. Initially, each of the host bacterium (non-pathogenic *Salmonella* or *E. coli*) is grown from a working bank sample (itself derived from the master bank) to a pre-determined optical density (OD) in an animal-product 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 and the filtrate is washed with a buffer consisting of 0.01M dipotassium phosphate and 0.01M magnesium phosphate, and concentrated by tangential flow filtration (TFF). Most of the endotoxins are expected to be removed during clarification and washing. The monophage solution is filter-sterilized.

Finally, after each monophage solution has passed the Quality Control (QC) specification steps (Table 2), they are blended and diluted to form SalmoPro<sup>®</sup> for commercialization, with each phage representing 50% of the minimum final product titer of  $1 \times 10^{10}$  PFU/mL. SalmoPro<sup>®</sup> is sterile filtered and packaged into sterile packaging components and placed in refrigerated storage (2-6°C). Quality Control (QC) is performed on each final batch (see Table 3). Only after passing QC tests is the batch released for sale.

SalmoPro<sup>®</sup> is diluted with water at the application site, to form the “working solution” with a maximum lytic activity of  $1 \times 10^9$  PFU/mL. Figure 1 is an overview of the manufacturing process.

**Figure 1: Overview of SalmoPro<sup>®</sup> Method of Manufacturing**



## **2.3 Food-Grade Material**

All components used in the manufacturing of SalmoPro<sup>®</sup> are animal-free and food grade.

## **Part 3 Dietary Exposure**

### **3.1 Application Rates**

For the dietary exposure estimation, the assumption is that SalmoPro<sup>®</sup> will be diluted and applied at the maximum rate of 1x10<sup>8</sup> PFU/g of food.

### **3.2 Dietary Intakes**

SalmoPro<sup>®</sup> 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](https://www.ers.usda.gov/data-products/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 SalmoPro<sup>®</sup> 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/](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
<b>Total</b>	<b>431.0</b>	<b>727.3</b>

### 3.3 Estimated Dietary Exposure to SalmoPro<sup>®</sup> Bacteriophages

The following calculation estimates the consumption of SalmoPro<sup>®</sup> when using a working solution of  $1 \times 10^9$  PFU/mL and applied at  $1 \times 10^8$  PFU/g of food:

Number of SalmoPro<sup>®</sup> phage per gram multiplied by the average daily per capita consumption in grams equals the total number of phages consumed per day.

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

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:

$$\frac{7.27 \times 10^{10} \text{ PFU} \times 9.02 \times 10^{-17} \text{ g}}{3000} = \mathbf{2.2 \text{ ppb}}$$

### 3.4 Estimated Dietary Exposure to SalmoPro<sup>®</sup>

The following calculation estimates the consumption of SalmoPro<sup>®</sup>:

SalmoPro<sup>®</sup> is diluted with water to a working concentration of  $1 \times 10^9$  PFU/mL and applied at a maximum rate of  $1 \times 10^8$  PFU/g of food. One gram of food is treated with 0.1 mL of SalmoPro<sup>®</sup>.

The average American consumes a total of 727.3 g of food daily that has been treated with SalmoPro<sup>®</sup> and will consume 72.73 mL of SalmoPro<sup>®</sup> at a concentration of  $1 \times 10^9$  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

As indicated in section 2.1.4, only 0.00009% of SalmoPro<sup>®</sup> is bacteriophages and the remainder 99.99991% is 0.01M dipotassium phosphate and 0.01M magnesium sulfate (0.00174g/mL  $\text{K}_2\text{HPO}_4$  and 0.00246g/mL  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ). Diluted SalmoPro<sup>®</sup> contains 0.000174g/mL  $\text{K}_2\text{HPO}_4$  and 0.000246g/mL  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .

The average American will consume an estimated 72.73 mL of SalmoPro<sup>®</sup> per day and each mL has 0.000174g  $\text{K}_2\text{HPO}_4$  and 0.000246g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .

$$72.73 \text{ mL} \times 0.000174 \text{ g} = \mathbf{0.0127 \text{ g dipotassium phosphate}}$$

$$72.73 \text{ mL} \times 0.000246 \text{ g} = \mathbf{0.0179 \text{ g magnesium sulfate}}$$

This amounts to 12.7 mg of dipotassium phosphate and 17.9 mg of magnesium sulfate 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.* (1966) 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<sup>2</sup>.

Complete removal of endotoxin during the production process of SalmoPro® 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 SalmoPro® purified lots produced. The level of Endotoxin in each of the 3 purified lots was less than 25,000 EU/mL. Using the maximum allowed for product release, we can calculate the daily consumption of endotoxins:

$$\frac{72.73 \text{ mL}}{\text{day}} \times \frac{2.5 \times 10^4 \text{ EU}}{\text{mL}} = \frac{\mathbf{1.82 \times 10^6 \text{ EU}}}{\text{day}}$$

Human saliva contains approximately 1 mg of endotoxins/mL (Leenstra et al., 1996) which is equivalent to 1x10<sup>6</sup> EU/mL. Saliva is produced at levels exceeding 500 mL/day, which amounts

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to  $5 \times 10^8$  EU/day. The maximum amount of SalmoPro<sup>®</sup> only constitutes 0.36% of the daily endotoxin load from saliva and is thus considered safe.



## Part 4 Self-limiting Levels of Use

The proposed use of SalmoPro<sup>®</sup> is as an antibacterial processing aid for foods that are at high risk to be contaminated with *Salmonella enterica*. The purpose of SalmoPro<sup>®</sup> is to significantly reduce or eliminate *Salmonella enterica* 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 *Salmonella enterica*.
- After the host bacteria *Salmonella enterica* contamination is depleted on the food, the phage will stop replicating and would gradually degrade; virions consist of only proteins and DNA.
- Phages are susceptible to a variety of environmental factors, including sunlight (Wommack, *et al.*, 1996), heat (Quiberoni, *et al.*, 2003), and UV light (Rigvava, 2012). 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.

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## Part 6 Narrative

Phagelux's determination of SalmoPro<sup>®</sup> as GRAS is based on scientific procedures and will be shown in the following sections.

### 6.1 Background on Salmonellosis

Salmonellosis is a common cause of food-borne diseases worldwide, causing diarrhea (sometimes bloody), fever, abdominal cramps and even life-threatening infections. *Salmonella* is considered to be one of the principal causes of zoonotic disease reported worldwide. *Salmonella enterica*, *Enteritidis* and *Typhimurium*, are responsible for the majority of the outbreaks, and most events are reported to be due to consumption of contaminated eggs and poultry, pig, and bovine meats, respectively. *Salmonella* is also a known spoilage bacterium in processed foods.

Each year in the United States, *Salmonella* causes approximately 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths. Direct medical costs are estimated to be \$2.4 billion annually, which constitutes a substantial economic hardship on national and local economies (Mead *et al.*, 1999).

*Salmonella* are also an international health risk, causing an estimates 93.8 million illnesses globally, and approximately 155,000 deaths, each year (Majowicz *et al.*, 2010). *Salmonella* infections are particularly severe in vulnerable persons such as young children, elderly people and immune-suppressed patients. According to the Food Safety and Inspection Service (FSIS) of the US Department of Agriculture (USDA), about 75% of the annual cases of human Salmonellosis are due to the consumption of contaminated poultry, beef and egg products ([http://www.cdc.gov/vital\\_sign/food\\_safety](http://www.cdc.gov/vital_sign/food_safety)). More recently, outbreaks have been associated with contamination of vegetables with fecal pathogens (Jain *et al.*, 2009).

Fluoroquinolones, such as Ciprofloxacin are strongly recommended for the treatment of severe infections. However, there are rising concerns with the increasing occurrence of multidrug-resistant *Salmonella* globally, particularly in Asia. The CDC is seeing resistance to ceftriaxone in about 3% of non-typhoidal *Salmonella* tested, and some level of resistance to ciprofloxacin in about 3%. About 5% of non-typhoidal *Salmonella* tested by CDC are resistant to 5 or more types of drugs (<http://www.cdc.gov/salmonella/index.html>).

Antibiotic-resistance will inevitably increase worldwide as the bacteria they are meant to kill mutate and multiply. 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.*, 2008; Friere-Moran *et al.*, 2011). As a result, regulatory agencies worldwide have shown a renewed interest in novel biocontrol measures; ([http://www.niaid.nih.gov/topics/antimicr...nts/\\_arstrategicplan2014.pdf](http://www.niaid.nih.gov/topics/antimicr...nts/_arstrategicplan2014.pdf)) and phages, are considered as the single most promising processing aid (Nilsson, 2014).

## 6.2 Lytic Phages are GRAS

### 6.2.1 Lytic versus Lysogenic: All lytic phages are by nature 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 & 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 (Brüssow & Kutter, 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 (Ackermann & DuBow, 1987). 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 *Salmonella*, *Campylobacter*, *Listeria*, *Escherichia*, and others. For example Microos' 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 & 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 *Salmonella*

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 *Salmonella*.

### **Studies on the Pre-harvest control of *Salmonella* spp:**

Several researchers demonstrated the use of bacteriophage as a pre-harvest intervention to decrease *S. enterica* concentration in poultry (Andreatti *et al*, 2007; Atterbury *et al*, 2007; Bardina *et al*, 2012). The administration of phages prior to challenge and the continuous dosing of phages were able to achieve a significant reduction of *Salmonella* in the animals over time. Bardina *et al*. used a cocktail of three bacteriophages (UAB\_Phi20, UAB\_Phi78 and UAB\_Phi87) against *S. Enteritidis* & *Typhimurium* in both mouse and chicken. The cocktail treatment achieved a 50% survival of the mouse model when it was administered simultaneously with infection and at 6, 24 and 30 h post-infection. A more significant reduction of *Salmonella* concentration in chicken cecum was observed when the cocktail was administered a day before infection followed by continuous dosing after infection.

These results were supported by other studies (Andreatti *et al*, 2007) in which cocktails of 4 different phages (CB4Ø) and 45 bacteriophages isolated from wastewater plant (WT45Ø) showed a significant reduction of *S. Enteritidis* in cecal tonsils of chicks after 24h post-infection

but persistent reduction was not observed after 48h post infection, suggesting that continuous dosing is required to sustain the efficacy of bacteriophage in reducing *Salmonella* colonization in poultry. Furthermore, several studies based on the combination use of bacteriophage and competitive exclusion products have been performed to reduce *Salmonella* colonization in experimentally induced infection in chickens (Toro *et al.*, 2005; Borie *et al.*, 2009). These studies concluded that the combination of both types of biocontrol agents (bacteriophage and exclusion products) can be an effective approach to reduce *Salmonella* colonization in poultry.

Interestingly, bacteriophage therapy can play a role in preventing horizontal transmission of the *Salmonella* between livestock, Lim *et al.* (2012) showed that  $\phi$ CJ07, a virulent bacteriophage, resulted in significant decrease in intestinal colonization of *S. Enteritidis* in both infected chicks and the uninfected cohabitating chicks.

### **Studies on the Post-harvest control of *Salmonella* spp:**

In the post-harvest control of *Salmonella*, promising results were obtained when bacteriophages were used to control the growth of *Salmonella* on ready-to-eat foods, such as chicken skin, pig skin, egg products, and cheese (Guenther *et al.*, 2009; Hooton *et al.*, 2011) and fresh produce. For example, Guenther *et al.* (2012) described the application of the bacteriophage FO1-E2, to control the growth of *Salmonella* Typhimurium in some ready-to-eat foods such as cooked and sliced turkey breast and egg yolk. Hungaro *et al.* (2013), used a bacteriophage cocktail and chemical agents such as dichloroisocyanurate, peroxy acetic acid and lactic acid to control *S. Enteritidis* on chicken skin under simulation of an industrial condition. The authors concluded that bacteriophages may be employed as an alternative biocontrol agent for *Salmonella* in poultry industrial setting due to the similar efficacy of the bacteriophage demonstrated in reducing the *S. Enteritidis* on chicken skin when compared to other chemical agents.

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.

As in previously described trials based on reduction in *E. coli* O157:H7 in lettuce, similar attempts with *S. Enteritidis* and *Typhimurium* serovars were made (Spricigo *et al.*, 2013). Fresh-cut romaine lettuce was contaminated by *Salmonella* *Enteritidis* or by *S. Typhimurium* ( $10^5$  CFU/mL for 5 min). During three-phage cocktail treatment at room temperature ( $10^9$  PFU/mL), the number of bacterial cells was evaluated after 30 and 60 min. In all examples, the phage cocktail significantly ( $P < 0.05$ ) reduced *Salmonella* concentration.

Cutting fresh fruits for commercial purposes deprives them of peel and rind, which constitute a natural barrier against bacterial pathogens. Leverentz *et al.* (2001) state that *Salmonella* *Enteritidis* populations can survive on fresh-cut melons and apples, showing increased growth with increasing temperature. The fruit slices after contamination with 25  $\mu$ L of *Salmonella* suspension ( $10^6$  CFU/mL) were treated with 25  $\mu$ L of a phage mixture ( $2 \times 10^{10}$  PFU/ mL, diluted before application to  $10^8$  PFU/mL) consisting of four lytic phages obtained from Intralytix, Inc. Slices were incubated at 5, 10 and 20°C, and the number of *Salmonella* cells was measured at 0, 3, 24, 48, 120 and 168 h after phage inoculation. During examination, phage persistence was

much higher on melon slices and decreased to a non-detectable level after 24 h on apple slices. Further investigation showed that low pH of apples (4.2) was a possible factor inhibiting phage survivability. In contrast, the *Salmonella* strain survived at all pH and temperature regimes. Moreover, at 20°C, its population started increasing 3 h after inoculation on both melon and apple slices. Phages were able to significantly reduce *Salmonella* populations only on melon slices (greater reduction than the use of chemical sanitizers), but no significant reduction of *Salmonella* was observed on the contaminated apple slices. Overall, phages seemed to be pH sensitive during treatment. More recently Zinno *et al.* (2014) demonstrated appreciable *S. Typhimurium* inactivation, in the order of 2 log cycles, using phage P22 in different food matrices: liquid eggs, energy drinks, whole and skimmed milk, apple juice, chicken breast and chicken mince.

**In conclusion**, most studies point to the fact that various factors determine the efficacy of the phage application such as lytic as opposed to lysogenic, stability of the phage(s) under physicochemical conditions of the food (pH, aW), under its storage conditions (temperature) and the ratio of phages to host cells (MOI). While efficacy of specific phages of *Salmonella* and *Campylobacter spp.* at refrigerated temperatures has been investigated, the required dose of phage is relatively high and may incur regulatory difficulties. The majority of studies examine the lytic activity of the phage at the optimum growth temperature of the host pathogen. The phage broad host range is also another important issue to consider in the design of phage applications. To avoid emergence of resistance, a phage cocktail is also important.

### 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.

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

K<sub>2</sub>HPO<sub>4</sub>: According to 21 CFR § 182.6285, dipotassium phosphate is generally recognized as safe when used in accordance with good manufacturing practice.

MgSO<sub>4</sub>·7H<sub>2</sub>O: Magnesium Sulfate is a GRAS substance according to 21 CFR § 184.1443.

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* and *Salmonella* production host strains are nonpathogenic and safe, see section 2.1.2 for details. In addition, the bacteria used for phage production is removed via filtration and goes through 2 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, SalmoPro<sup>®</sup> was determined to be generally recognized as safe by Phagelux (Canada) Inc. through scientific procedures.

## 6.4 Safety

SalmoPro<sup>®</sup> is a mixture of 2 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* and *Salmonella* host bacteria (LPS is a component of the outer membrane of Gram-negative bacteria). The non-pathogenic *E. coli* and *Salmonella* host bacteria was tested for absence of undesirable genes.

During the manufacturing process, the clarification and washing ensures a final concentration of less than 25,000 EU/mL in a  $1 \times 10^9$  PFU/mL phage preparation, as assessed by QC procedure for each lot of SalmoPro<sup>®</sup> (Table 3).

### 6.4.2 Phages are Non-toxic

All available data indicate that the oral consumption of 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-Chenoufi *et al.*, 2004; Bruttin & Brussow, 2005). In our hands (Murthy *et al.*, 2002; 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 (Gomez & 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 (Wommack & Cowell, 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, 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 (Gautier *et al.*, 1995), meat (Atterbury *et al.*, 2003; Hsu *et al.*, 2002), with fermented foods like wine (Poblet-Icart *et al.*, 1998), yogurt (Kilic *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  $7 \times 10^5$  PFU/g (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 (Suarez *et al.*, 2002). Also Campylobacter phages have been isolated at levels of  $4 \times 10^6$  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 & Donkersloot, 1995), feces (Gantzer *et al.*, 2002; Grabow *et al.*, 1995), saliva (Bachrach *et al.*, 2003) and vagina (Kilic *et al.*, 2001). Phages were shown to be present in municipal water supplies of large European cities, indicating resistance to physico-chemical methods of purification of drinking water (Weber-Dabrowska *et al.*, 2014). 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 (<http://www.cfsan.fda.gov/rdb/opa-g218.html>).

#### 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 *et al.*, 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) (Ho *et al.*, 2011; Chaturongakul & Ounjai, 2014). 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 & Slach, 2011), while vitamin B<sub>12</sub> uptake protein BtuB is used by T5-like phages (Kim & 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 against 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 antiviral defense systems (toxin-antitoxin, abortive infection) and operate synergistically in order to increase the overall resistance to phage infection (Oliveira *et al.*, 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 phages 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 methyltransferase (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**

<b>Antiviral mechanisms</b>		<b>Phage evasion tactics</b>
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 <i>ral</i> , 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 (Sarker *et al.*, 2012; McCallin *et al.*, 2013), and topical application has not shown any adverse effects (Wright *et al.*, 2009; Merabishvili *et al.*, 2009). Other internal

organs, including the bloodstream, are however not natural environments for phages, and it has been suggested that phages may modulate both the innate and the adaptive immune system when administered intravenously (Merrill *et al.*, 2006). They could affect free-radical production and phagocytosis (Przerwa *et al.*, 2006).

Phages may inhibit interleukin (IL-2), tumor necrosis factor and, to some extent, Interferon-gamma (Gorski *et al.*, 2012; Dabrowska *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 (Merrill *et al.*, 2006; Skurnik *et al.*, 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 BP-63 and LVR16A was sequenced and deposited in the GenBank. Accession number: KM366099 for BP-63 and MF681663 for LVR16A.

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 BP-63 and LVR16A sequences demonstrated the lack of harmful or undesired genes against a panel of virulence or transduction genes identified in GenBank.

#### **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, including three GRAS approved phage products against *Salmonella*:

##### **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 CFR§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).

# Phagelux

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- 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 *Pseudomonas syringae* pv. *tomato*, is EPA-registered for use on tomatoes and peppers. AgriPhage 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.67986-1)
- 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).

## 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.

## Salmonex™

- Salmonex™, 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 Gallinarum* (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 to control *Salmonella* on poultry products (GRN No. 603).

## 6.6 Efficacy data at the intended levels of use

The literature reports on multiple studies concerning the application of bacteriophages on chicken and turkey carcasses for the reduction of *Salmonella* spp. (Higgins et al., 2005; Fiorentin et al., 2005; Zinno. et al., 2014). Goode et al., (2003) studied the efficacy of lytic bacteriophages

in reducing *Salmonella spp.* on chicken skin and showed a rapid 2 log reductions after storage for 48h, as well as rapid eradication of *S. Enteritidis* at low levels of contamination. Wichard et al., (2003) reported a significant reduction (1.8-2.1 log) of *S. typhimurium* contamination of chicken frankfurters when treated with phage Felix-O1. Bigwood et al. (2008) showed that Phage P7 significantly reduced *Salmonella typhimurium* levels by 2 log units at 5°C and by 5.9 log units at 24°C in raw and cooked beef. Efficacy increased when the phage: bacteria ratio was increased to 10 000:1 and host density was high. Treatment with phage FO1-E2 ( $3 \times 10^8$  PFU/g of food) reduced viable counts of *S. Typhimurium* from turkey deli meat (Guenther et al., 2012).

Sharma *et al.*, (2015) showed a 1.3 log reduction in *Salmonella* load when phages were applied to the surface of turkey breast. However, no significant reduction of *Salmonella* Heidelberg ( $P > 0.05$ ) was observed in ground turkey when turkey meat pieces inoculated with *Salmonella* Heidelberg were surface treated with phage preparation ( $10^7$  PFU/g) before grinding. These findings indicate that the bacteriophage preparation was **effective in reducing *Salmonella* on turkey breast cutlets as a surface treatment but did not cause any reduction of *S. Heidelberg* in ground turkey.**

Moreover, the USDA-FSIS has recently approved the use of a *Salmonella* lytic bacteriophage preparation (*SalmoFresh*<sup>TM</sup>) during processing of raw and ready-to-eat poultry products with phage concentration up to  $10^7$  PFU/g in the finished product. The product was able to achieve  $> 1$  log unit (a maximal reduction of 1.9 log unit) reduction of susceptible strains. Their results agree with previously reported studies, where *Salmonella* did not grow at 4°C (therefore the phage is not amplified), and there was no prolonged phage activity after the initial application (Guenther et al., 2012). They clearly demonstrate 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.

Based on the above results, we designed multiple comprehensive challenge studies to determine whether SalmoPro<sup>®</sup> would significantly reduce the population of different *Salmonella* strains. We show that the application of SalmoPro<sup>®</sup> at a maximum rate of  $1 \times 10^8$  PFU/g of food is effective in controlling *Salmonella* strains (see Appendix I).

## 6.7 Summary and Basis for GRAS

SalmoPro<sup>®</sup> is a *Salmonella* specific cocktail of two naturally occurring monophages (BP-63 and LVR16A). A number of bacteriophage products for the biocontrol of pathogens have previously been GRAS-approved. The current SalmoPro<sup>®</sup> phage product is equivalent to other *Salmonella* specific phage preparations that were already GRAS-approved.

Based on genetic and biologic/chemical analysis as well as experimental challenges, scientific data are showing that the individual phages contained in SalmoPro<sup>®</sup> are safe:

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



SalmoPro<sup>®</sup> is also shown to be effective in reducing *Salmonella* on many types of food (Appendix 1).

Both Phagelux and OmniLytics have reviewed the available data and information and are 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 GRAS-approved phage products, SalmoPro<sup>®</sup> should also be considered GRAS.**

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## Part 7 List of Supporting Data and Information

### 7.1 Appendices (Not Generally Available)

Appendix I: Efficacy Studies of SalmoPro<sup>®</sup> on Foods

### 7.2 References (Generally Available)

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## **APPENDIX I: EFFICACY STUDIES OF SALMOPRO<sup>®</sup> ON FOOD**

**Test Substance:** SalmoPro<sup>®</sup> bacteriophages

**Products Tested:**

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

**Treatment Amounts:** SalmoPro<sup>®</sup> was applied to the surfaces of the products tested at the concentration of  $1 \times 10^8$  PFU (Plaque Forming Units) per gram.

**Labeling Requirements:** None under the accepted conditions of use

SalmoPro<sup>®</sup> consists of a mixture of equal concentrations of two *Salmonella*-specific lytic bacteriophages. SalmoPro<sup>®</sup> is intended for use as an antimicrobial processing aid to control *Salmonella* on food, when applied to food surfaces up to  $1 \times 10^8$  PFU (Plaque Forming Units) per gram of food.

**Efficacy:** SalmoPro<sup>®</sup> has been shown to be effective in significantly reducing *Salmonella* on food.

<b>Products Tested</b>	<b>Study</b>	<b><i>Salmonella</i> Reduction</b>	<b>Log Reduction</b>	<b>Significant</b>
Raw chicken breast	50-RP-00008	90%	1.00	Yes
Spinach	50-RP-00009	97%	1.51	Yes
Crab	50-RP-00010	96%	1.37	Yes
Pre-cut apples	50-RP-00011	94%	1.21	Yes
Deli sliced honey baked ham	50-RP-00012	69%	0.51	Yes
Eggs	50-RP-00013	99%	2.10	Yes
Salmon	50-RP-00014	92%	1.12	Yes
Ground beef	50-RP-00015	88%	0.92	Yes

# Project Summary

## Title of Report:

**Evaluation of the ability of SalmoPro® to reduce Salmonella contamination in experimentally contaminated chicken breast**

**Document # 50-RP-00008 A**

## Report Approval

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

Analyst/Lead: Kelley Burtch, Laboratory Manager

(b) (6)

Signature

Date

12/22/17

Supervisor: Ryan Bringhurst, Senior Scientist

(b) (6)

Signature

Date

12/22/17

# SalmoPro® reduction on chicken breast

Scientist Name(s): Kelley Burtch, Ryan Bringham

Date(s) of Testing: 12/19/17 thru 12/20/17

Relevant Notebook Page(s): KGB-017-080 and KGB-017-081

## Purpose / Abstract

The purpose of this study is to test the efficacy of reducing *Salmonella* on raw chicken breast using SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g.

## Results Summary and Conclusions

Results of Applying SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g on experimentally contaminated raw chicken breast. One way ANOVA ( $\alpha = 0.05$ ) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
SalmoPro®	3	3.63E+05	90%	Yes	0.004
PBS	3	3.60E+06			

SalmoPro® can significantly reduce viable *Salmonella* levels on experimentally contaminated chicken by 90% in 30 min at room temperature when used at  $1 \times 10^8$  PFU/g.

## Materials and Methods

Challenge Organism:

- 08-575A (*Salmonella* Kentucky, isolated on raw chicken from a local market)

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 SalmoPro® in PBS to a titer of  $10^9$ . SalmoPro® (94-117002) has a titer of  $4 \times 10^{10}$ , so this lot was diluted 1:40 to prepare the test solution.
- 6) Apply 1 mL of diluted SalmoPro® 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  $\mu$ L of  $10^{-2}$  and  $10^{-3}$  dilutions for samples and  $10^{-3}$  and  $10^{-4}$  for controls on Salmonella ChromAgar.
- 11) Incubate at 37°C overnight.
- 12) Perform above test in triplicate.
- 13) Also, perform above without inoculating or applying SalmoPro® to assess background colony load.

**Data**

Raw data from colony counts.

Sample	Replicate	Count	Portion	Plate	Titer (CFU/mL)	Average Titer	
SalmoPro	1	31	1	4	3.10E+05	3.63E+05	1.00
	2	35	1	4	3.50E+05		
	3	43	1	4	4.30E+05		
Control	1	47	1	5	4.70E+06	3.60E+06	
	2	30	1	5	3.00E+06		
	3	31	1	5	3.10E+06		
Negative		0	1	1	0.00E+00		

# Project Summary

## Title of Report:

**Evaluation of the ability of SalmoPro® to reduce Salmonella contamination in experimentally contaminated spinach**

**Document # 50-RP-00009 A**

## Report Approval

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

Analyst/Lead: Kelley Burtch, Laboratory Manager

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12/22/17

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Supervisor: Ryan Bringhurst, Senior Scientist

(b) (6)

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12/22/17

Date

# SalmoPro® reduction on spinach

Scientist Name(s): Kelley Burtch, Ryan Bringham

Date(s) of Testing: 12/21/17 thru 12/22/17

Relevant Notebook Page(s): KGB-017-082 and KGB-017-083

## Purpose / Abstract

The purpose of this study is to test the efficacy of reducing *Salmonella* on bagged baby spinach using SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g.

## Results Summary and Conclusions

Results of Applying SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g on experimentally contaminated spinach. One way ANOVA ( $\alpha = 0.05$ ) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
SalmoPro	3	5.47E+04	97%	Yes	< 0.0001
PBS	3	1.76E+06			

SalmoPro® can significantly reduce viable *Salmonella* levels on experimentally contaminated spinach by 97% in 30 min at room temperature when used at  $1 \times 10^8$  PFU/g.

## Materials and Methods

Challenge Organism:

- 08-575A (*Salmonella* Kentucky, isolated on raw chicken from a local market)

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 SalmoPro® in PBS to a titer of  $10^9$ . SalmoPro® (94-117002) has a titer of  $4 \times 10^{10}$ , so this lot was diluted 1:40 to prepare the test solution.
- 6) Apply 1 mL of diluted SalmoPro® 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  $\mu$ L of  $10^{-2}$  and  $10^{-3}$  dilutions for samples and  $10^{-3}$  and  $10^{-4}$  for controls on *Salmonella* ChromAgar.
- 11) Incubate at 37°C overnight.
- 12) Perform above test in triplicate.
- 13) Also, perform above without inoculating or applying SalmoPro® to assess background colony load.

**Data**

Raw data from colony counts.

Sample	Replicate	Count	Portion	Plate	Titer (CFU/mL)		Average Titer	LOG Drop
SalmoPro	1	24	1	3	2.40E+04		5.47E+04	1.51
	2	60	0.5	3	1.20E+05			
	3	20	1	3	2.00E+04			
Control	1	102	0.5	4	2.04E+06		1.76E+06	
	2	71	0.5	4	1.42E+06			
	3	91	0.5	4	1.82E+06			
Negative		0	1	1	0.00E+00			

# Project Summary

## Title of Report:

**Evaluation of the ability of SalmoPro® to reduce Salmonella contamination in experimentally contaminated crab**

**Document # 50-RP-00010 A**

## Report Approval

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

Analyst/Lead: Kelley Burtch, Laboratory Manager

(b) (6)

Signature

12/22/17

Date

Supervisor: Ryan Bringhurst, Senior Scientist

(b) (6)

Signature

12/22/17

Date



# Project Summary

## SalmoPro® reduction on crab

**Scientist Name(s):** *Kelley Burtch, Ryan Bringham*  
**Date(s) of Testing:** 12/21/17 thru 12/22/17  
**Relevant Notebook Page(s):** KGB-017-082 and KGB-017-083

### Purpose / Abstract

The purpose of this study is to test the efficacy of reducing *Salmonella* on cooked crab legs using SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g.

### Results Summary and Conclusions

Results of Applying SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g on experimentally contaminated crab. One way ANOVA ( $\alpha = 0.05$ ) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
SalmoPro	3	8.70E+04	96%	Yes	< 0.0001
PBS	3	2.05E+06			

SalmoPro® can significantly reduce viable *Salmonella* levels on experimentally contaminated crab by 96% in 30 min at room temperature when used at  $1 \times 10^8$  PFU/g.

### Materials and Methods

Challenge Organism:

- 08-575A (*Salmonella* Kentucky, isolated on raw chicken from a local market)

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 SalmoPro® in PBS to a titer of  $10^9$ . SalmoPro® (94-117002) has a titer of  $4 \times 10^{10}$ , so this lot was diluted 1:40 to prepare the test solution.
- 6) Apply 1 mL of diluted SalmoPro® 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  $\mu$ L of  $10^{-2}$  and  $10^{-3}$  dilutions for samples and  $10^{-3}$  and  $10^{-4}$  for controls on *Salmonella* ChromAgar.
- 11) Incubate at 37°C overnight.
- 12) Perform above test in triplicate.
- 13) Also, perform above without inoculating or applying SalmoPro® to assess background colony load.

# Project Summary

<b>Data</b>
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Raw data from colony counts.

Sample	Replicate	Count	Portion	Plate	Titer (CFU/mL)	Average Titer	LOG Drop
SalmoPro	1	54	0.5	3	1.08E+05	8.70E+04	1.37
	2	69	1	3	6.90E+04		
	3	42	0.5	3	8.40E+04		
Control	1	103	0.5	4	2.06E+06	2.05E+06	
	2	105	0.5	4	2.10E+06		
	3	100	0.5	4	2.00E+06		
Negative		0	1	1	0.00E+00		

# Project Summary

## Title of Report:

**Evaluation of the ability of SalmoPro® to reduce Salmonella contamination in experimentally contaminated pre-cut apples**

**Document # 50-RP-00011 A**

## Report Approval

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

Analyst/Lead: Kelley Burtch, Laboratory Manager

(b) (6)

Signature

12/22/17

Date

Supervisor: Ryan Bringhurst, Senior Scientist

(b) (6)

Signature

12/22/17

Date

# SalmoPro® reduction on pre-cut apples

Scientist Name(s): Kelley Burtch, Ryan Bringham

Date(s) of Testing: 12/21/17 thru 12/22/17

Relevant Notebook Page(s): KGB-017-082 and KGB-017-083

## Purpose / Abstract

The purpose of this study is to test the efficacy of reducing *Salmonella* on pre-cut apples using SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g.

## Results Summary and Conclusions

Results of Applying SalmoPro® at a concentration of  $1 \times 10^8$  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
SalmoPro	3	9.13E+04	94%	Yes	< 0.0001
PBS	3	1.48E+06			

SalmoPro® can significantly reduce viable *Salmonella* levels on experimentally contaminated apples by 94% in 30 min at room temperature when used at  $1 \times 10^8$  PFU/g.

## Materials and Methods

Challenge Organism:

- 08-575A (*Salmonella* Kentucky, isolated on raw chicken from a local market)

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 SalmoPro® in PBS to a titer of  $10^9$ . SalmoPro® (94-117002) has a titer of  $4 \times 10^{10}$ , so this lot was diluted 1:40 to prepare the test solution.
- 6) Apply 1 mL of diluted SalmoPro® 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  $\mu$ L of  $10^{-2}$  and  $10^{-3}$  dilutions for samples and  $10^{-3}$  and  $10^{-4}$  for controls on *Salmonella* ChromAgar.
- 11) Incubate at 37°C overnight.
- 12) Perform above test in triplicate.
- 13) Also, perform above without inoculating or applying SalmoPro® to assess background colony load.

**Data**

Raw data from colony counts.

Sample	Replicate	Count	Portion	Plate	Titer (CFU/mL)	Average Titer	LOG Drop
SalmoPro	1	35	0.5	3	7.00E+04	9.13E+04	1.21
	2	40	0.5	3	8.00E+04		
	3	62	0.5	3	1.24E+05		
Control	1	72	0.5	4	1.44E+06	1.48E+06	
	2	73	0.5	4	1.46E+06		
	3	77	0.5	4	1.54E+06		
Negative		0	1	1	0.00E+00		

# Project Summary

## Title of Report:

**Evaluation of the ability of SalmoPro® to reduce Salmonella contamination in experimentally contaminated deli sliced honey baked ham**

**Document # 50-RP-00012 A**

## Report Approval

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

Analyst/Lead: Kelley Burtch, Laboratory Manager

(b) (6) 

Signature

12/22/17  
Date

Supervisor: Ryan Bringhurst, Senior Scientist

(b) (6) 

Signature

12/12/17  
Date

# SalmoPro® reduction on deli sliced honey baked ham

Scientist Name(s): Kelley Burtch, Ryan Bringham

Date(s) of Testing: 12/21/17 thru 12/22/17

Relevant Notebook Page(s): KGB-017-082 and KGB-017-083

## Purpose / Abstract

The purpose of this study is to test the efficacy of reducing *Salmonella* on deli sliced honey baked ham using SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g.

## Results Summary and Conclusions

Results of Applying SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g on experimentally contaminated ham. One way ANOVA ( $\alpha = 0.05$ ) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
SalmoPro	3	5.87E+05	69%	Yes	0.006
PBS	3	1.88E+06			

SalmoPro® can significantly reduce viable *Salmonella* levels on experimentally contaminated ham by 69% in 30 min at room temperature when used at  $1 \times 10^8$  PFU/g.

## Materials and Methods

Challenge Organism:

- 08-575A (*Salmonella* Kentucky, isolated on raw chicken from a local market)

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 SalmoPro® in PBS to a titer of  $10^9$ . SalmoPro® (94-117002) has a titer of  $4 \times 10^{10}$ , so this lot was diluted 1:40 to prepare the test solution.
- 6) Apply 1 mL of diluted SalmoPro® 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  $\mu$ L of  $10^{-2}$  and  $10^{-3}$  dilutions for samples and  $10^{-3}$  and  $10^{-4}$  for controls on *Salmonella* ChromAgar.
- 11) Incubate at 37°C overnight.
- 12) Perform above test in triplicate.
- 13) Also, perform above without inoculating or applying SalmoPro® to assess background colony load.

**Data**

Raw data from colony counts.

Sample	Replicate	Count	Portion	Plate	Titer (CFU/mL)	Average Titer	LOG Drop
SalmoPro	1	32	1	4	3.20E+05	5.87E+05	0.51
	2	62	1	4	6.20E+05		
	3	82	1	4	8.20E+05		
Control	1	82	0.5	4	1.64E+06	1.88E+06	
	2	113	0.5	4	2.26E+06		
	3	87	0.5	4	1.74E+06		
Negative		2	1	0	2.00E+00		



# Project Summary

## Title of Report:

**Evaluation of the ability of SalmoPro® to reduce Salmonella contamination in experimentally contaminated eggs**

**Document # 50-RP-00013 A**

## Report Approval

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

Analyst/Lead: Kelley Burtch, Laboratory Manager

(b) (6)

Signature

12/22/17  
Date

Supervisor: Ryan Bringhurst, Senior Scientist

(b) (6)

Signature

12/22/17  
Date

# SalmoPro® reduction on eggs

Scientist Name(s): Kelley Burtch, Ryan Bringham

Date(s) of Testing: 12/21/17 thru 12/22/17

Relevant Notebook Page(s): KGB-017-082 and KGB-017-083

## Purpose / Abstract

The purpose of this study is to test the efficacy of reducing *Salmonella* on eggs using SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g.

## Results Summary and Conclusions

Results of Applying SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g on experimentally contaminated eggs. One way ANOVA ( $\alpha=0.05$ ) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
SalmoPro	3	2.37E+04	99%	Yes	0.03
PBS	3	3.00E+06			

SalmoPro® can significantly reduce viable *Salmonella* levels on experimentally contaminated eggs by 99% in 30 min at room temperature when used at  $1 \times 10^8$  PFU/g.

## Materials and Methods

Challenge Organism:

- 08-575A (*Salmonella* Kentucky, isolated on raw chicken from a local market)

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 SalmoPro® in PBS to a titer of  $10^9$ . SalmoPro® (94-117002) has a titer of  $4 \times 10^{10}$ , so this lot was diluted 1:40 to prepare the test solution.
- 6) Apply 1 mL of diluted SalmoPro® 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  $\mu$ L of  $10^{-2}$  and  $10^{-3}$  dilutions for samples and  $10^{-3}$  and  $10^{-4}$  for controls on *Salmonella* ChromAgar.
- 11) Incubate at 37°C overnight.
- 12) Perform above test in triplicate.
- 13) Also, perform above without inoculating or applying SalmoPro® to assess background colony load.

**Data**

Raw data from colony counts.

Sample	Replicate	Count	Portion	Plate	Titer (CFU/mL)	Average Titer	LOG Drop
SalmoPro	1	30	1	3	3.00E+04	2.37E+04	2.10
	2	9	1	3	9.00E+03		
	3	32	1	3	3.20E+04		
Control	1	40	1	5	4.00E+06	3.00E+06	
	2	60	0.5	4	1.20E+06		
	3	38	1	5	3.80E+06		
Negative		0	1	1	0.00E+00		

# Project Summary

## Title of Report:

**Evaluation of the ability of SalmoPro® to reduce Salmonella contamination in experimentally contaminated salmon**

**Document # 50-RP-00014 A**

## Report Approval


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

Analyst/Lead: Kelley Burtch, Laboratory Manager

(b) (6)  
  
Signature

12/22/17  
Date

Supervisor: Ryan Bringhurst, Senior Scientist

(b) (6)  
  
Signature

12/22/17  
Date

# SalmoPro® reduction on salmon

Scientist Name(s): Kelley Burtch, Ryan Bringham

Date(s) of Testing: 12/21/17 thru 12/22/17

Relevant Notebook Page(s): KGB-017-082 and KGB-017-083

## Purpose / Abstract

The purpose of this study is to test the efficacy of reducing *Salmonella* on salmon filets using SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g.

## Results Summary and Conclusions

Results of Applying SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g on experimentally contaminated salmon. One way ANOVA ( $\alpha = 0.05$ ) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
SalmoPro	3	1.61E+05	92%	Yes	0.004
PBS	3	2.15E+06			

SalmoPro® can significantly reduce viable *Salmonella* levels on experimentally contaminated salmon by 92% in 30 min at room temperature when used at  $1 \times 10^8$  PFU/g.

## Materials and Methods

Challenge Organism:

- 08-575A (*Salmonella* Kentucky, isolated on raw chicken from a local market)

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 SalmoPro® in PBS to a titer of  $10^9$ . SalmoPro® (94-117002) has a titer of  $4 \times 10^{10}$ , so this lot was diluted 1:40 to prepare the test solution.
- 6) Apply 1 mL of diluted SalmoPro® 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  $\mu$ L of  $10^{-2}$  and  $10^{-3}$  dilutions for samples and  $10^{-3}$  and  $10^{-4}$  for controls on *Salmonella* ChromAgar.
- 11) Incubate at 37°C overnight.
- 12) Perform above test in triplicate.
- 13) Also, perform above without inoculating or applying SalmoPro® to assess background colony load.

**Data**

Raw data from colony counts.

Sample	Replicate	Count	Portion	Plate	Titer (CFU/mL)	Average Titer	LOG Drop
SalmoPro	1	42	0.25	3	1.68E+05	1.61E+05	1.12
	2	38	0.25	3	1.52E+05		
	3	41	0.25	3	1.64E+05		
Control	1	40	0.25	4	1.60E+06	2.15E+06	
	2	53	0.25	4	2.12E+06		
	3	68	0.25	4	2.72E+06		
Negative		0	1	1	0.00E+00		

# Project Summary

## Title of Report:

**Evaluation of the ability of SalmoPro® to reduce Salmonella contamination in experimentally contaminated ground beef**

**Document # 50-RP-00015 A**

## Report Approval

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

Analyst/Lead: Kelley Burtch, Laboratory Manager

(b) (6)  
[Redacted Signature]  
Signature

12/22/17  
Date

Supervisor: Ryan Bringhurst, Senior Scientist

(b) (6)  
[Redacted Signature]  
Signature

12/22/17  
Date

# SalmoPro® reduction on ground beef

Scientist Name(s): Kelley Burtch, Ryan Bringham

Date(s) of Testing: 12/21/17 thru 12/22/17

Relevant Notebook Page(s): KGB-017-082 and KGB-017-083

## Purpose / Abstract

The purpose of this study is to test the efficacy of reducing *Salmonella* on ground beef using SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g.

## Results Summary and Conclusions

Results of Applying SalmoPro® at a concentration of  $1 \times 10^8$  PFU/g on experimentally contaminated ground beef. One way ANOVA ( $\alpha = 0.05$ ) was used to determine significance.

Sample	Replicates	Mean CFU/mL	% reduction	Significant?	P-value
SalmoPro	3	4.20E+05	88%	Yes	0.0004
PBS	3	3.53E+06			

SalmoPro® can significantly reduce viable *Salmonella* levels on experimentally contaminated ground beef by 88% in 30 min at room temperature when used at  $1 \times 10^8$  PFU/g.

## Materials and Methods

Challenge Organism:

- 08-575A (*Salmonella* Kentucky, isolated on raw chicken from a local market)

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 SalmoPro® in PBS to a titer of  $10^9$ . SalmoPro® (94-117002) has a titer of  $4 \times 10^{10}$ , so this lot was diluted 1:40 to prepare the test solution.
- 6) Apply 1 mL of diluted SalmoPro® 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  $\mu$ L of  $10^{-2}$  and  $10^{-3}$  dilutions for samples and  $10^{-3}$  and  $10^{-4}$  for controls on *Salmonella* ChromAgar.
- 11) Incubate at 37°C overnight.
- 12) Perform above test in triplicate.
- 13) Also, perform above without inoculating or applying SalmoPro® to assess background colony load.



**Data**

Raw data from colony counts.

Sample	Replicate	Count	Portion	Plate	Titer (CFU/mL)	Average Titer	LOG Drop
SalmoPro	1	34	1	4	3.40E+05	4.20E+05	0.92
	2	43	1	4	4.30E+05		
	3	49	1	4	4.90E+05		
Control	1	37	1	5	3.70E+06	3.53E+06	
	2	39	1	5	3.90E+06		
	3	30	1	5	3.00E+06		
Negative		0	1	1	0.00E+00		

1. Confirm that the address of the notifier is 6100 Royalmount, Montreal as stated on page 1 of the notice and not 1600 Royalmount, Montreal as stated in section 1.2.

This was a typo; the address is: 6100 Royalmount, Montreal

2. The manufacturing section (Section 2.2) lists magnesium phosphate as one of the components of the wash buffer and presumably the suspension/storage buffer; yet in sections 2.1.4, 3.5 and 6.3 you mention magnesium sulfate. Please confirm whether two different buffers were used or if one buffer was used and also state which of the magnesium salts was used.

This was also a typo; the buffer uses magnesium sulfate, not magnesium phosphate:

“After a determined time of incubation, the culture is clarified by filtration to remove bacteria and the filtrate is washed with a buffer consisting of 0.01M dipotassium phosphate and 0.01M magnesium sulfate, and concentrated by tangential flow filtration (TFF).”

3. In Table 3, your endotoxin assay is performed using a  $10^9$  PFU/mL sample while your product will be sold at  $10^{10}$  PFU/mL concentration and diluted to achieve a concentration of  $10^8$  PFU/ g of food. You also calculate that the maximum amount of you product that could be possible consumed is  $7.27 \times 10^{10}$  PFU/mL. Please provide a comment on the choice of the endotoxin test PFU concentration.

We request changing the language to the following:

“< 250,000 EU/mL for concentrated product containing  $1 \times 10^{10}$  PFU/mL”

This is the same ratio as <25,000 EU/mL for concentrated product containing  $1 \times 10^9$  PFU/mL, but adjusted to the concentration of the final product.

4. In section 2.3 you state that all components used in manufacturing of your product are animal-free and food grade, however, you do not mention whether there are any allergens present in final product. Please comment on the potential for any of the major allergens being found in your final product.

“The final SalmoPro<sup>®</sup> product contains no preservatives, known allergenic substances, or additives.”

5. Please confirm that the Wichard et al. (2003) citation (line #2, page 36) is actually Whichard et al. (2003).

This was a typo; the correct spelling is Whichard

## SalmoPro™

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**SECTION 1: Identification**

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**PRODUCT NAME:** SalmoPro™  
**FDA GRAS #:** 752  
**MANUFACTURER:** OmniLytics, Inc.  
**ADDRESS:** 9100 South 500 West, Sandy, Utah 84070  
**PHONE:** 801.746.3600  
**TOLL FREE:** 866.285.2644  
**FAX:** 801.746.3461

**PRODUCT USE:** SalmoPro™ is intended for use as an antimicrobial processing aid to control *Salmonella* on food, when applied to food surfaces up to 1x10<sup>8</sup> PFU (Plaque Forming Units) per gram of food.

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**SECTION 2: Hazard(s) Identification**

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As bacteriophages are not hazardous, toxicology information is based on the non-hazardous inert buffer solution (0.01M dipotassium phosphate & 0.01M magnesium sulfate) that makes up 99.99991% of SalmoPro™.

**Classification according to the Hazard Communication Standard (HCS):**

SalmoPro™ is not a hazardous substance or mixture

**Hazard Statement:**

SalmoPro™ 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

## SalmoPro™

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**SECTION 3: Composition/Information on Ingredients**

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**Chemical Name:** Bacteriophages active against *Salmonella* species  
**Common Name:** SalmoPro™  
**CAS #:** Not applicable  
**FDA GRAS #:** 752  
**Active Ingredient:** 0.00009% (Bacteriophages)  
**Inert Ingredients:** 99.99991% (0.01M dipotassium phosphate & 0.01M magnesium sulfate)

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**SECTION 4: First-Aid Measures**

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**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 ~~15-20~~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:** ~~Wear a mask or respirator.~~ If inhaled, supply fresh air.  
Consult doctor if symptoms persist.

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

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**SECTION 5: Fire-Fighting Measures**

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SalmoPro™ is Non-Flammable.

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

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**SECTION 6: Accidental Release Measures**

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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. SalmoPro™ is not a hazard.

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**SECTION 7: Handling and Storage**

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**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

## SalmoPro™

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 corrugated paper packaging.

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**SECTION 8: Exposure Controls/Personal Protection**


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**Engineering Controls:** ~~When using as an aerosol, provide exhaust ventilation to minimize airborne concentrations of vapors. No specialized engineering controls required.~~

**Respiratory Protection:** ~~Use a mask or respirator and avoid spray mist when ventilation is not adequate. Use under ventilated conditions. No specialized ventilation required.~~

**Ventilation:**

**Personal Protective Equipment:** Use a lab coat, Long Sleeved Shirt, Long Pants, Waterproof Gloves, Waterproof Shoes plus socks.

**Eye Protection:** Avoid contact with eyes. Use eye protection.

**Hygienic Practices:** Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.

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**SECTION 9: Physical and Chemical Properties**


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## 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:** 0.995 - 1.000 g/ml

**Solubility:** Fully Miscible

**Partition Coefficient:** Not Determined

**Autoignition Temperature:** Product is not selfigniting

**Decomposition Temperature:** Not Determined

**Viscosity:** Not Determined

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**SECTION 10: Stability and Reactivity**


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**Reactivity:** Stable

**Chemical Stability:** Stable

**Hazardous Reactions:** None known

**Conditions to Avoid:** No known conditions to avoid.

# SAFETY DATA SHEET

SDS Revision Date: May 10, 2018

## SalmoPro™

**Incompatible Materials:** None known  
**Hazardous Decomposition Products:** No dangerous decomposition products known.

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### SECTION 11: Toxicology Information

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As bacteriophages are not hazardous, toxicology information is based on the non-hazardous inert buffer solution (0.01M dipotassium phosphate & 0.01M magnesium sulfate) that makes up 99.99991% of SalmoPro™.

**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

SalmoPro™ does not contain any known hazards or have any toxic effects.

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### SECTION 12: Ecological Information

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SalmoPro™ has no known hazards to any ecological systems.

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### SECTION 13: Disposal Considerations

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**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.

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### SECTION 14: Transport Information

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

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### SECTION 15: Regulatory Information

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SalmoPro™ is generally recognized as safe (GRAS).

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### SECTION 16: Other Information

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**Date of Preparation:** May 10, 2018

# SalmoPro™

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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