

Potential for biocontrol of food-borne pathogens with *Bacteriovorax* sp. and implications for food safety

Modesto Olanya and Gary Richards

USDA Agricultural Research Service, Eastern
Regional Research Center, Wyndmoor, PA and
USDA-ARS, Dover, DE

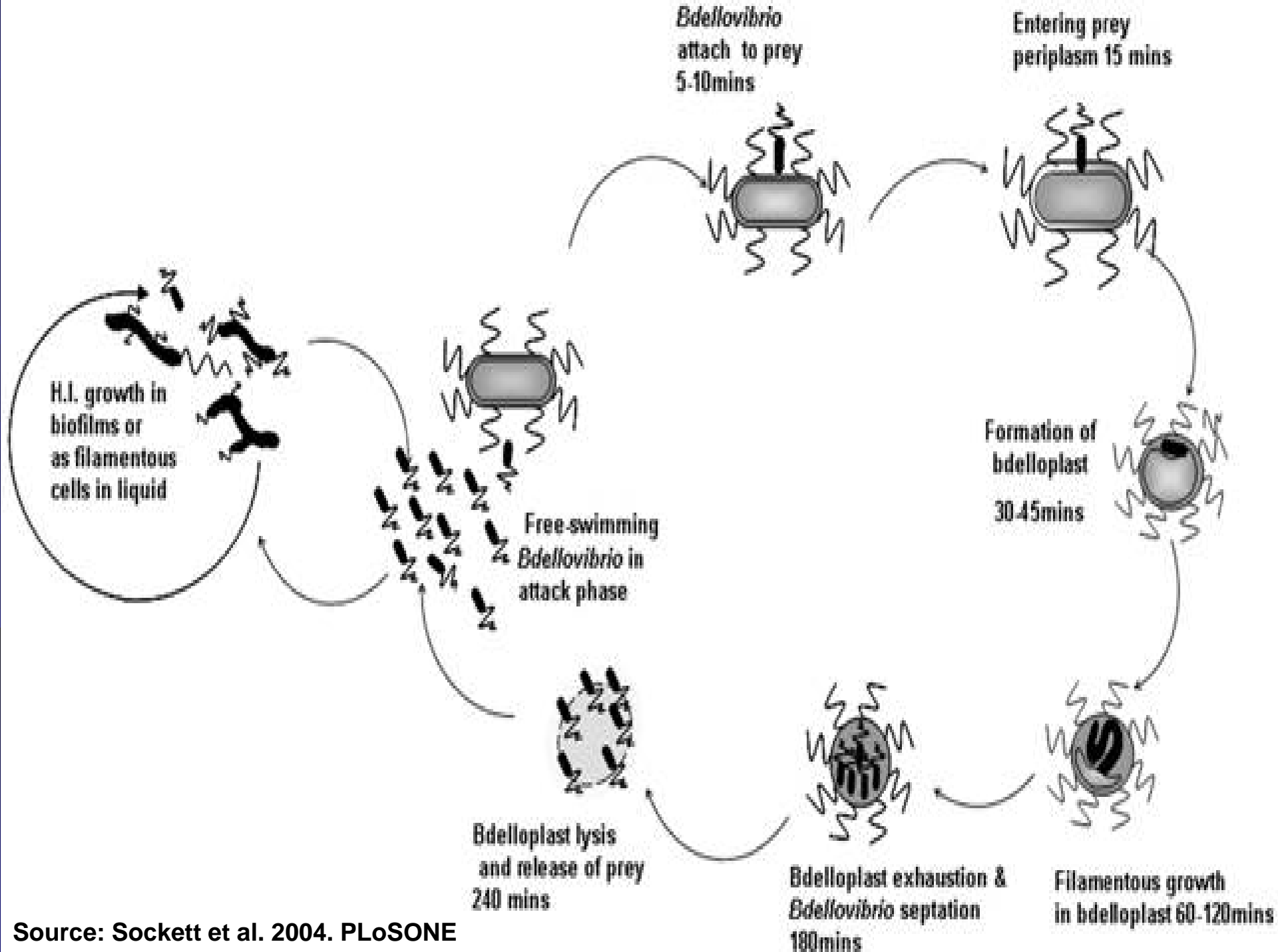


Comparison of predatory prokaryotes

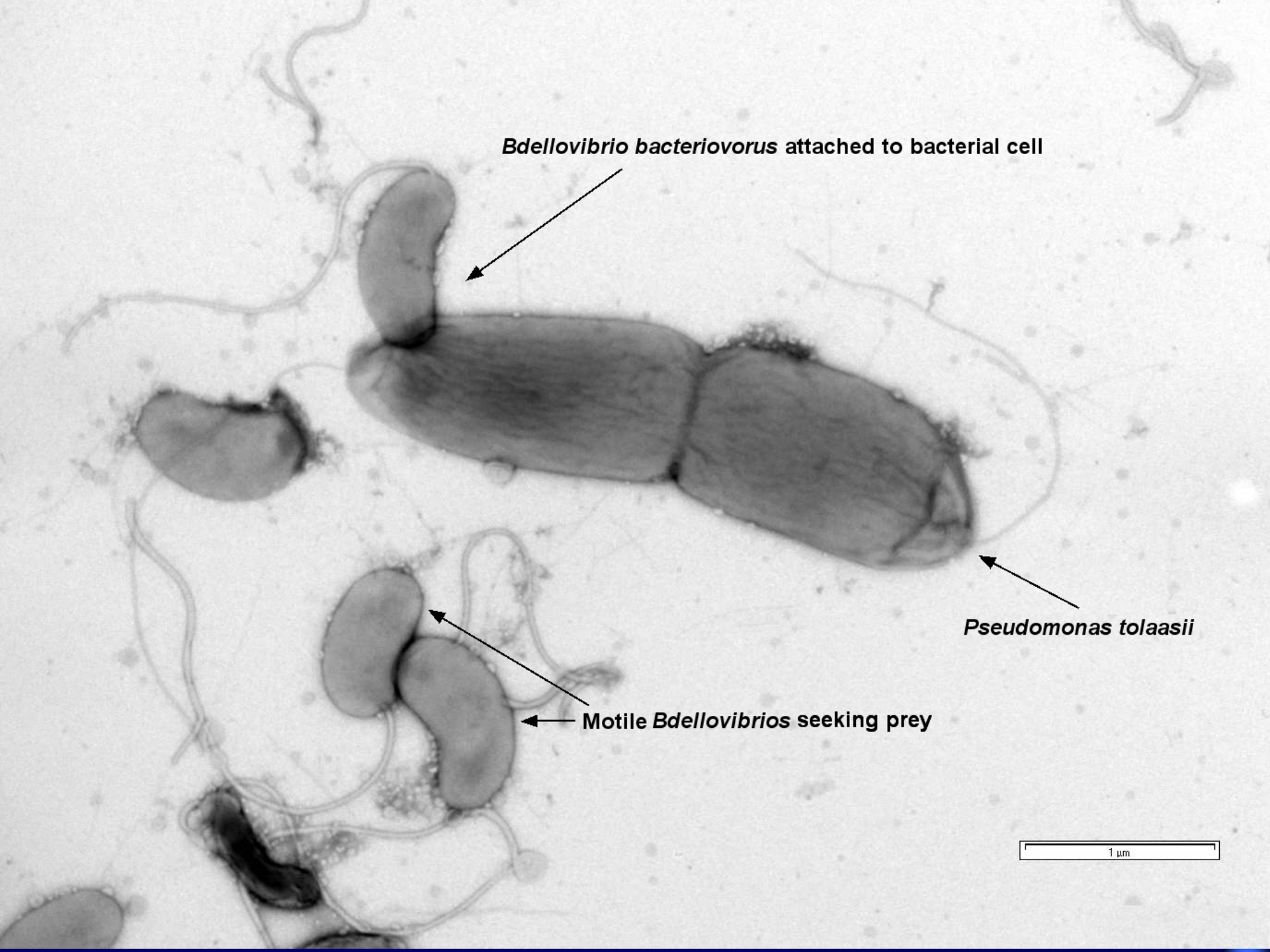
Parameters	<i>Bdellovibrio / Bacteriovorax</i>	<i>Vampiricoccus</i>	<i>Daptobacter</i>
Occurrence	Seawater, soil, water	Fresh water	Fresh water
Size & morphology	0.35 x 1.2 μm , curved rods	0.6 μm , ovoid	0.5 x 1,5 μm Straight rods
Motility	Single polar sheath flagellum	Non-motile	Single polar, un- sheathed flagellum
Site in prey	Periplasmic space	Epibiotic – attached to cell wall	Endobiotic / cytoplasm
Reproduction	Bdelloplasts, segmentation	Binary fission	Binary fission
Prey hosts	Gram negative bacteria	<i>Chromatium</i> sp., phototrophs	Chromatiaceae, phototrophs
Host specificity	Obligate / host independent forms	Obligate	Obligate

Adapted: from Guerreo *et al.* 1986





Source: Sockett et al. 2004. PLoS ONE



Bdellovibrio bacteriovorus attached to bacterial cell

Pseudomonas tolaasii

Motile *Bdellovibrios* seeking prey

1 μ m

Biocontrol of plant pathogens with predatory bacteria

Plant pathogens	Host	Disease	Reference
<i>Pseudomonas glycinea</i>	Soybean	Bacterial blight	Scherff, 1972
<i>Burkholderia glumae</i>	Rice	Grain rot	Song 2004
<i>Xanthomonas oryzae</i>	Rice	Sheath blight	Uematsu, 1980
<i>Erwinia carotovora</i>	Potato slices	Soft rot	Epton <i>et al.</i> 1990

Biocontrol of foodborne pathogens with predatory bacteria

Predator	Pathogen	Host / Media	Reference
<i>Bdellovibrio bacteriovorus</i>	<i>Pseudomonas tolaasii</i>	Mushroom (pilei brown spot)	Saxon <i>et al.</i> 2014
<i>Bacteriovorax</i> sp.	<i>Vibrio vulnificus</i> / <i>V. parahaemolyticus</i>	Oysters / seawater	Richards <i>et al.</i> 2012
<i>B. bacteriovorus</i>	<i>E. coli</i> O157:H7	<i>In vitro</i> assay	Sockett 2009
<i>B. bacteriovorus</i>	<i>Staphylococcus aureus</i>	Human epithelial cells	Monappa <i>et al.</i> 2014
<i>B. bacteriovorus</i>	Drug-resistant Gram negative bacteria	<i>In vitro</i> assay	Kadouri <i>et al.</i> 2013
<i>B. bacteriovorus</i>	<i>P. aeruginosa</i> and <i>S. aureus</i>	<i>In vitro</i> assay	Valerio <i>et al.</i> 2014

Objectives

- To optimize the plaque assay and assess the predation of *E. coli* O157:H7 and other foodborne pathogens by *Bacteriovorax* sp. (Predatory bacteria).
- To evaluate the effect of temperatures on the predation of *E. coli* O157:H7 and other foodborne pathogens by *Bacteriovorax* sp. and subsequently their potential as biocontrol agents.





OPERATION

The Water Bath should be set on a surface that is level and in an area that is free from drafts and wide ambient temperature variation. All bath models are operated as follows:

1. Power requirements are located on label on rear panel. Plug into a suitable grounded electrical outlet.
2. Select a bath fluid that is **NOT** corrosive or flammable. Distilled water is **NOT** recommended. The following liquids are **NOT** recommended and may cause damage to the unit:
 - Deionized water
 - Hard Tap Water
 - Acid photoreduction (acetic)
 - Most photoreduction (acetic)
 - Most photoreduction (acetic)
 - Bleach (sodium hypochlorite)
 - Solutions with strong acids, fluorides, chlorides, iodides, cyanides or sulfur
 - Solutions with halides, fluorides, chlorides, iodides, cyanides or sulfur
3. Fill bath with liquid so that the level is approx. 1/2" to 3/4" from the top when samples are placed in the bath.

4. Wait for the temperature to stabilize at the desired level. The display shows the actual temperature. The display may be used to adjust the set point. The display may be used to adjust the set point. The display may be used to adjust the set point.
5. Use a flat blade screwdriver to rotate the Safety Set Knob to the maximum setting (rotate the knob fully clockwise).
6. Press the Set/Menu button. The current set point will be displayed and the desired set point will be displayed. The current set point will be displayed and the desired set point will be displayed.
7. After the bath has stabilized, the Heat Indicator light comes on and of the controller. At this point, the Safety Indicator light comes on and of the controller.



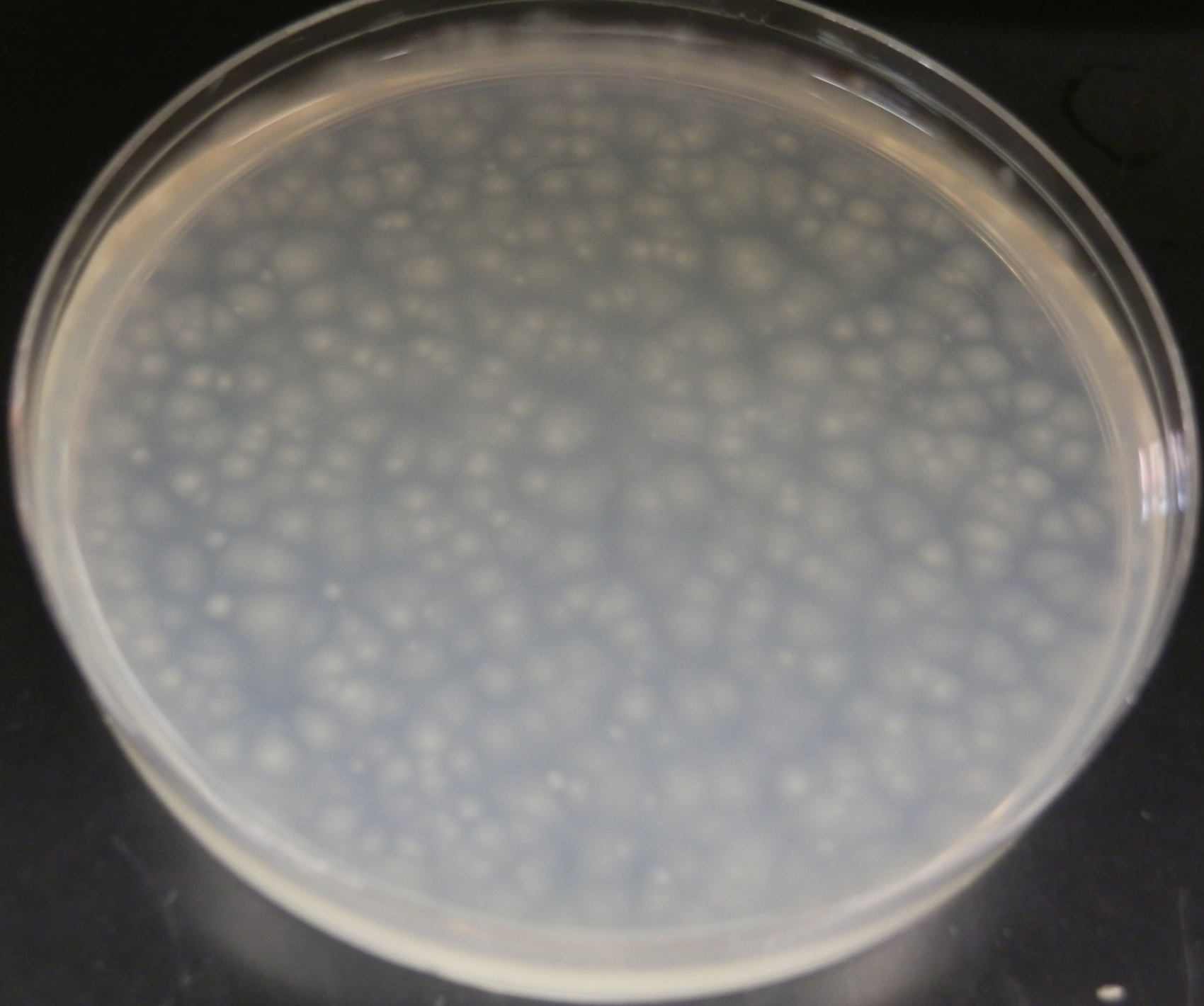


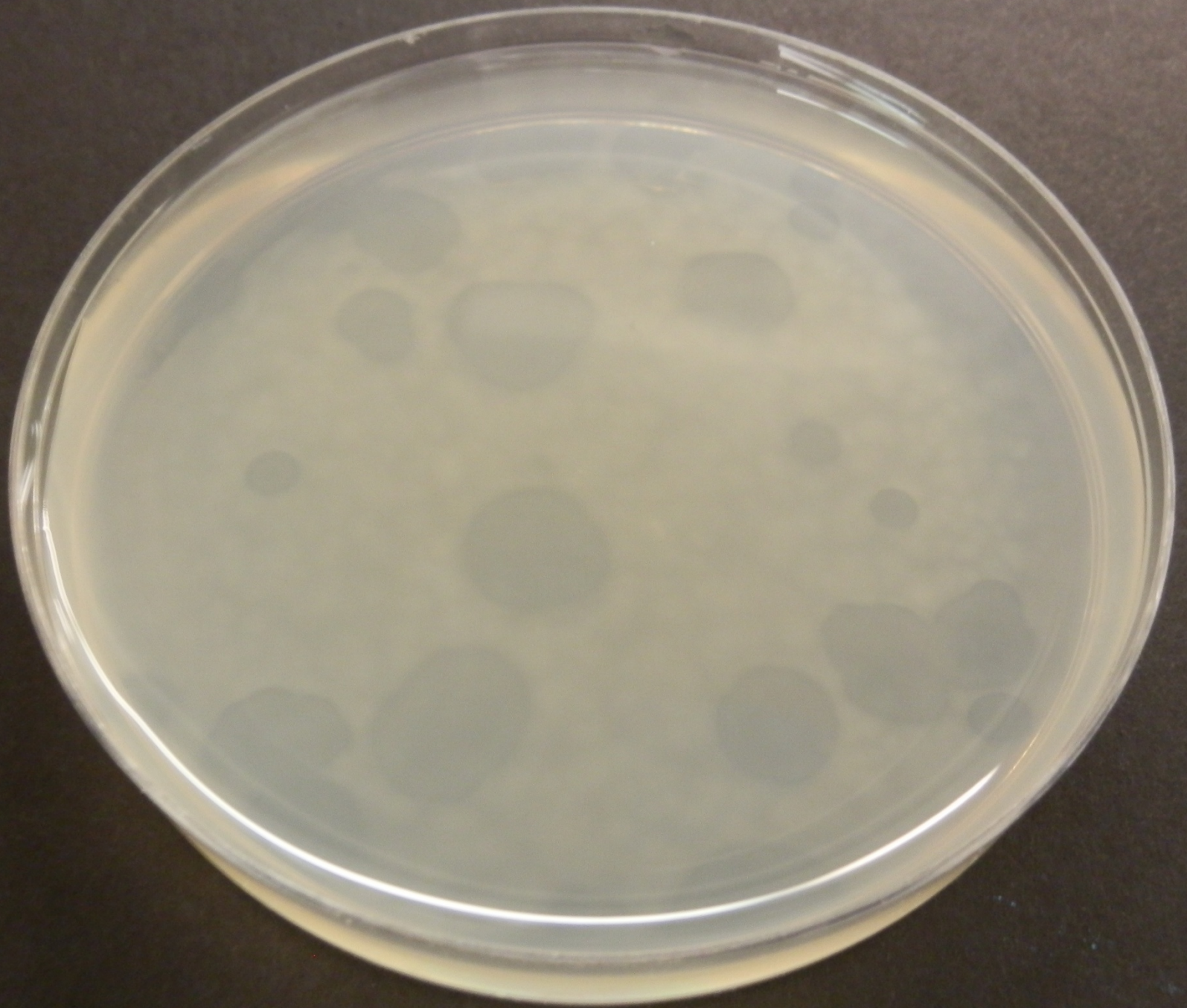
RESULTS

Optimization of Plaque assay

Temperature effects on plaque formation and predation of bacteria host cells







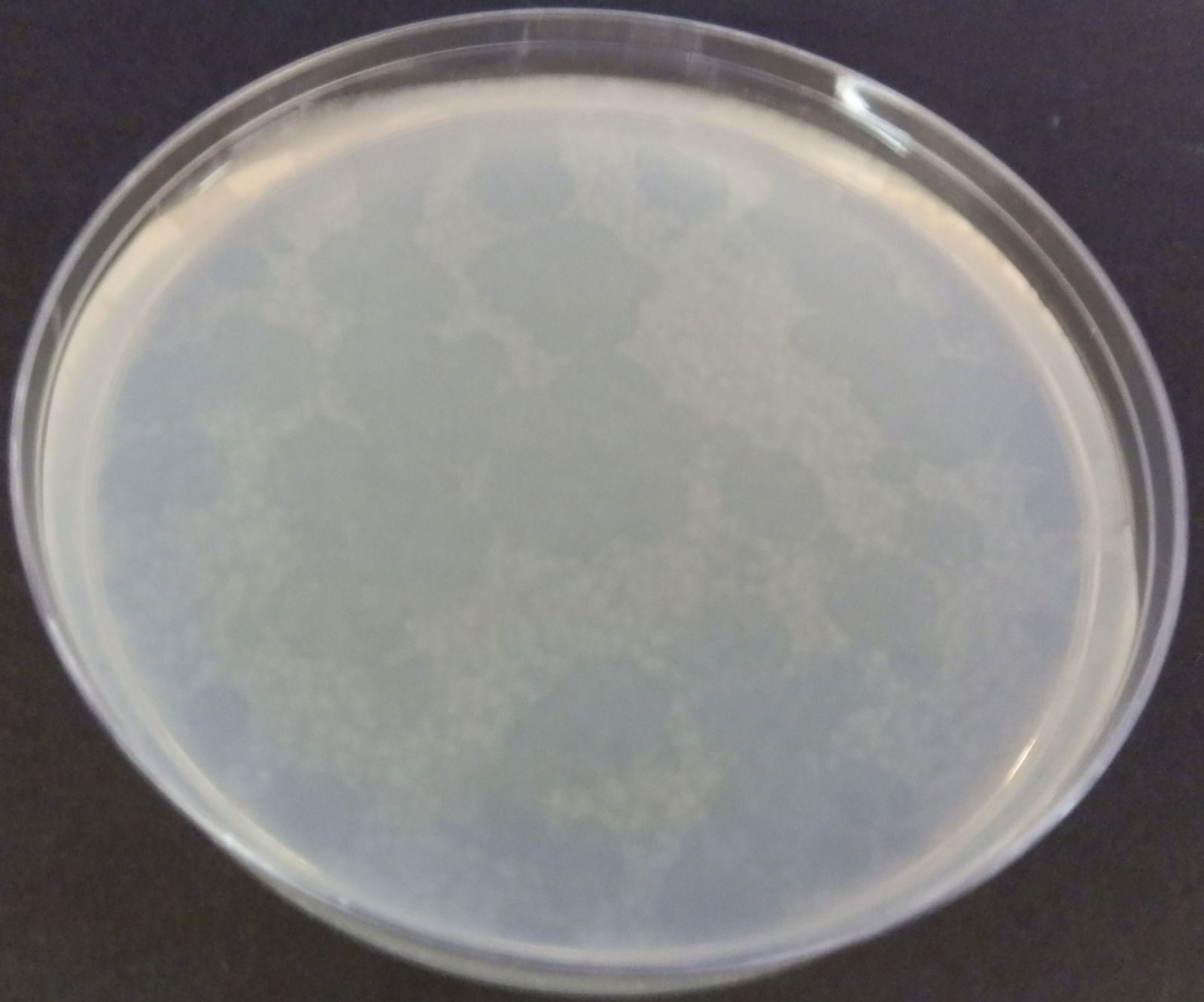
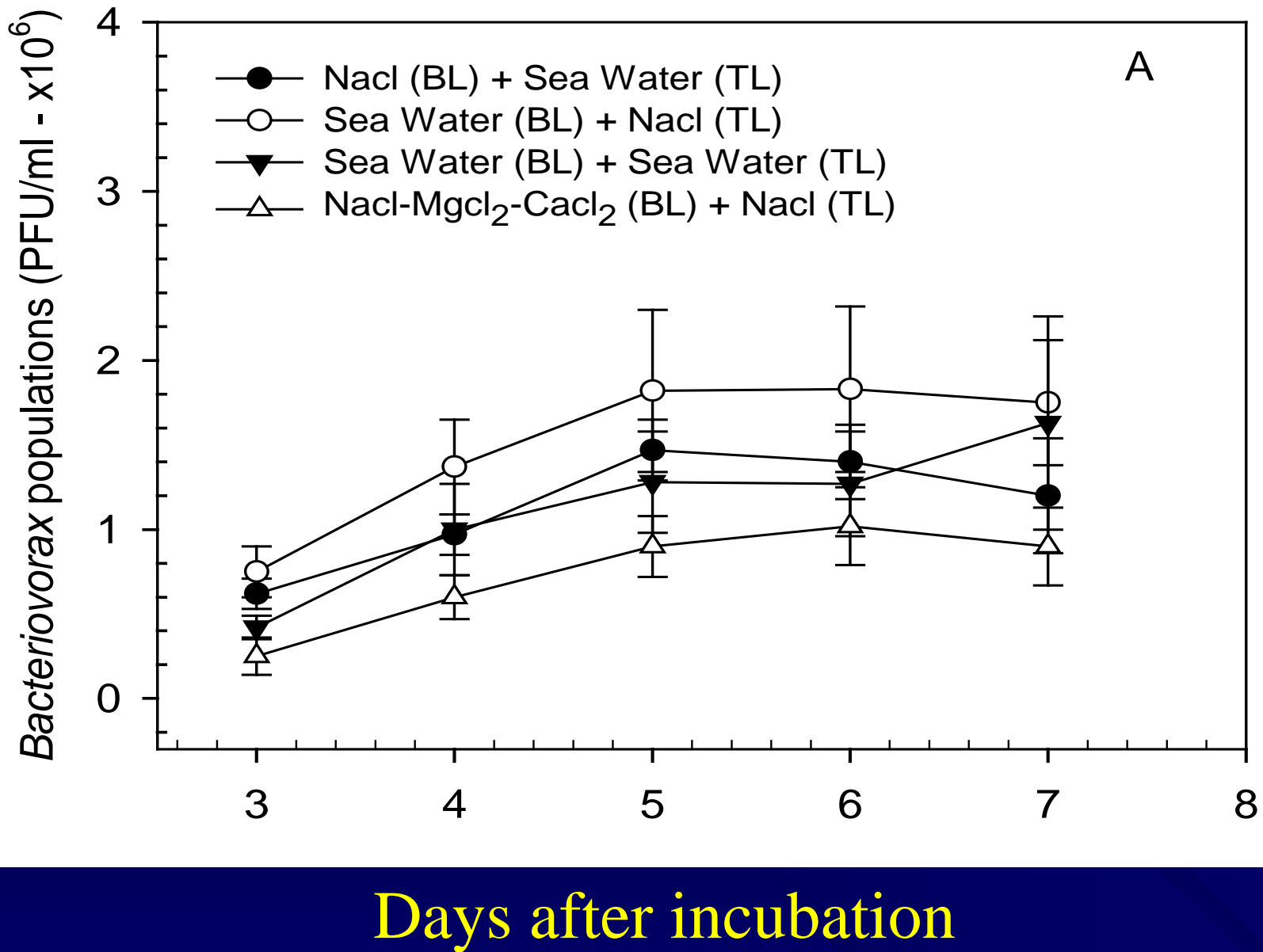
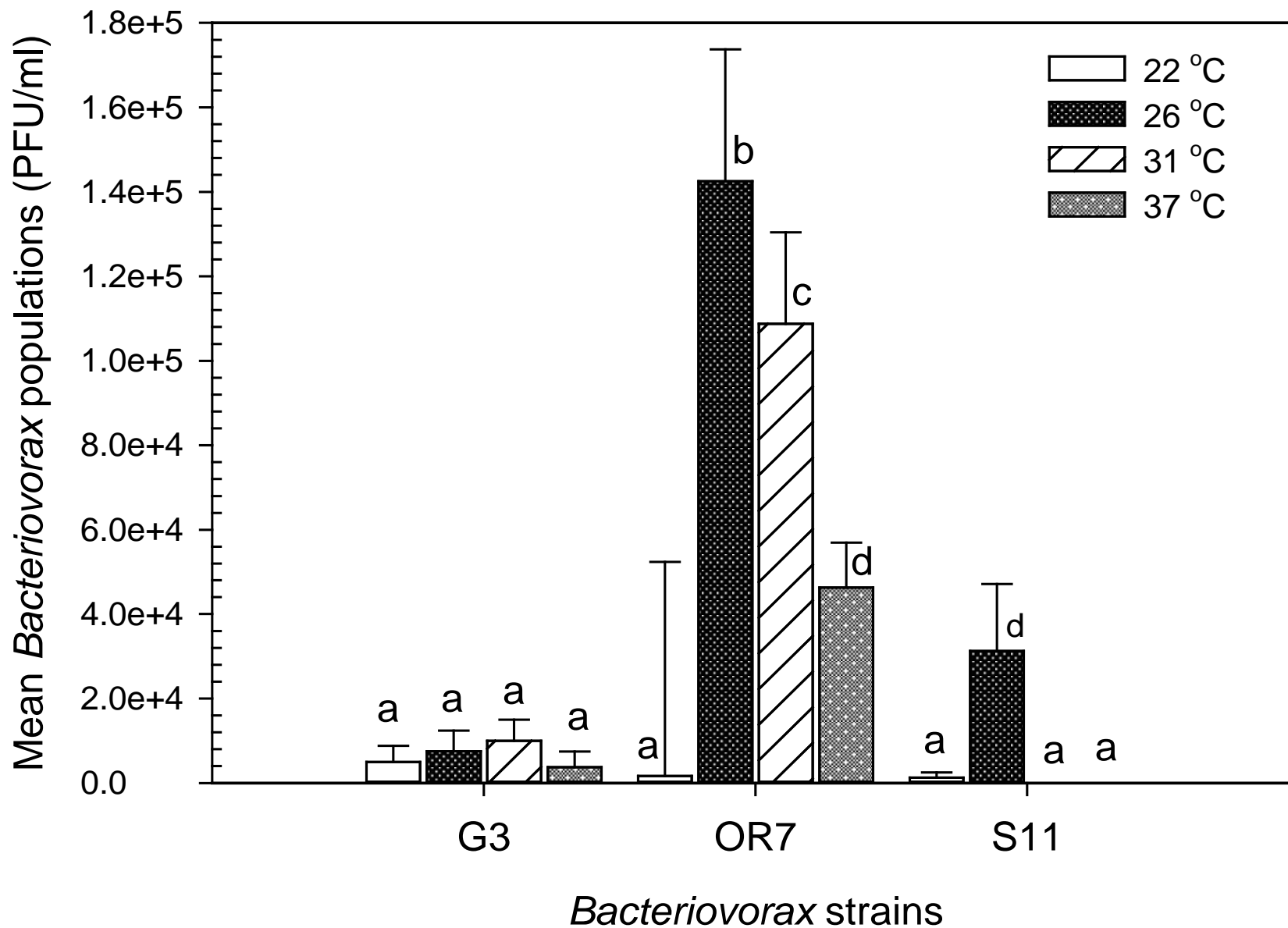


Table 1. Amendment of polypeptone peptone medium with seawater, NaCl, and salts of divalent cations on plaques by *Bacteriovorax* strains on *E. coli* O157:H7

Treatments	G3 (PFU/ml)	S11 (PFU/ml)
NaCl (BL)+SW (TL)	$1.13 \times 10^6 \pm 0.11 \times 10^6$	$0.44 \times 10^6 \pm 0.10 \times 10^6$
SW (BL)+NaCl (TL)	$1.50 \times 10^6 \pm 0.17 \times 10^6$	$0.25 \times 10^6 \pm 0.06 \times 10^6$
SW (BL)+ SW (TL)	$1.22 \times 10^6 \pm 0.26 \times 10^6$	$0.09 \times 10^6 \pm 0.02 \times 10^6$
NaCl-MgCl ₂ -CaCl ₂ (BL) + NaCl (TL)	$1.22 \times 10^6 \pm 0.26 \times 10^6$	$1.22 \times 10^6 \pm 0.26 \times 10^6$
LSD _(0.05)	1.47×10^6	0.15×10^6
BL=bottom layer, TL=top layer of PP20	1 μL of <i>Bvx</i> & 1 ml of <i>Ec</i>	







Summary

- The plaque assay was optimized for quantifying *Bacteriovorax* sp. on lawns of *E. coli* O157:H7 host cells.
- The predation of *E. coli* O157:H7 host cells by *Bacteriovorax* (Bvx) sp. was documented, indicating their biocontrol potential.
- Plaque forming units (PFU/ml) on PP20 amended with Seawater ranged from 0.56×10^6 (OR7) to 2.07×10^6 (S11).
- Plaque numbers on Polypeptone peptone (PP20) medium amended with NaCl and divalent salts (Ca^{2+} and Mg^{2+}) varied.

Summary

- Growth of pathogens and Bvx on PP20 devoid of seawater suggest that modified assay may be suitable in assessing efficacy of *Bacteriovorax* in biocontrol studies.
- Storage temperature effects on plaque development varied with *Bacteriovorax* spp. (Bvx) as the optimum temperature for OR7 and S11 was 26 °C.
- Biocontrol recorded at various temperatures imply that reductions of foodborne pathogens may be possible under diverse conditions (on produce & food contact surfaces).

Acknowledgements

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