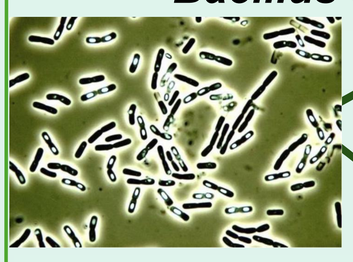
Synergism between bacteriophage and nisin for biocontrol of multidrug-resistant *Bacillus cereus* in food

Marwa Nabil Sayed Abdelaziz[1,2], Yoshimitsu Masuda[1], Ken-ichi Honjoh[1], and Takahisa Miyamoto[1]

[1] Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan
[2] Department of Food Hygiene, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Giza 12618, Egypt

Introduction

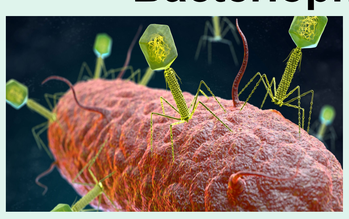
Bacillus cereus sensu stricto



Emetic toxin → Diarrhea toxin → **Food-borne illness**

Spores → **Stress tolerance**

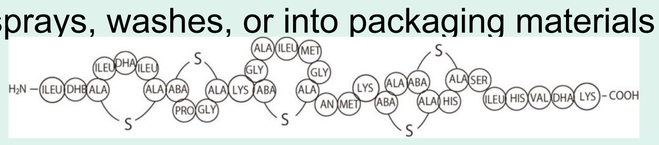
Bacteriophage (phage)



Bacteriophage as a food additive

- Generally recognized as safe (GRAS) by the FDA
- Effective against antibiotic-resistant bacteria
- No effect on taste, texture, smell or color of food
- No effect on beneficial microflora and nutritional value
- Applied as sprays, washes, or into packaging materials

Nisin

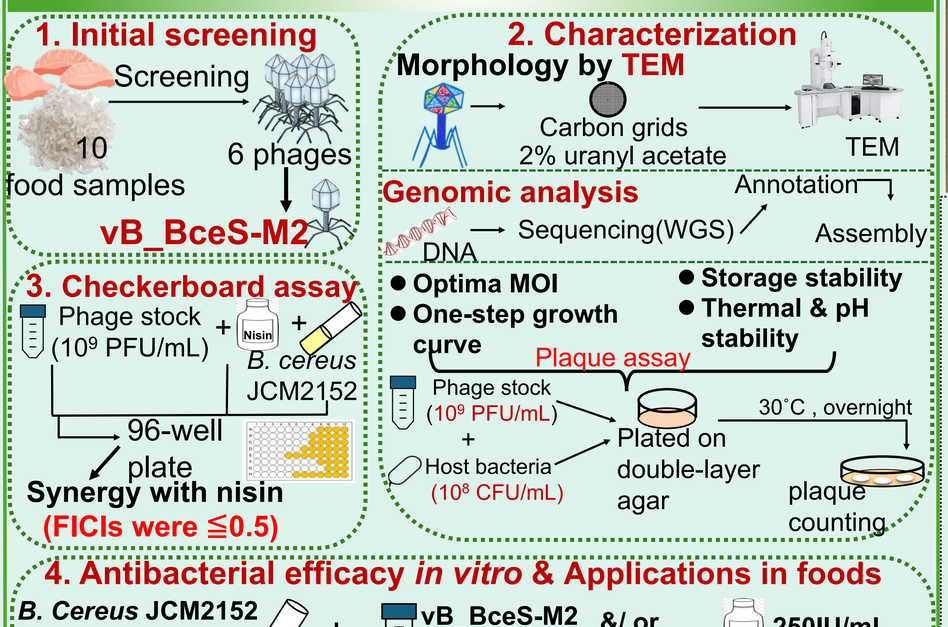


- Bacteriocin, an antimicrobial heat-stable peptide
- Food preservative
- Generally recognized as safe (GRAS) by the FDA
- Synergistic effects with other preservatives

Objectives

Isolation of Bacteriophages against *B. cereus s. s.* strain.
Select the best ***Bacillus cereus s. s.* lytic phage**.
Antibacterial effects of phage combined with nisin *in vitro*
Application of the combination in different foods.

Methods



Genome map of vB_BceS-M2

Circular double-stranded DNA
56,482 bp
114 ORFs

- ❑ No antibiotic resistance genes
- ❑ No virulence-related factors
- ❑ No lysogeny-related genes

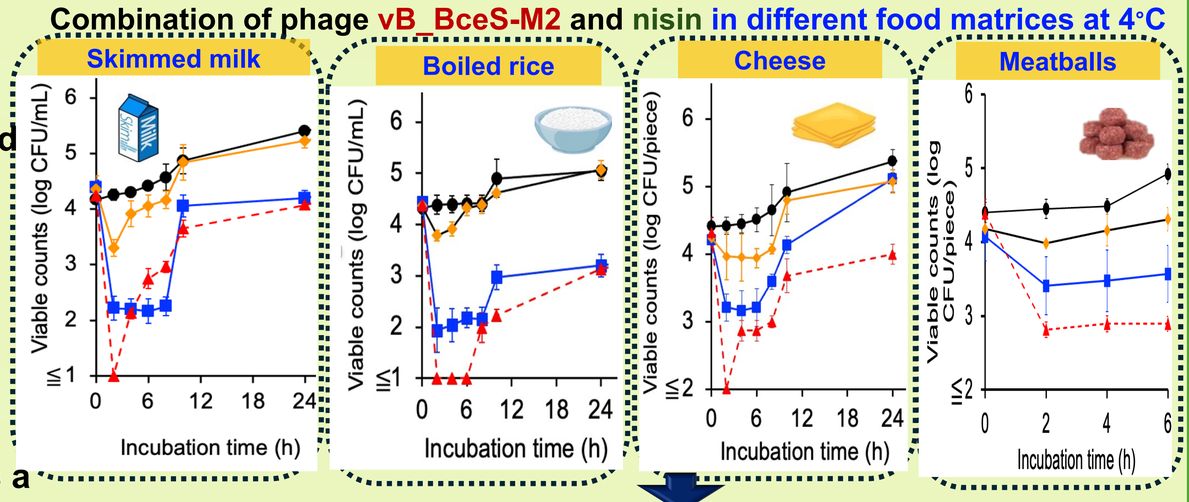
Phage structure and packaging
DNA replication
Host lysis
Hypothetical protein

Conclusion

Phage vB_BceS-M2

- **Broad host range**
- **Effective to toxigenic and multidrug- resistant *B. cereus s. s.***
- **Siphovirus morphotype**
- **Stable across a broad spectrum of temperatures and pH values**
- **A promising candidate as a biocontrol agent combined with nisin against *B. cereus s. s.* strains in different foods**

Combination of phage vB_BceS-M2 and nisin in different food matrices at 4°C



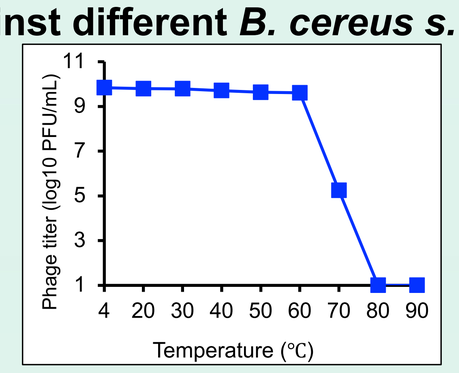
A combination of phage vB_BceS-M2 and nisin was effective to reduce viable count and retarded the regrowth of *B. cereus* in various food matrices at 4°C

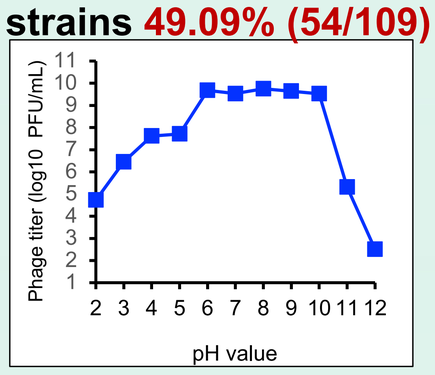
Results

Phage vB_BceS-M2

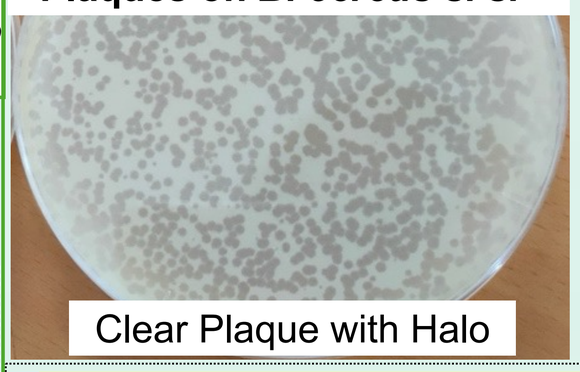
wide host range against different *B. cereus s. s.* strains 49.09% (54/109)

- **Short latent period: 20min**
- **large burst size: 613PFU/cell**
- **Stable from 4 to 60 °C for 1h**
- **Stable at pH from 6 to 10**
- **The optimal MOI = 1**
- **Survived for 10 weeks at -80 °C in 20% glycerol**

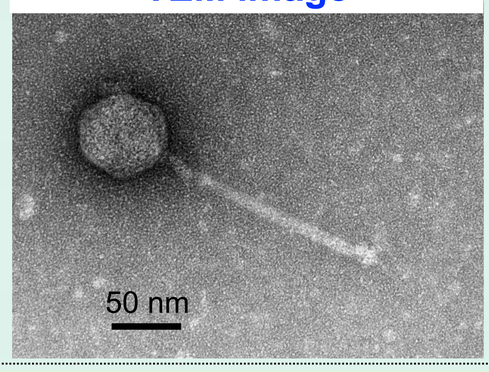




Plaques on *B. cereus s. s.*



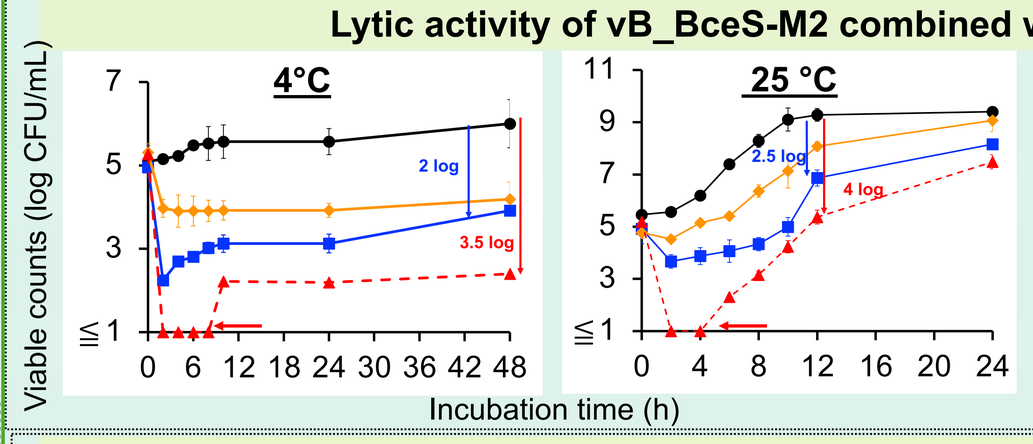
TEM image



Isometric head
(diameter = 60 nm)
Long non-contractile tail
(length ~170 nm)

Siphovirus morphotype

Lytic activity of vB_BceS-M2 combined with nisin *in vitro*



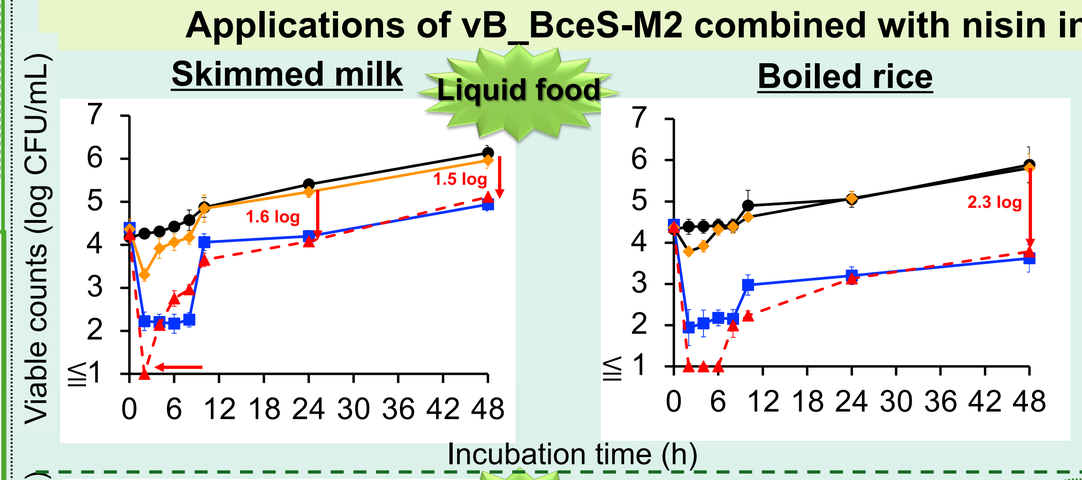
●, Control ■, Phage MOI= 10^3 ◆, Nisin 250 IU/mL ▲, Phage MOI= 10^3 + Nisin 250 IU/mL

Phage MOI= 10^3 + Nisin 250IU/mL inhibited *B. cereus* JCM2152^T

4°C
- Under detection limit for 8h
- 3.5 logs difference after 48h

25°C
- Under detection limit for 4h
- 4 logs difference after 12h

Applications of vB_BceS-M2 combined with nisin in different foods at 4°C

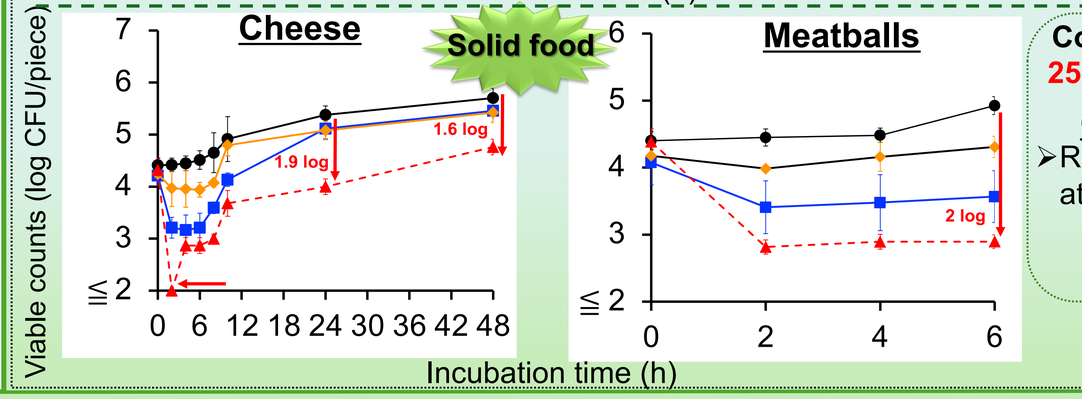


Combination of Phage MOI= 10^3 + Nisin 250 IU/mL inhibited *B. cereus* JCM2152^T

Skimmed milk
- Regrowth started at 4h

Boiled rice
- Regrowth started at 8h

The inhibition was better in boiled rice than in skimmed milk



Combination of Phage MOI= 10^3 + Nisin 250 IU/mL inhibited *B. cereus* JCM2152^T

Cheese
- Regrowth started at 8h

Meatballs
- No significant growth for 6h

The combination was effective in both solid foods

●, Control; ■, Phage MOI= 10^3 ; ◆, Nisin 250 IU/mL ; ▲, Phage MOI= 10^3 + Nisin 250 IU/mL