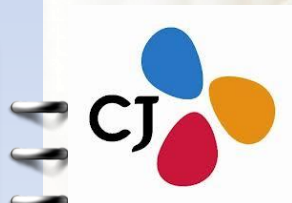




Bacteriophages: The alternatives to antibiotics for animal feeds



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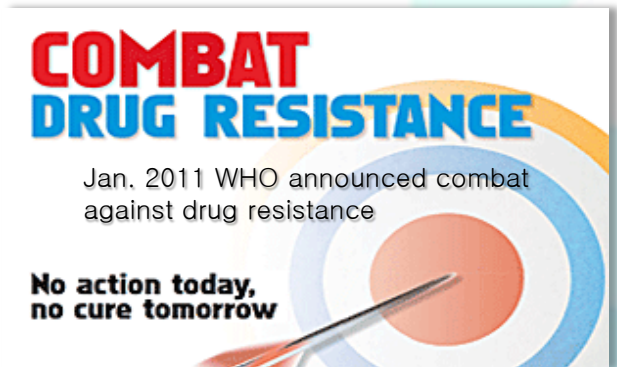
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1. Introduction

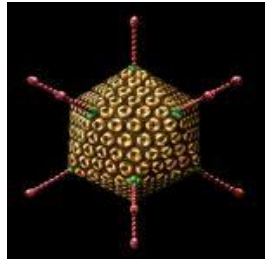
Background

- Emergence of antimicrobial resistance (super-bacteria) worldwide
- In 2006, antimicrobials banned for feed additives in EU
- In 2011, antimicrobials banned for feed additives in Korea
- WHO announced COMBAT against drug-resistance in 2011
- Bacteriophage is ubiquitous in nature and foods.



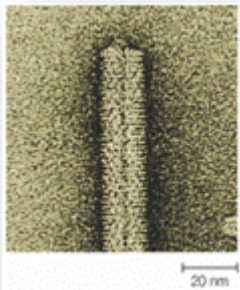
1. Introduction cont'd

Bacteriophage is virus infecting specific bacteria



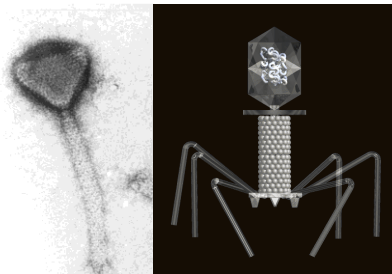
Animal virus

ex.) Influenza, AIDS



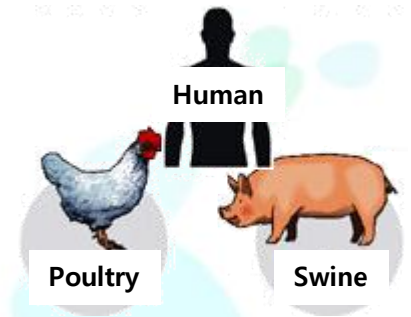
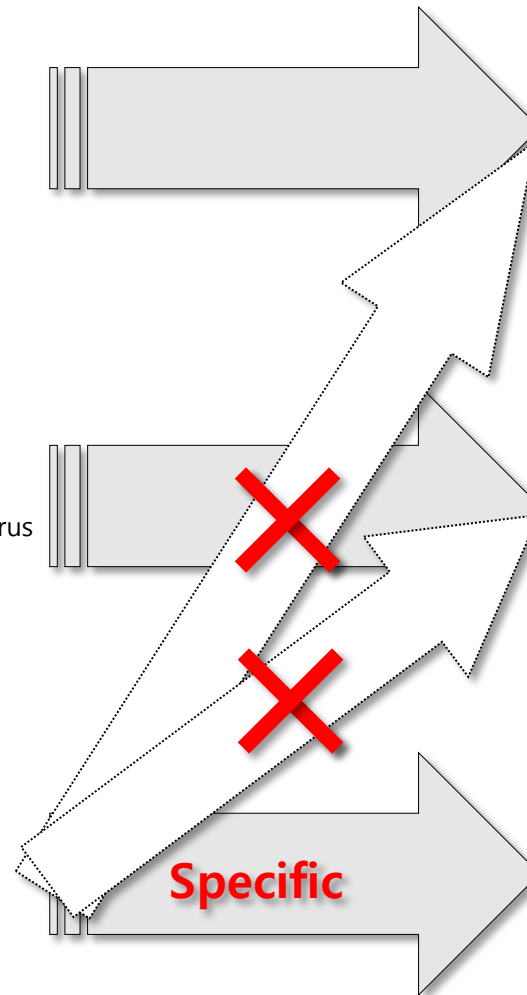
Plant virus

ex.) Tobacco mosaic virus



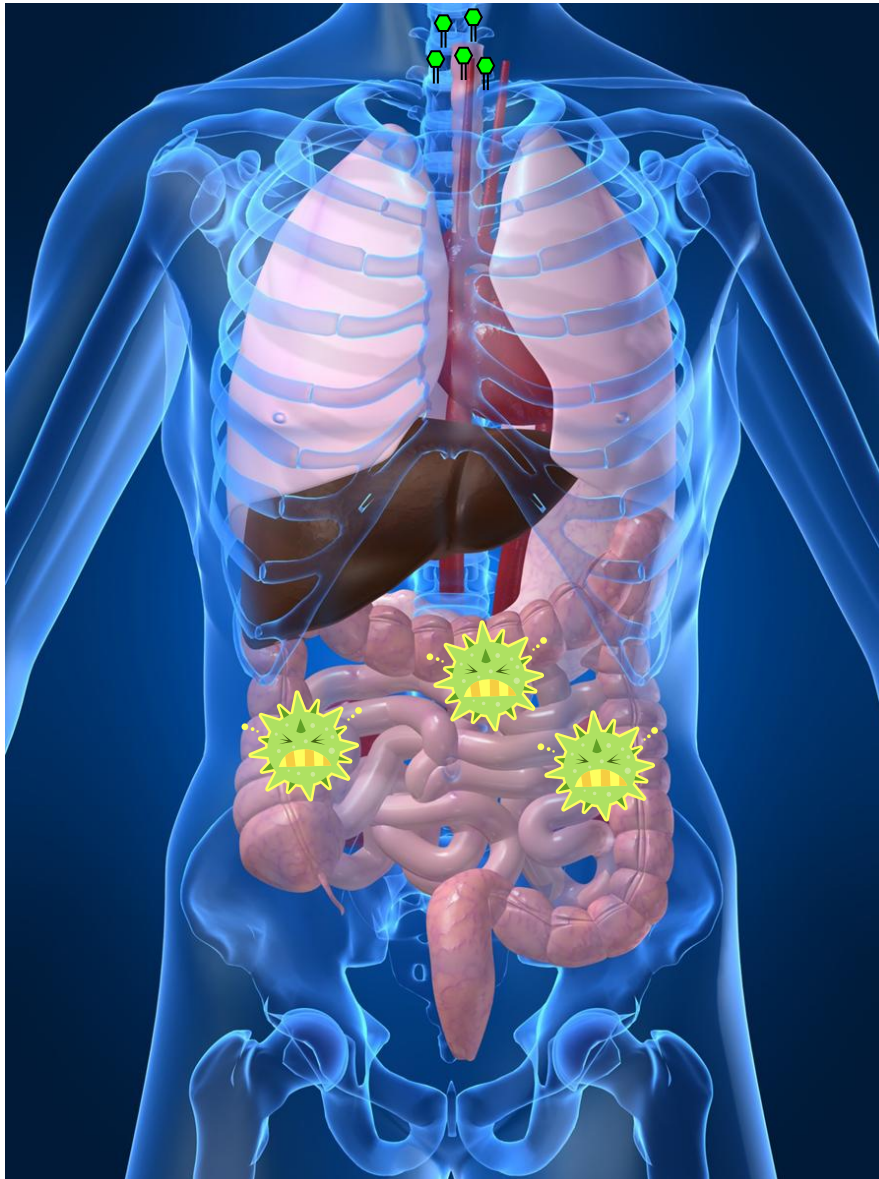
Bacteriophage

ex.) T1, T2



1. Introduction cont'd

Bacteriophage was used as an antibiotics in 1920s



In 1917, Felix d'Herelle called it "bacteriophage" (bacteria-eater in Greek) and he used phage to cure human diarrhea

Republic of Georgia has been using phage therapy

In 2006, US FDA approved *Listeria* phage mixture for using in Ready-to-Eat meat & poultry

In 2010, US Organic Material Review Institute (OMRI) registered *Listeria* phage on OMRI list

In 2011, US FDA approved *E. coli* phage for using in food

1. Introduction cont'd

Safety issue on bacteriophage

Natural (functional) aspects

- Phage requires specific receptor only existing on bacterial surface (e.g. Lipopolysaccharide, TA, Peptidoglycan)
- Phage capsid does not have animal cell membrane degrading function

Historical aspects

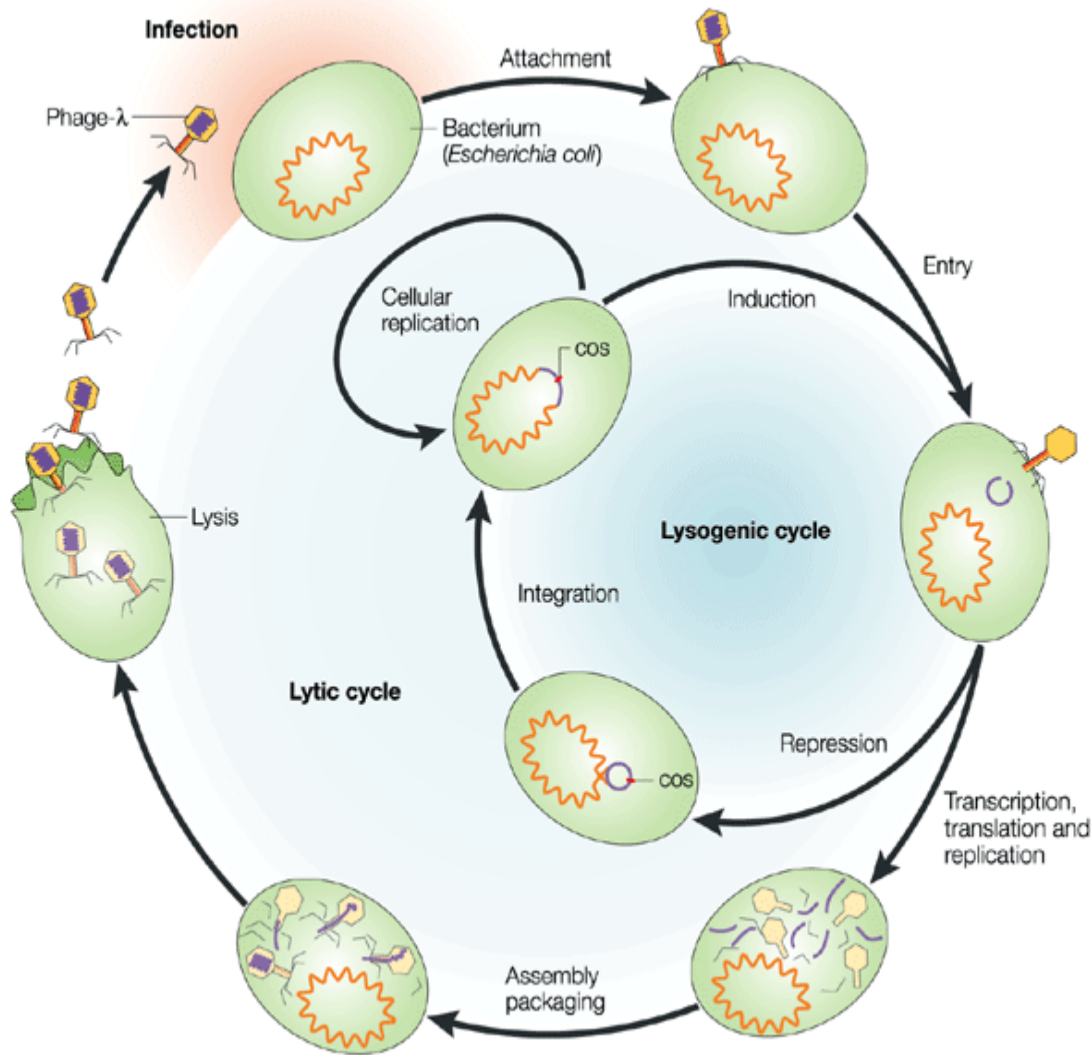
- Felix D'Herelle developed phage solution to cure human diarrhea in 1917
- Phage solution was supplied to all Russian soldiers as an antibiotics in World War II
- Georgia has been using phage in hospital from 1940s up to now

US FDA/USDA approval of phage usage on foods

- In 2006, US FDA approved *Listeria* phages for using on food
- In 2010, *Listeria* phages were registered on Organic Material Index (OMRI)
- In 2011, US FDA approved *E. coli* phage for using on Food

1. Introduction cont'd

Life cycle of bacteriophage



Lytic Cycle

- ✓ Viral DNA destroys cell DNA, takes over cell functions and destroys the cell.
- ✓ The Virus replicates and produces progeny phages.
- ✓ Virulent viral infection takes place.

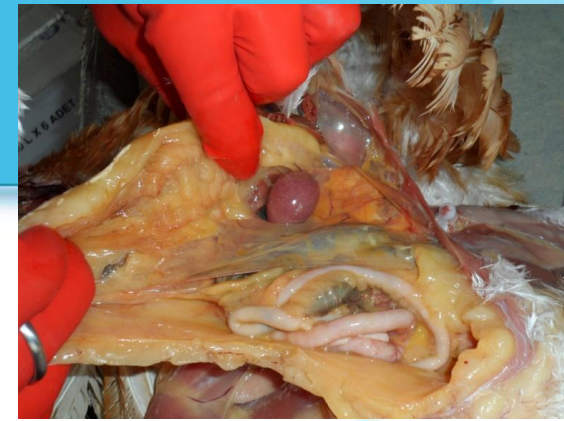
Lysogenic Cycle

- ✓ Viral DNA merges with cell DNA and does not destroy the cell.
- ✓ The virus does not produce progeny.
- ✓ Temperate viral replication takes place.

2. Drug-resistance vs. phage

S. Gallinarum vs SG bacteriophage

(*S. Gallinarum* causes fowl typhoid in poultry)



Antibiotics	Disc	<i>Salmonella</i> Gallinarum isolates from layers						
		#5	#6	#7	#9	#10	#12	#13
Spiramycin	SP100	S	R	R	S	R	R	S
Neomycin	N30	S	S	S	S	S	S	S
Colistin sulfate	CT10	R	R	R	R	R	R	R
Apramycin	APR15	S	S	S	S	S	S	S
Cephalothin	KF30	S	S	S	S	S	S	S
Chloramphenicol	C30	S	S	S	S	S	S	S
Gentamycin	CN10	S	S	S	S	S	S	S
Bacitracin	B10	R	R	R	R	R	R	R
Erythromycin	E15	S	R	R	S	R	R	S
Amoxycillin/clavulanic acid	AMC30	S	S	S	S	S	S	S
Oxolinic acid	OA2	S	R	R	S	R	R	S
Florfenicol	FFC 30	S	S	S	S	S	S	S
Doxycycline	DO30	S	S	S	S	S	S	S
Tetracycline	TE30	S	S	S	S	S	S	S

BIOTECTOR®-S1 for poultry	NG (+++)	NG (+++)	NG (+++)	NG (+++)	NG (+++)	NG (+++)	NG (+++)	NG (+++)
------------------------------	-------------	-------------	-------------	-------------	-------------	-------------	-------------	-------------

R: resistance, S: susceptible, NG: no growth, (+++): complete lysis of target bacteria, (++) : few bacteria survived (+): few bacteria lysed, (-): no bactericidal effect

2. Drug-resistance vs. phage cont'd

ETEC vs ETEC bacteriophage



(Enterotoxigenic *E. coli* causes watery diarrhea in swine)

Antibiotics Isolates	AMP	AMC	Apramycin	Collistin	Tetracycline	Gentamicin	Cephalothin	Chloramphenicol	BIOTECTOR®-S1 for swine
ETEC7 (F4)	R	S	S	S	R	S	R	R	NG (+++)
ETEC22 (F6)	IR	S	S	R	S	S	R	S	NG (+++)
ETEC9 (F4)	IR	S	S	R	R	S	R	S	NG (+++)
ETEC60 (F4)	R	R	R	R	R	R	R	R	NG (+++)
ETEC62 (F4)	R	IR	R	S	R	R	R	S	NG (+++)
ETEC93 (F6)	R	S	R	S	R	R	R	R	NG (+++)
ETEC74 (F4)	R	S	S	S	R	R	R	R	NG (+++)
ETEC18 (F5)	R	IR	S	S	IR	S	IR	S	NG (+++)
ETEC8 (F4)	S	S	S	S	R	S	R	S	NG (+++)
ETEC80 (F5)	R	R	S	R	R	S	R	R	NG (+++)
ETEC4(F4)	IR	IR	S	R	R	S	R	S	NG (+++)
ETEC20 (F4)	S	S	S	S	R	S	IR	S	NG (+++)

AMP: ampicillin, AMC: amoxacillin, R: resistance, IR: intermediate resistance, S: susceptible, NG: no growth
 (+++): complete lysis of target bacteria, (++) : few bacteria survived, (+): few bacteria lysed, (-): no bactericidal effect

3. SG bacteriophage in poultry

SG bacteriophage: SG challenge trial - Broiler

1. TITLE :

To examine the preventive efficacy of BIOTECTOR® S1 as a feed additives against challenge with *Salmonella gallinarum* in broilers

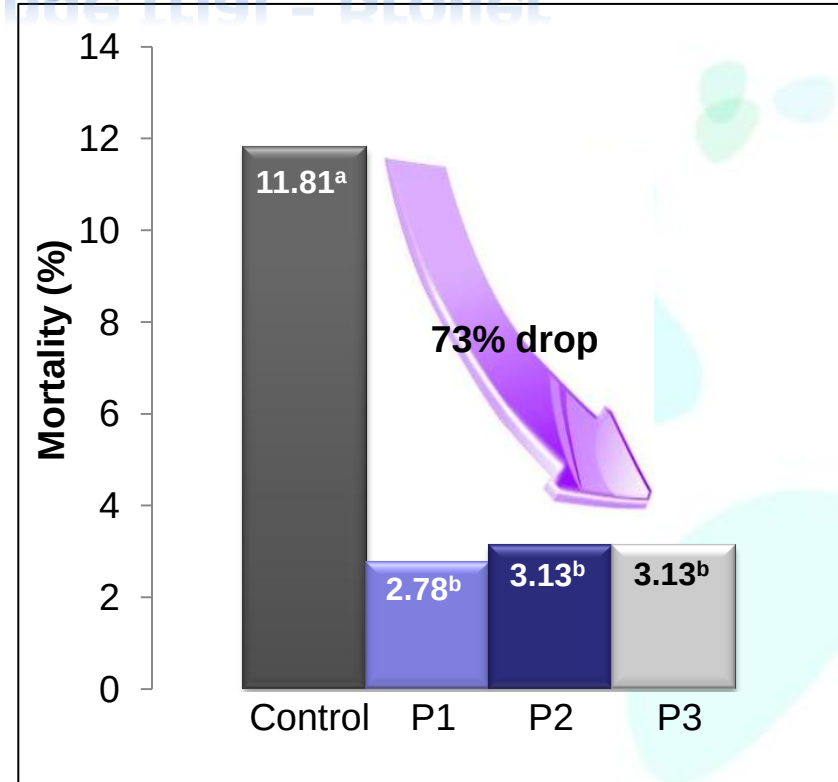
2. DURATION : 3 weeks

3. MATERIALS & METHODS :

1) Animals : Total 136 *Salmonella gallinarum*-free Broilers (5-wks old male Ross)

2) Treatments : 4 Treat X 34 Heads

3) SG challenge : SG KP-93 strain 5×10^6 cfu/ml/head



		Heads	Treatment
Challenge	Control	34	Basal Diet
	T1	34	Basal Diet + BIOTECTOR S1 (5×10^7 PFU/kg)
	T2	34	Basal Diet + BIOTECTOR S1 (1×10^8 PFU/kg)
	T3	34	Basal Diet + BIOTECTOR S1 (2×10^8 PFU/kg)

SG bacteriophage: SG challenge trial - Broiler breeder

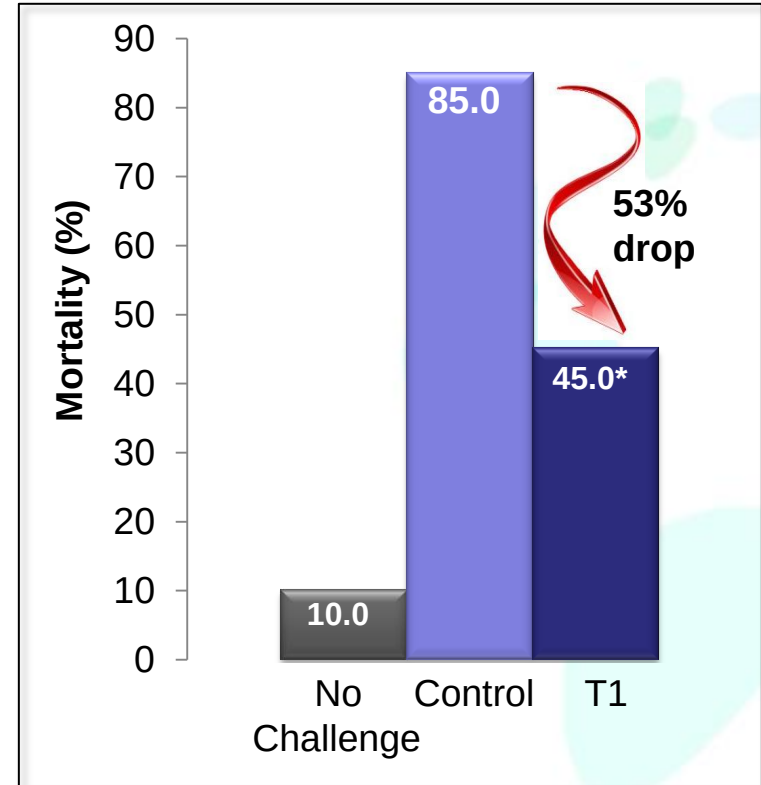
1. TITLE :

To examine the preventive efficacy of BIOTECTOR® S1 as a feed additives against challenge with *Salmonella gallinarum* in broiler breeders

2. DURATION : 4 weeks

3. MATERIALS & METHODS :

- 1) Animals : Total 60 *Salmonella gallinarum*-free Broiler breeders (67-wks old Ross)
- 2) Treatments : 3 Treat X 20 Heads
- 3) SG challenge : SG KP-93 strain 5×10^7 cfu/ml/head



* $P < 0.05$ by Fisher's exact test

		Heads	Treatment
No Challenge		20	Basal Diet
Challenge	Control	20	Basal Diet
	T1	20	Basal Diet + BIOTECTOR S1 (1 X 10 ⁶ PFU/kg)

3. SG bacteriophage in poultry cont'd

SG bacteriophage: SG challenge trial – Hy line brown Layer

1. TITLE :

To examine the preventive efficacy of BIOTECTOR® S1 s a feed additives against challenge with *Salmonella gallinarum* in layers

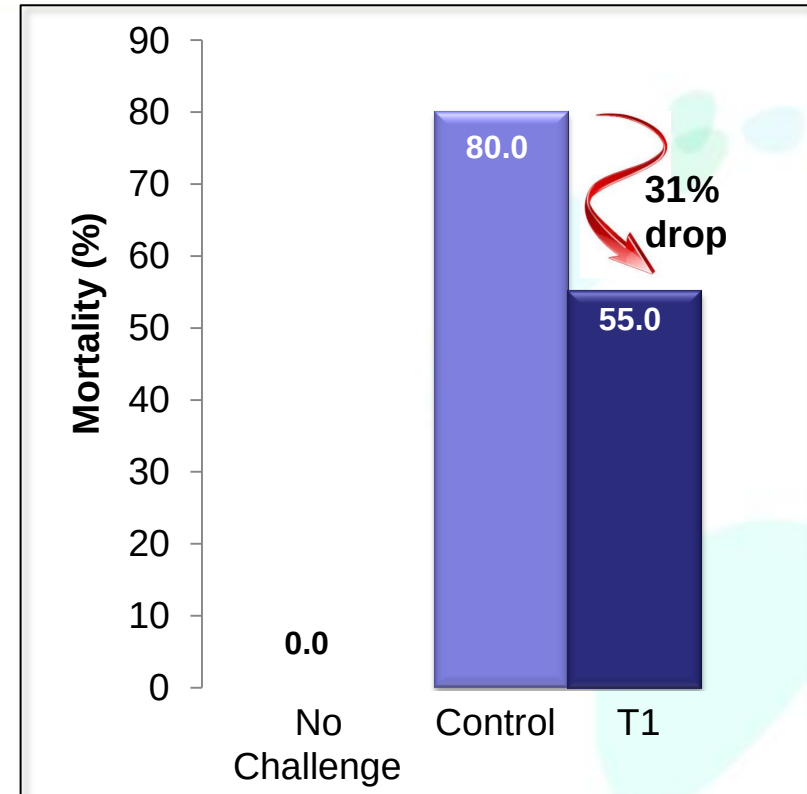
2. DURATION : 4 weeks

3. MATERIALS & METHODS :

1) Animals : Total of 60 *Salmonella gallinarum*-free Layers (6-wks old Hy-line Brown)

2) Treatment : 3 Treat X 20 Heads

3) SG challenge : SG KP-93 strain 5×10^8 cfu/ml/head



		Heads	Treatment
No Challenge		20	Basal Diet
Challenge	Control	20	Basal Diet
	T1	20	Basal Diet + BIOTECTOR S1 (1 X 10 ⁶ PFU/kg)

3. SG bacteriophage in poultry cont'd

SG bacteriophage: SG challenge trial – Old Lohmann Layer

1. TITLE :

To examine the preventive efficacy of BIOTECTOR® S1 as a feed additives against challenge with *Salmonella gallinarum* in layers

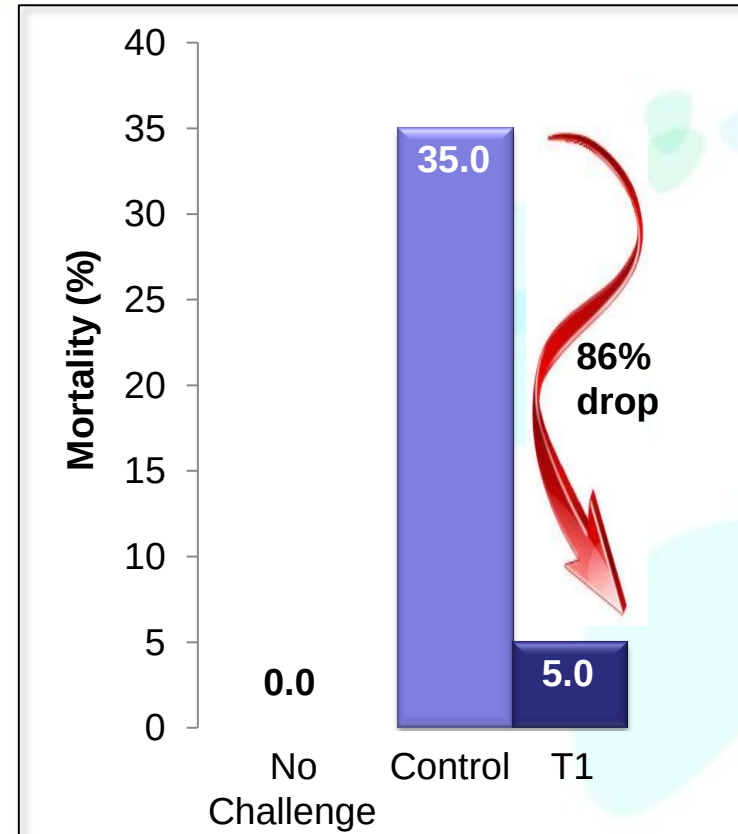
2. DURATION : 4 weeks

3. MATERIALS & METHODS :

1) Animals : Total 60 *Salmonella gallinarum*-free Layers (6-wks old Lohmann Brown)

2) Treatment : 3 Treat X 20 Heads

3) SG challenge : SG KP-93 strain 5×10^8 cfu/ml/head



		Heads	Treatment
No Challenge		20	Basal Diet
Challenge (Dissemination)	Control	20	Basal Diet
	T1	20	Basal Diet + BIOTECTOR S1 (1 X 10 ⁶ PFU/kg)

SG bacteriophage: Layer performance trial

1. TITLE :

Supplementation of BIOTECTOR® as a feed additives on the performance in Layers

2. DURATION : 10 weeks

3. MATERIALS & METHODS :

1) Animals : Total 120 *Salmonella gallinarum*-free Layers (Hy-line Brown)

2) Treatments : 3 Treat X 4 Rep X 10 Heads

	Heads	Treatment
Control	40	Basal Diet
T1	40	Basal Diet + BIOTECTOR S1 (1×10^6 pfu/kg)
T2	40	Basal Diet + BIOTECTOR S1 (1×10^8 pfu/kg)

3) Criteria investigated :

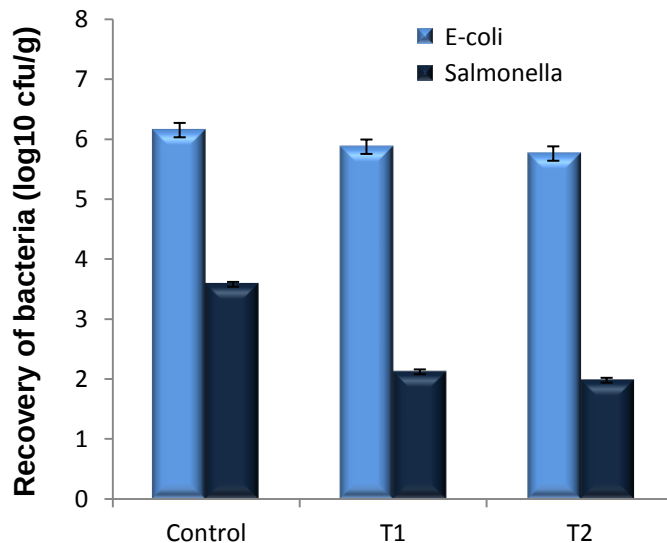
- Egg production, Egg weight, Feed intake, FCR
- Organ weight, Blood composition, Cecal microflora

SG bacteriophage: Layer performance trial

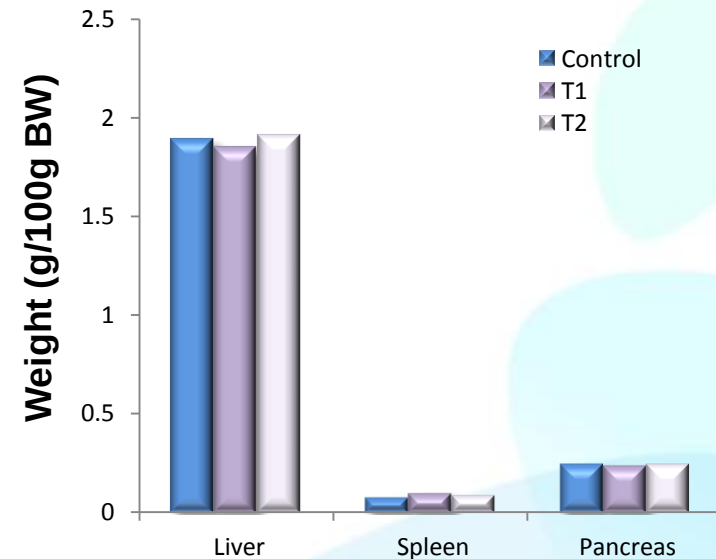
1. Performance results

	Control	T1	T2
Egg Production (%)	87.5 ^b	87.7 ^b	90.6 ^a
Egg Weight (g/egg)	64.8	65.5	65.4
Egg Mass (g/day/bird)	56.8 ^b	57.6 ^b	59.2 ^a
Feed Intake (g/day/hen)	113.6	114.1	115.7
FCR (feed/gain)	2.01	1.99	1.96

2. Cecal microflora



3. Organ weight



SG bacteriophage: comparison among phage, antibiotics etc

1. TITLE :

To examine the efficacy of BIOTECTOR® S1 on horizontal transmission of *Salmonella gallinarum* in layers

2. DURATION : 9 weeks

3. MATERIALS & METHODS :

1) Animals : Total of 135 *Salmonella gallinarum*-free brown layers (6-wks old Hyline Brown)

2) Treatment : 4 Treat X 30 Heads

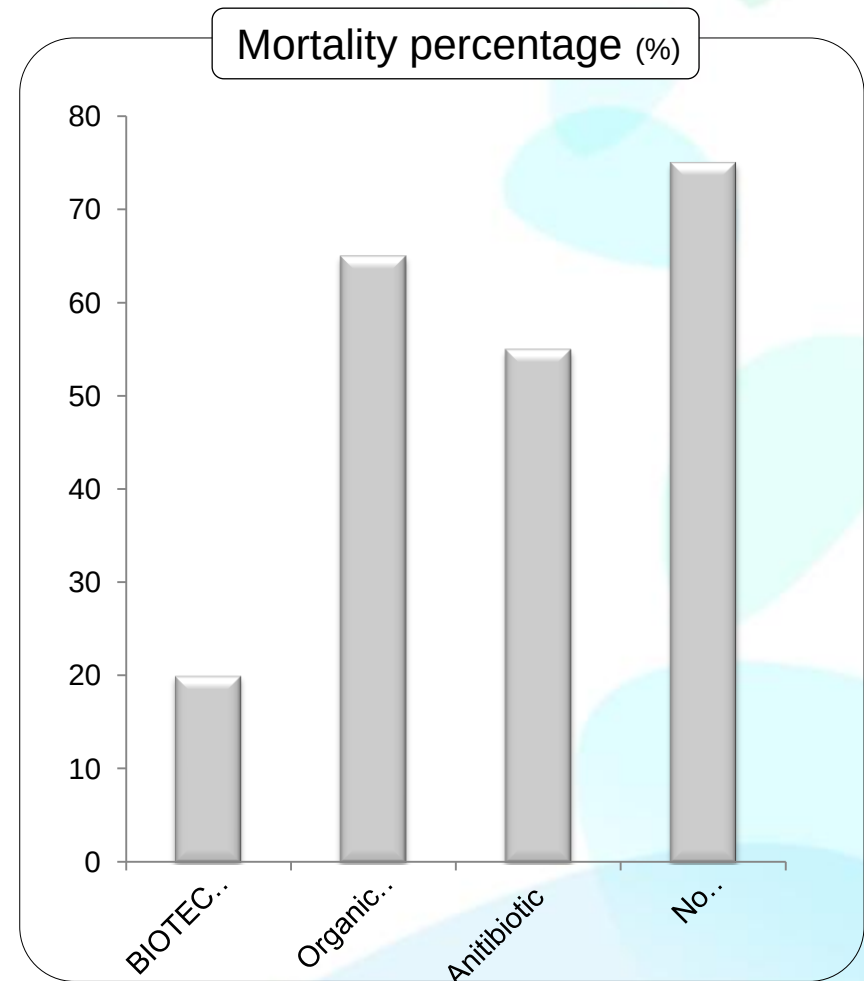
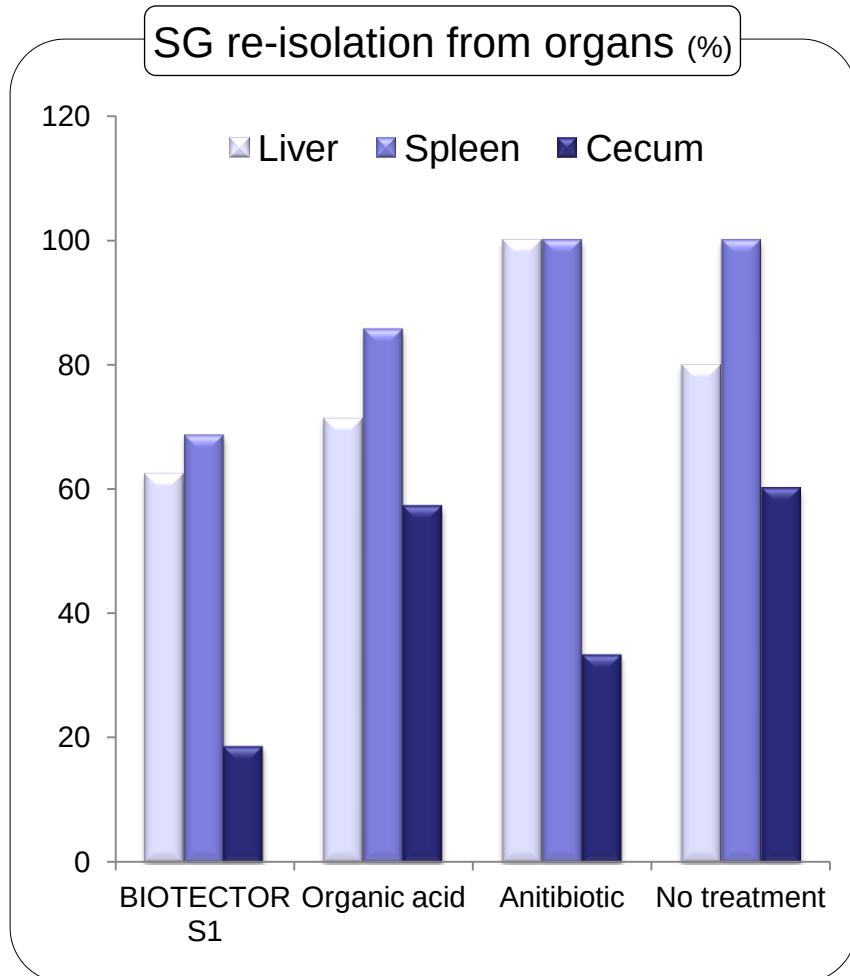
Control X 15 Heads

3) SG challenge : SG KP-93 strain 5×10^8 CFU/head

	Head s	Treatment	
		10 heads	20 heads
Negative	15	No challenge w/ SG	
Group 1	30	Challenged w/ SG	BIOTECTOR S1
Group 2	30	Challenged w/ SG	Antibiotics
Group 3	30	Challenged w/ SG	Organic acid
Group 4	30	Challenged w/ SG	No treatment

SG bacteriophage: comparison among phage, antibiotics etc

Performance results



SG bacteriophage: commercial farm trial

1. House Type and Size : Windowless, 400,000 heads

2. House Location and ages :
Layer pullets : 16 ~ 23 weeks



3. Preliminary Inspection

	Results
serological analysis	SG 8/10
other disease	N/D

4. Feeding Trial and Dosage

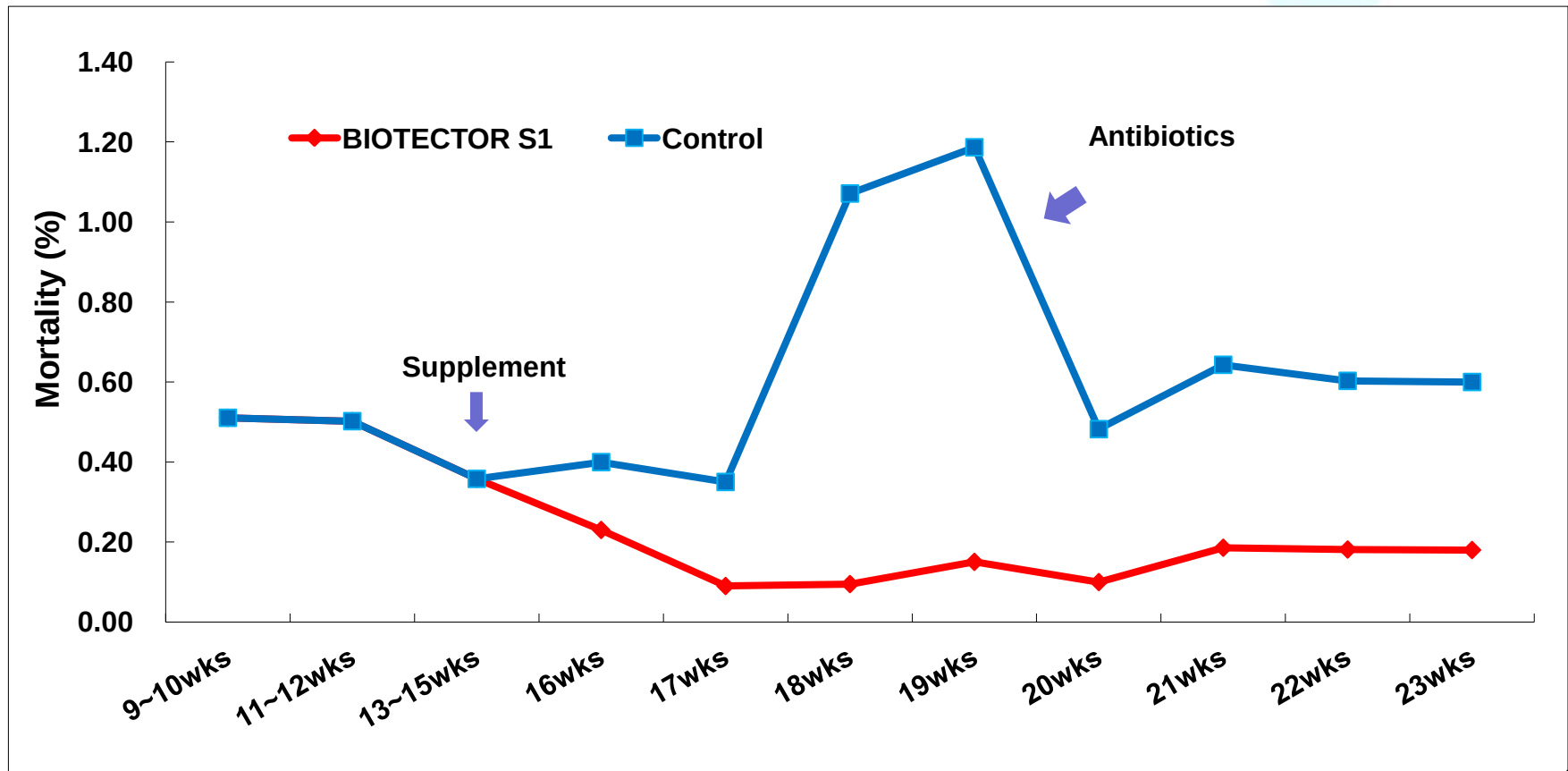
	Trial periods	Dosage
Control	8 weeks	-
BIOTECTOR S1		1.0 kg / ton of feed

3. SG bacteriophage in poultry cont'd

SG bacteriophage: commercial farm trial

1. Weekly Mortality in Layer after supplement of BIOTECTOR®

- 1) Control : 0.67 % (AVG)
- 2) SG phage: 0.15 % (AVG)

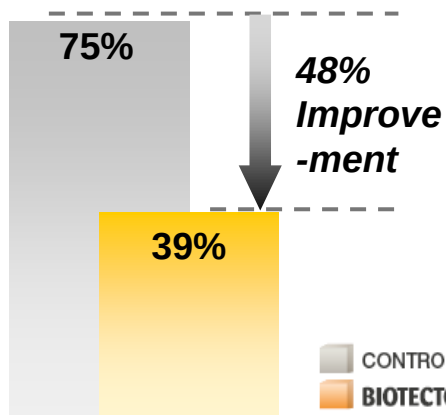


3. SG bacteriophage in poultry cont'd

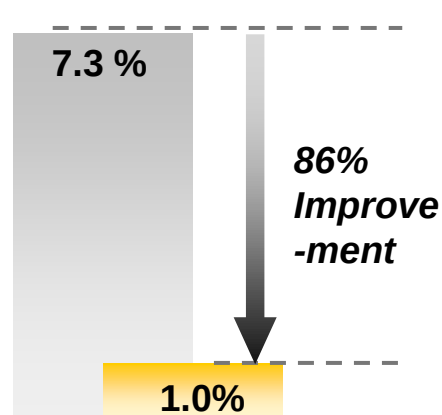
SG bacteriophage: customer benefit

Mortality

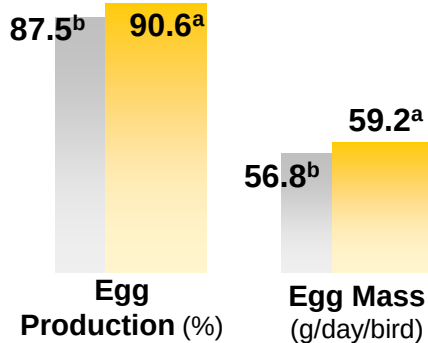
Lab / Univ. trial



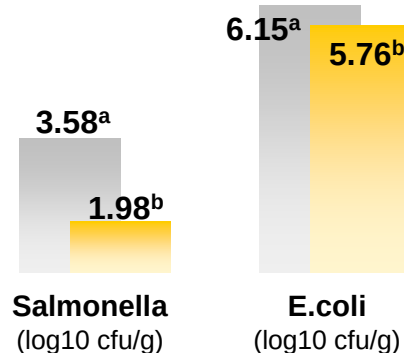
Commercial Trial



Performance

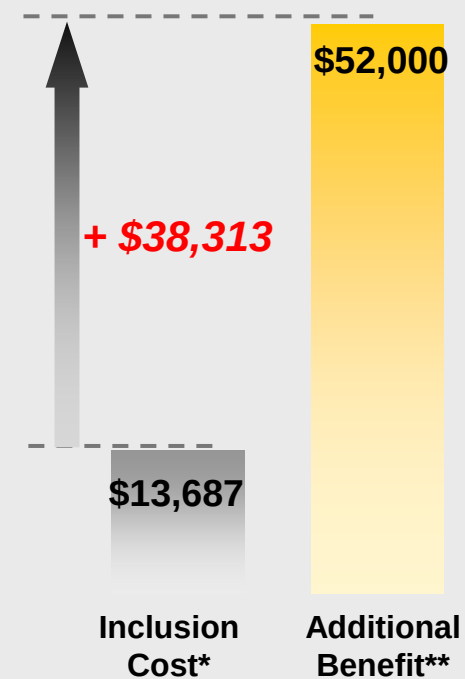


Microflora



Benefit

- 100,000 birds / Egg production 80%
- Improvement of mortality 48%



* Inclusion level : 500 gm/ ton of feed

** Standard mortality (Hy-line) : 3.70%→1.92%
Additional egg production : 520,000 ea x \$0.1

4. Conclusions

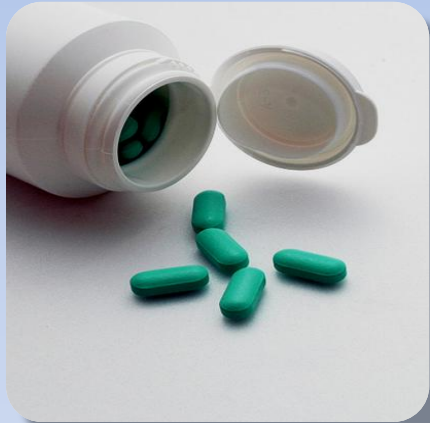
Phage is from nature → organic material

Phage targets specific bacteria (pathogen)

Bactericidal mechanism is clear

Phages is effective to control drug-resistant bacteria

Phage is one of the most promising agents to replace antibiotics



The answer is in nature.
Enemy's enemy in nature is our friend!

Question?

You can get CJ BIOTECTOR® brochure
during coffee break