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## Artificial Evaluation of Lytic Bacteriophage to Restrain those Development from *Ralstonia syzygii* subsp. *indonesiensis*, the Fresh Bacterium of Vegetable Sag Affliction

Irda Safni

Department of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia

\* Corresponding Author: **Irda Safni**

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### Abstract

Bacterial sag ailment, the individual of the big afflictions of vegetables, made by *Ralstonia syzygii* subsp. *indonesiensis* (Injury to the wrist). Many works have existed created to control bacterial fail affliction, containing material control, projectiles for weaponry, and the use of bacteriophages. Premature studies have proved that bacteriophage request in ruling plant afflictions is a fast-extending extent and has excellent potential to supplant synthetic arrangements. This study aims to decide the potential of lytic bacteriophage in restraining the progress of Repetitive stress injury artificial. This study secondhand a Non-Factorial, Entirely Randomized Design accompanying 3 replications and 6 situation levels: R1 (Injury to the wrist remove 1 outside bacteriophage), R2 (Repetitive stress injury disconnect 1 accompanying bacteriophage), R3 (Injury to the wrist disengage 2 outside bacteriophage), R4 (Injury to the wrist segregate 2 accompanying bacteriophage), R5 (Injury to the wrist separate 3 outside bacteriophage), and R6 (Injury to the wrist disconnect 3 accompanying bacteriophage). The results presented that bacteriophage take care of humiliate the Injury to the wrist people at 24 hours by look at the ocular mass (OD) worth of 600 nm intuitiveness and increasing on NA medium utilizing the spread-plate procedure. Best choice situation was at R6 accompanying a public of  $1 \times 10^8$  cfu/ml Injury to the wrist. This research plans that bacteriophage has the potential to restrain the development of Injury to the wrist, that causes vegetable bacterial fail ailment, artificial.

**Keywords:** bacteriophage, bacterial fail affliction, artificial assay, vegetable, *Ralstonia syzygii* subsp, *indonesiensis*

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### 1. Introduction

Vegetable is an main relating to plants possession in Indonesia and general. Distinguished to different herb result, vegetables have a extreme average yield, but it fluctuates done yearly <sup>[1]</sup>. reports total vegetable result in Northward Sumatra in 2020 was 1.22 heap tons accompanying result centers in Karo Regime that has a result of 703.6 thousand tons. The condition and abundance of vegetable yields maybe decreased by various cultivational challenges, in the way that virus scourge and plant afflictions. Bacterial fail ailment created by *Ralstonia solanacearum* variety complex is a bigger ailment that influences vegetable plants in sweltering nations, in the way that Indonesia, likewise substitute-equatorial and sober atmospheres. The type *Ralstonia* is deliberate expected ultimate main plant pathogenic bacterial class <sup>[2]</sup>. *R. solanacearum* class complex has sustained taxonomic changes; phylotype IV that infects the kin Solanaceae is produced for one bacterium *Ralstonia syzygii* subsp. *indonesiensis* <sup>[3]</sup>. Many works have existed fashioned to control bacterial sag produced for one pathogenic germ *R. syzygii* subsp. *indonesiensis* (Repetitive stress injury), containing land cleanliness, equalized breeding, and utilizing bactericides in the way that medicines and law enforcement officer-located compounds. Still, medicines and law enforcement officer fighting have happened labeled and typified in many plant-pathogenic microorganisms <sup>[4]</sup>. Additionally, the use of completely clean projectiles for weaponry can influence human well-being and adulterate land regions cause bactericides leave synthetic residues. Accordingly, various alternative measures are now being tested and examined, to a degree the use of biofumigant <sup>[5]</sup> and organic powers, containing *Trichoderma koningii* <sup>[6]</sup> and the opposing microorganisms *Bacterium subtilis* <sup>[7]</sup>. Another alternative now being widely grown is the request of bacteriophages, that are viruses that contaminate and destroy bacterial containers straightforwardly. Bacteriophages live and copy in unicellular structures, different additional viruses that duplicate in the materials of multicellular creatures <sup>[8]</sup>. Bacteriophages have happened secondhand as hopeful decontaminating powers to control plant afflictions because the early 20th centennial by way of their extreme precision, lack of negative affect persons and mammals, and lack of referring

## 2. Materials and Methods

### 2.1. Seclusion of the Bacterial Bacterium from Vegetable Plant

Microorganisms were unique from vegetable plant samples presenting syndromes of complete plant sag by acclimating means of <sup>[18]</sup>. Seclusion was completed activity by deriving the ooze (bacterial bulk) from polluted vegetable plant stems. The polluted stems were uncluttered utilizing running water, and therefore the surface was completely clean by spraying 70% intoxicating. Polluted plant stems were cut and immersed into clean water as far as the ooze debuted and enhanced seeable. The ooze was ridged accompanying vitamin agar (NA) (complaint extract 10 g, peptone 10 g, NaCl 5 g, agar 15 g/L) medium in a Petri tray and hatched at 28 °C for 24-28 hours. The bacterial communities were moved into the almost-discriminating radio, casein peptone and oxygen-tetrazolium chloride (CPG-TZC) (casein body of rushing waters 1 g, peptone 10 g, organic compound composed of carbon 5 g, agar 15 g, 1% tetrazolium/L), and hatched for 4 days at 28 °C.

### 2.2. Labeling of Bacterial Bacterium

These bacterial pathogens private from the vegetables from 3 areas, Karo locality, Northward Sumatra, Indonesia (Barus Julu, Simpang Inala and Sukanalu villages), were labeled by their semantic, corporeal, and biochemical traits. The surging test of observant the bacterial ooze in the vascular plant fabric was acted as per plan of <sup>[19]</sup>. This spilling test is written as a helpful backlash when the ooze discharges from the vascular fabric <sup>[19]</sup>. The bacterial communities were noticed established their color, shape, magnitude, surface, advancement and border. Grandam staining was acted, and the development was noticed at 27 °C and 40 °C. The potassium hydroxide (KOH) test was acted in accordance with form of <sup>[20]</sup>. The community of proven strains was agitated into the answer of two to three drops of 3% KOH utilizing a clean loop for 5 s. If the KOH resolution enhanced sticky later 5 seconds, as proved by a fiber-like muck following u.s. city, the test was written as beneficial. The vigor hydrolysis test was acted utilizing an agar medium as detailed by <sup>[21]</sup> to decide the strength of the microorganisms to produce sure exoenzymes, containing  $\alpha$ -amylase and oligo-1,6-glucosidase, to hydrolyse vigor. The agar medium held individual prescription of CPG medium (casein body of rushing waters 1 g, peptone 10 g, organic compound composed of carbon 5 g, agar 15 g/L containing 0.2% dissolved vigor). The proven strains were variegated upon the medium and hatched at 28 °C just before burdensome development happen. The plates were engulfed accompanying iodine resolution (iodine 1 g, potassium iodide 2g, water purified by distillation, 100 ml). The exercise of hydrogen tests was established means of <sup>[22]</sup>. The Hayward's basic medium held peptone, 1 g; ammonium dihydrogen phosphate (without water), 1 g; potassium chloride, 0.2 g; magnesium sulfate heptahydrate (without water), 0.2 g; agar, 3 g; bromothymol sad, 0.08 g; and water purified by distillation 1 L; pH 7.0-7.1. Various loops of recently of age ideas were immunized aseptically into the carbohydrate radio plates, containing and oxygen, and oxygen and hydrogen. The plates were hatched at 28 °C for four days. The colour change from green to yellow was written as a definite response.

### 2.3. Advancement of Bacteriophage

The bacteriophage disengage  $\phi$ BTF1 was calm from the Lab of Plant Affliction, Ability of Farming at Academy of Sumatera Utara from study of <sup>[17]</sup>. The insulate  $\phi$ BTF1 was recognized by <sup>[23]</sup> as Ordo Caudovirales and Offspring Myoviridae. Bacteriophage advancement was acted in accordance with arrangement of <sup>[24]</sup>. The distinct community of bacterial bacterium Injury to the wrist was immunized into the food soup (NB) medium and hatched at 37 °C for 24 hours just before it attained OD600nm=1. Individual hundred microliters of Injury to the wrist breedings were argue the 100  $\mu$ l of phage light in weight in a clean hose and hatched at 37 °C for 30 brief time period. Therefore 5 ml of simple agar (0,2% agar oppose NB medium) at 47 °C was additional into the combinations, spewed into the NA medium plates and hatched at 37 °C for 24 hours.

The bacteriophage cleansing was transported established the system of <sup>[25]</sup>. Memorial was detached utilizing a Pasteur hose; the memorial was before argue 2-3 ml of 25% Ringers solid (CaCl<sub>2</sub> 0.02 g, KCl 0.03 g, NaCl 0.6 g, sodium give milk 0,31 g/L) or seasoning of magnesium (SM). Phage delay was vortexed and abandoned for 5-10 brief time period at range hotness. The delay was before centrifuged at 4°C for 25 record and frequent doubly. The light in weight was winnowed utilizing a 0.22  $\mu$ m absorbent winnow, and before stocked as stock.

### 2.4. Memorial Assay

Memorial assay was acted utilizing the drop design (spot test) to decide the vicinity of bacteriophages, that is registered for one presence of the horizon lysis ring of bacteriophage contamination, and get clean communities of bacteriophages from Injury to the wrist isolates. Having 50 of something microliters of bacterial ideas of Repetitive stress injury (108 CFU/mL) were spread on the NA and CPG-TZC publishing utilizing a trio bottle bar as far as dry. The bacteriophage lysate was trickled upon the surface of NA and TTC news by 2  $\mu$ l utilizing a micropipette and hatched for 24-48 hours at 28 °C <sup>[26]</sup>. The experiment was recurring six occasions.

### 2.5. Bacteriophage Mass

A phage plague is a clear district made in a yard of containers on account of lysis by phage. At a depressed variety of contamination (MOI), a container is polluted accompanying a sole phage and lysed, issuing offspring phage that can wordy to adjacent containers and pollute ruling class; This process is frequent just before eventually happening in a circular field of container lysis in a cloudy terrace of containers <sup>[27]</sup>. Memorial assay is acted by devious the number of polluted viruses apiece presence of a lysis district or clear district in the gentle agar radio from various something for dunking succession of bacteriophage. The bacteriophage mass was premeditated utilizing the multilevel something for dunking means by <sup>[28]</sup>. A total of 0.1 ml of bacteriophage stock was increased 0.9 ml of physical salty (until 10-6 something for dunking). Each something for dunking (10-1 to 10-6), 200  $\mu$ l of bacteriophage was captured expected increased 6 ml of NB holding Injury to the wrist civilization and homogenize. The delay was therefore spilled into the NA medium and copied 6 opportunities and hatched at 37 °C for 24 employment recruiting and management. The

Plaques made on the Petri cup were noticed, and the number of bacteriophage communities was deliberate accompanying the following recipe:

Number of bacteriophages = number of plaques x something for dunking level <sup>[29]</sup> (1)

## 2.6. Artificial Assay of Culture Decline of Bacterial Bacterium

The new civilizations of the bacterial bacterium were additional into a new food soup (NB) medium fortified accompanying 5 mM CaCl<sub>2</sub> and MgCl<sub>2</sub>; it was hatched in a cubes brooder at 200 rpm at 37°C just before it attained an OD of 600 = 1 ( $\leq 1 \times 10^8$  cfu/mL). Individual hundred bacterial breedings in each something for dunking were increased 72 ml of the new NB medium. The phage stock was thinned utilizing SM safeguard (NaCl 5g, MgSO<sub>4</sub>·7H<sub>2</sub>O 2g, Tris-ClpH7 550ml, jam 5ml/L) until 108 pfu/mL. The bacteriophage delay was amounted to the bacterial ideas and hatched in the cubes brooder at 20-30 rpm at 37 °C. Attention of the OD600 was completed activity on the breeding all stage just before lysis happen (a decrease in the OD of each host microorganisms) utilizing a spectrophotometer accompanying NB medium. The bacterial educations were discussed the alike effectively situations and enhanced a control situation. The results were noticed each 1 hr for 24 employment recruiting and management <sup>[17]</sup>.

## 2.7. Bacteriophage Contamination Era

Scrutiny of the bacteriophage contamination phase against the bacterial bacterium was completed activity to decide the lysis or lysogenic phase middle from two points situations, that was acted utilizing the absorbance advantage from turbidimetric predictions. If the tumor of the bacterial bacterium resumes to increase later the request of bacteriophages, therefore phage duplication is determined to happen lysogenically, and if it is with the order reversed, the phages are pronounced to have lytic duplication <sup>[23]</sup>.

## 3. Results and Discourse

### 3.1. Labeling of Bacterial Bacterium

The plant from what or which place the bacterial bacterium was unique had/shown sag manifestations in all plant but the leaves were still green in colour (Smallest amount 1A). It was noticed that three vegetable samples granted beneficial dribbling and burn in the vascular bundle and vegetable container. The polluted vegetable vegetable had a dark-dirty coloured show the vegetable ship when cut across (Smallest amount 1b). Oil to silver bulk still came into view in the vegetable ship, and the vegetable enhanced rot (Smallest amount 1b). The polluted stem base of the plant was cut and immersed in clean water in a clear Cup jar just before the cloudy silver ooze discharged (Composite fruit 1c). In the pouring test, three vegetable plant samples accompanied beneficial bacterial ooze pouring in clear water.

**Table 1:** Community Semantic Traits of Repetitive stress injury

Phenotypic profiles	Rsi 1	Rsi 2	Rsi 3
Gram reaction	-ve	-ve	-ve
Bacterial shape	rod	rod	rod
Growth at 27 °C	+ve	+ve	+ve
Growth at 40 °C	-ve	-ve	-ve
Potassium hydroxide test	+ve	+ve	+ve
Starch hydrolysis	-ve	-ve	-ve
Oxidative acid from:	+ve	+ve	+ve
Lactose	+ve	+ve	+ve
Maltose	+ve	+ve	+ve
Glucose	+ve	+ve	+ve

All three Injury to the wrist isolates presented a certain backlash to the Gramam staining test, potassium hydroxide test, oxygen result from organic compound composed of carbon, sweet substance, and hydrogen, and displayed a negative response in vigor hydrolysis (Table 2). In the hotness subtlety test, the test isolates presented helpful and usual progress at 27°C but acted frustrate some potential progress at 40°C (Table 2).

The bacterial bacterium that was private from the vegetable plant presenting sag manifestations was rooted as Injury to the wrist, specifically on the presentation of dribbling and burn in the vascular bundle and vegetable container. R. solanacearum variety complex is the only sag-encouraging bacterium that produces ooze <sup>[30]</sup>. These distinguishing traits were situated semantic and biochemical traits.

### 3.2. Bacterial Mass

The maximal aggregation of bacteriophage state was about something for dunking 10-6 accompanying a total of 8.65 x 10<sup>7</sup> pfu/ml, and hostile bacteriophage state was about 10-1 something for dunking accompanying a total populace of 2.78 x 10<sup>2</sup> pfu/ml (Table 3). The computation was established

optic attention, that is, manipulative the number of plaques that arose on the compassionate agar radio. The taller the something for dunking level, the larger the aggregation of the bacterial plaques.

### 3.3. People Decline of Ralstonia syzygii subsp. indonesiensis artificial

The curve style in situation outside bacteriophage request (R1, R3, and R5) demonstrated that bacterial tumor resumed to increase until 24 hours of scrutiny (Composite fruit. 2). R5 situation (Repetitive stress injury disconnect 3) accompanied the chief number of Injury to the wrist people last of attention (24 hours) (149.33 x 10<sup>8</sup> cfu/ml) attended by R3 situation (Repetitive stress injury confine 2) (151.3 x 10<sup>8</sup> cfu/ml) and R1 situation (Injury to the wrist insulate 1) (120.33 x 10<sup>8</sup> cfu/ml), individually. Nevertheless, all situations accompanying bacteriophage request (R2, R4, and R6) revealed a decline in Repetitive stress injury populace. R6 situation (Injury to the wrist sequester 3) started to show a falling birthrate at the 20th moment of scrutiny and attained rude number of Injury to the wrist public (1 x 10<sup>8</sup> cfu/ml) at 24 hours of note. Additional bacteriophage situations

(Repetitive stress injury disconnect 1/ R1 and detach 2/R2) likewise accompanied a falling birthrate in the end of remark (24 hours), that were  $3.33 \times 10^8$ cfu/ml.

### 3.4. The Contamination Era of Bacteriophage

The use of bacteriophage  $\phi$ BTF1 take care of defeat the community of Injury to the wrist as proved by ocular bulk principles in the awareness of 600 nm (Composite fruit 2 & Composite fruit 3). The decrease in the public of Injury to the wrist driven that the bacteriophage disconnect  $\phi$ BTF1 is a lytic bacteriophage that had sustained the lytic biological clock. In the beginning note ending, the Repetitive stress injury evolved evenly till 16 hours, but the tumor of the Injury to the wrist in the situations of R2 (segregate 1), R4 (insulate 2) and R6 (separate 3) moderately dropped off from the OD principles middle from two points 1-1.5 to beneath 1 last of the scrutiny ending (24 hours).

This study shows that the use of bacteriophage insulate  $\phi$ BTF1 commit lower the progress of the Rsi culture under artificial condition apiece increase of bacteriophage mass and the decrease of Rsi state. This preliminary artificial study desires that bacteriophage maybe a hopeful alternative organic power to control bacterial sag affliction of vegetable. In a former study, bacteriophage confine  $\phi$ BTF1, that was secondhand in this place study, was establish to restrain the contamination of *R.szygii* subsp. *celebesensis*, that cause ancestry affliction in joker [23]. The current study under artificial condition more certified the effectiveness concerning this phage disengage in restraining the study of human population of bacterial fail bacterium of vegetable (Rsi). In isolates that were not used accompanying bacteriophages, the diagram proved an increase in the Rsi peoples cause the microorganisms evolved in the acceptable medium and skilled were no powers that shy their development.

Phage sequester  $\phi$ BTF1 is a lytic bacteriophage that includes the duplication of viruses utilizing a host container to produce more viruses; they therefore escape and lyse the host container. Strong lytic endeavor is necessary to nearly control the bacterial sag bacterium *Ralstonia solanacearum* [31]. During the contamination era of the lytic phage, enzymes, chiefly holins and lysins, are created [32]. Lysin enzymes are very active chemical compound that attack individual of the four key peptidoglycan linkages to goal the uprightness of the bacterial container obstruction [33]. Lysin builds up in the cytoplasm all the while phage incident in the contaminated microorganisms expected for phage development. When holin particles are introduced into the cytoplasmic sheath at a innately prearranged importance, patches are made that yet cause statement sheet failure, admitting the cytoplasmic lysin to approach the peptidoglycan, superior to container lysis and the release of offspring phage [34, 35]. Small RNA and DNA phages use phage-encrypted proteins to upset bacterial host enzymes complicated in peptidoglycan result, superior to an wrong congregation of container obstruction and concluding lysis [36, 37].

Bacteriophages are prokaryotic viruses present plentifully completely surroundings and maybe erect unspecified area the potential host-microorganisms are present [38]. A total number of 1032 virions of bacterial viruses are present in the surroundings, and this number is 100 opportunities in addition the complete number of now typified microorganisms [39]. This extensive incident of bacteriophages presents the excellent benefits of confining

bureaucracy in character for plant affliction control purposes. A prior study utilizing lytic bacteriophages to control bacterial sag ailment confirmed that bacteriophages are well host distinguishing, and lytic bacteriophages manage weaken the pathogenic sagging action of *R. solanacearum* in attractive woman seedlings in addition to vegetable vegetable [40]. In hothouse and field tests, attractive woman plants doctored accompanying differing phage consolidations further shortened the incident of the bacterial sag *Ralstonia* ailment by 80% [41]. These studies submitted that sole-phage uses to bacterial fail affliction were well fixed accompanying powerful lytic action for persuasive administration of phage fighting.

### 4. Conclusion and Recommendation

The request of bacteriophage was capable to humble the state of Rsi, increasing on NA medium utilizing the spread plate arrangement, at 24 hours of attention by look at the ocular mass (OD) advantage of 600 nm awareness. By the use of lytic bacteriophage  $\phi$ BTF1, Rsi disconnect, 3 confine had the slightest culture ( $1 \times 10^8$ cfu/ml). This research desires that lytic bacteriophage has excellent potential to restrain the progress of vegetable bacterial sag bacterium Rsi artificial and can be persisted accompanying *in vivo* experiment to decide skilled is an effect of bacteriophages request to control Rsi. So the results of the research maybe secondhand as fundamental news to control bacterial fail ailment in vegetable plants.

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