Investigation Potency Serratia marcecens Indigenoes in Controlling Udbatta Disease that is Caused by Ephelis oryzae at Invitro Test

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Abstract. One of the obstacles in increasing rice production in Indonesia is pests of plant diseases such as the attack of Udbatta diseases caused by Ephelis oryzae. This disease attacks the panicles so that the panicles can not grow and do not produce at all. The current use of biological agents has considerable attention worldwide. This treatment is more popular because of the increased public awareness of biological safety issues and environmental health issues, with respect to phytotoxicity due to the excessive use of synthetic pesticides. Environmental differences in the tropics are a very sharp effect on diseases and bacterial biocontrol agents. So that effective isolates as biological control agents are those originating from the diseases environment (indigenoes). Therefore, in this research, developed S. marcecens indigenoes to control the Udbatta diseases. The purpose of this research is to know the ability of S. marcecens indigenoes as biological controlling agents of Udbatta diseases. The test was performed by knowing the production of the extracellular enzyme by S. marcecens indigenoes so that the bacteria could potentially act as plant disease control. The test was done invitro test. From the results of the study it is known that S. marcescens indigenus secretes chitinase, protease and cellulase enzymes. These three enzymes can destroy the fungal cell wall of E. oryzae causing udbatta disease, destroying cellulase and protein, thus potentially as biological agents of Udbatta disease. Inhibition of S. marcescens indigenous bacteria on the growth of E. oryzae fungus with the method of in-vitro test is from 58.8% to 62,2 %.

Keywords: *Serratia marcecens, Udbatta diseases, Ephelis oryzae.*

1 Introduction

Nowadays, Udbatta diseases which are caused by *Ephelis oryzae* infection begin to haunt the rice farmer. Its infection may affect the tassel that leads to unproductive plants. Udbatta disease attacks the panicle, where a whitish grayish fungus encloses a panicle so that a panicle can not develop (Figure 1).

WMA-2 2018, January 24-25, Padang, Indonesia
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DOI 10.4108/eai.24-1-2018.2292407

Figure 1. Rice field that was attacked by udbatta disease in west Sumatera

Pathogenic Infected rice strands cause the flower to glue so one on the panicle axis and filled with the spores causing the panicle to become hard and no seed can be formed. This results in a yield loss of up to 100% of the infected panicle. In some cases, this disease can be known before the panicles come out with the shrinking of the white-colored flags appearing on the leaf midrib [1].

Although the disease can cause high yield loss, it is rarely controlled. Control that can be done is by seed treatment by soaking with warm water at temperature 54oC for 10 minutes, or by heating directly. Conventionally, this disease is controlled by seed treatment using fungicide such as Agrosan, because soaking the seeds with hot water is difficult to be adopted by farmers, hence the use of a fungicide is a control that many done [1].

Microorganisms are developed to control Udbatta diseases by using *Serratia* sp, it indicates positive potential in controlling the pest and blight. *Serratia marcescens* which is called red bacteria in Indonesia is a gran-negative entomopathogenic bacterium from the Enterobacteriaceae family. *Serratia* sp can be easily found in the natural surrounding especially in soil, water, and infected insect. [2] characterized and identified the isolated *Serratia* sp. which was taken from the soil and infected insect, they found 7 strains of Serratia which were then classified into 2 species; they are *S. plymuthica* and *S. functicola*

S. proteamaculans subs. Quinovorans has a straight cell, negative gran, it doesn't form spore, and it budges with peritrichus facultative anaerobes which is then kept on incubation for 24 hours in 30^0C temperature. Its colony diameter is 1-3mm with a round and glisten shape that is opaque. Its biochemistry characteristic: it can live on plants from various carbon sources such as glucose, sucrose, selubiosa, and malate. Strain Serratia produces chitinase, gelatinase, and lysine; its colony is shaped like convex that contains red pigment. The pigment from *S. marcescens* is a secondary metabolite which is known as prodigiosin from tetrapyrrole family that contains 4-methoxy-2,2-bipyrolle [3].

Prodigiosin with both economic and commercial values possess multifunction nature as an antibacterial, antifungal, antiprotozoal [4] cytotoxic [5], anti-tumor [6], as medicine, ant malarial, ant diabetic, antioxidant, non-steroidal anti-inflammatory and as cloth dye for wool and silk [7] .

People in West Sumatra are not familiar with *Serratia* sp, it is the reason why collecting the information regarding the indigenous *Serratia* sp in West Sumatra is need.

Petersen & Tisa [8] estated that environment diversity in tropical areas will give a direct effect on the population of insect and insect pathogenic bacteria. [9] argued that the increasing activities in finding new isolates from insect pathogenic bacteria will improve the invention of new isolates with high insecticide effectiveness as the source of genetic supply. [10]

The purposes of this research are to know the ability of *S. marcecens* indigenoes as biological controlling agents of Udbatta diseases.

2 The Material and Method

The investigation was conducted in West Sumatra in 4 districts: the samples were brought to the biology and chemistry laboratory of the Payakumbuh Agriculture Polytechnic and agriculture laboratory of Andalas University.

2.1 Exploration of *Serratia* sp.

Exploration toward Serratia sp. in West Sumatra was conducted in 4 districts including Agam, Solok, Tanah Datar, and Limapuluh Kota. The exploration of Serratia entomopathogenic was done by collecting pests that were infected by Serratia sp. Transect method was applied in collecting the sample, it had 100 meters long which was adapted with the surrounding topography; 4 replications on 5 random diagonal spots were done in supporting the exploration. Each transect had the infected insects with Serratia sp, the insects would suffer the following symptoms: no appetite, diarrhea, the insect tend to avoid the sun, and it changes color to red. The infected insects were then brought to the laboratory to be investigated by using colder boxes; the investigation was conducted based on its regional sample.

2.2 Isolates Insulation

Insulation was done by sterilizing the superficies of the insect's body, it was conducted by plunging the body to disinfectant that contains 95% ethanol for 2 minutes and then with 5.25 % NaClO for 3.5 minutes. Next, the insect's body was kept on 10 % Sodium thiosulfate for 3 and half minutes to dispel the chlorine; it was then sterilized with water for three times. The insect's body was put on sterilized petri dish to dissect the body on its dorsal part, insect's haemolymph was separated by using capillary pipette and kept on 2 ml sterilized water. The solvent was poured to King;S B, it was incubated for 2 days in 25-27 oC temperature. The sole colony which was taken from the incubation would change color to red; it was taken with a sterilized toothpick and grown in Petri cub with a similar medium. Each petri dish contains 10 sole colonies from different isolates of each districts, they were incubated for 24 hours in 25-27 oC temperature. The growing isolates were numbered and transferred to Eppendorf vial (vol. 1 ml) with sterile distilled water; it was kept for the next investigation [11].

2.3 Characterization and Isolates Identification of *Serratia* sp.

Characterization was done by seeing the morphology characteristic (Colonies, cells and flagella) as well as physiological and biochemical. Descriptive method was applied in completing the characterization by taking the information from literature such as [12]–[14].

The colony has some morphology characteristics; the description appears when both cell and flagella are observed for 3 days after the inoculation on Kings^{B} medium. Morphology investigation is possible with Smirnoff dyed technique to observe the shape, color, surface, edge

shape, and its diameter. Morphology characteristics that are investigated are the shape of the cell and the presence of spore. The shape of its flagella is observed by applying Leifson's method [15] that was placed at the slide will test salt reaction. Bacteria that were incubated for 2 days are mixed together [11]. Then the investigation was conducted by focusing on conglomeration on KOH 3%.

2.4 Carbohydrate Metabolism Test (Sucrose, Inositol, and Arabinose, Utilization Citrate)

2 days old bacterium isolates were inoculated on petri dish with 10 ml ammonium salt sugars base with 0,1 ml sucrose, inositol or arabinose solvent. It is incubated for 2 days in room temperature [16] Isolates ability in using the carbohydrate (Sucrose, inositol and arabinose) is shown through the change of color from blue to yellow (positive reaction)

2.5 The Potency of Extra Cellular Enzymes Secretion

The potency of extracellular enzymes production (chitinase, protease, and cellulose) identifies Rizo-bacterial isolates affectivity as antagonist agents. The capability of extracellular enzymes secretion on Rizo-bacterial was evaluated in a qualitative approach, by using culture isolates *S. marcescens* which is grown on TSA medium for 48 hours.

The qualitative method was used to analyze the activation of chitinase by applying an approach by [17] [18][19], using colloidal chitin in creating the medium. It was made from rajungan chitin (20 gram) which was reconstituted with HCl concentrate (400 ml) and incubated for 24 hours in 24^0C temperature. The suspension was then strained by glass wall and filtrate which was added with cold aquades 200 ml and NaOH 12 N until it reached pH 7,0. The filtrate was centrifuged with 7500 rpm for 15 minutes, and refrigerated on 4^0C for 24 hours. The supernatant was discarded while the colloidal chitin pellets were mixed with cold aquades (200 ml) which then re-centrifuged on 7500 rpm for another 15 minutes on 4^oC temperature for 24 hours. The deposition was the colloidal chitin that acts as a chitin medium. In investigating the activity of Serratia sp, the medium needed to contains 0,2 % colloidal chitin, it was sterilized with autoclave medium on a petri dish (O 9 cm). A hole was made on the medium by using cork-borer and then added with 0,2 ml suspension isolates of the tested *Serratia* sp. The medium was tested trough incubation for 4 days on 24^0C temperature to create a halo around the hole of the bacterial suspension.

In analyzing the activation, a qualitative method with Carboxymethyl Cellulose (CMC) substrate was applied. It was made from 4 main substances including A solvent: NaCl, K2HPO4, CMC, and aquades (400 ml). CMC was slowly mixed with aquades, then shaken with 100 rpm for 24 hours on 50 ^{0}C and B solvent : MgSO4 (1.0 M), C solvent : Na2HPO4 , NH4Cl , glycerol, Yeast Ekstrak, gelatin and aquades (100 ml), and D solvent D : 7,5 % (W/V) $CaCl_2$. All substances were sterilized with an autoclave for 20 minutes. A and C solvent was slowly mixed which was then added with 1,0 ml B solvent and 1,0 ml d solvent until it became homogeny. The blend was kept on a petri dish (O 9 cm), perforated with cork-borer, added with 0,2 ml tested Rizo-bacterial isolates suspension, incubated for 3 days in 28^oC temperature. After completing the incubated procedure, the medium was added with congo-red dye around 10 ml or (0,1%), re-incubated for 10 minutes, and then cleaned with NaCl (1,0 M) for 15 minutes until it created a halo around the hole of the bacterial suspension [13]

The analysis of protease activity was also conducted in a qualitative approach by using gelatin substrate, the medium was made from gelatin (4 g) reconstituted with 50 ml sterilized

aquades which was then sterilized with an autoclave. The tested medium was placed on a petri dish (O 9 cm), it was then perforated with cork-borer and then added with 0,2 ml suspension isolates of the tested *Serratia* sp. it was incubated for 3 days in incubation room with 28^0C temperature. After the incubation procedure complete, it was soaked with a saturated solution of ammonium sulfate (5 ml) until it created a halo around the hole of bacterial suspensions [12].

2.6 Identification toward *Serratia* sp. Isolates

The test was conducted in the agriculture laboratory at Andalas University, Padang; while the DNA tested was done by sending the samples to Charonpopan Jakarta.

2.7 Rizo Bacterium's Inhibitory toward *E.oryzae* that Cause Udbatta diseases

Inhibitory test of *Serratia* sp toward pathogen fungus *E. oryzae* that caused Udbatta diseases on invitro was done by selecting the isolates that act as bio-control agent. The fungus was classified as a seed-borne pathogen that infects the rice plant during the seeding or sowing. The medium section of 0,5 cm solid PDA with pathogenic fungal hyphae was used as both inoculums and inoculated on a petri dish that filled with fresh PDA. The strip of inoculums was put 3cm away from the edge of the cup, the cultur was incubated in room temperature around 26-28^0C for 42 hours. The tested *Serratia* sp. isolates were scratched lengthwise with 3cm away from the edge of the cup against the growing pathogen. The testing was done for 3 times, while the observation needed to be done every day to record the growth of pathogen and Rizo bacterium's inhibitory by applying the following formula:

$$DH = \frac{R1 - R2}{R1} \times 100\% \quad \text{.....(1)}$$

Explanation
R1 = the range between pathogen growth to the edge of petri dish
R2 = the range between pathogen growth toward Rizo-bachterium

The inhibitory data were taken from fad analysis by applying statistic program SAS, while the difference in inhibitory median between tested treatment and multiple range test was conducted with Duncan with 5% real extent

2.8 Investigation Procedure

The bred bacteria that were 3 days old were centrifuged at 3500 rpm for 15 minutes. The supernatant was disposed of while the pellets were suspended with sterilized aquades and then re-centrifuged with the same procedure. The supernatant was disposed of while the pellets were suspended with sterilized aquades on dilution 10.

3 Result And Discussion

3.1 Result

3.1.1 The Exploration toward *Serratia* sp.

The exploration was done in 4 districts including 1) Payakumbuh, 2) Tanah Datar 3). Solok 4) Agam. The exploration data shows that Serratia sp. can only be found in 2 districts, they are

Payakumbuh and Solok. While both Tanah Datar and Agam are recorded to have no Serratia sp bacterium, even after the re-exploration.

3.1.2 Isolation and Purification of *Serratia* sp. from Host Insect

Serratia sp. that are taken from Payakumbuh and Solok were isolated and purified on King b medium, the result is described on the following image

a  b 

Figure. 2. Picture a. *Serratia* sp. from Payakumbuh, Picture b *Serratia* sp. from Solok

The growth of *Serratia* sp. colony which is taken from Payakumbuh has brownish red color when it is put on medium, while *Serratia* sp. from Solok has more bright red color (picture 2a and 2b)

3.1.3 Physiology and Biochemistry Characteristic

Physiology and biochemistry characteristics on both *Serratia* sp isolates are presented on the following table.

Table1. Physiology and biochemistry characteristic on indigenous *Serratia* sp.

Physiology and biochemistry characteristic	**Observation result**
Gram reaction	negatif (-)
Cell shape	basil, without flagela and spora
Arabinosa	Positif (+)
Sukrosa	Positif (+)
Manitol	Positif (+)
Utilization citric	Positif (+)

3.1.4 Identification of *Serratia* sp.

Identification toward Payakumbuh *Serratia* sp.

After sensitivity classification toward both biologist plate and sequences rRNA, it is found that indigenous *Serratia* sp from Payakumbuh is *Serratia marcescens* strain jx1 16S ribosomal RNA gene, partial sequence Length=1372 which is then named as *S. marcecens* PYK (Figure 3)

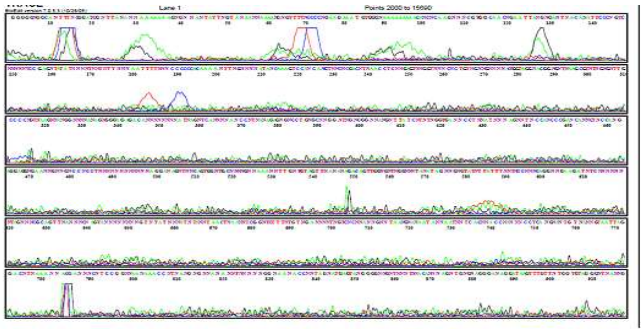

Figure 3. The result of biologist plate and sequences rRNA from PYK

***Serratia* sp. from Solok**

Sensitivity classification toward both biologist plate and sequences rRNA indicates that indigenous *Serratia* sp. from Solok is *Serratia marcescens* strain DAP35 16S ribosomal RNA gene, partial sequence Length=1461 which is then named as *Serratia marcecens* SLK (Figure 4)

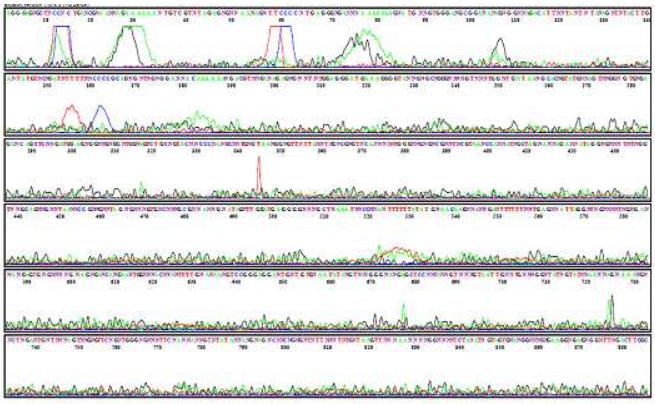

Figure 4. The result of biologist plate and sequences rRNA from SLK

The description of excretion of extracellular enzymes on indigenous *S. marcecens* is shown in Table 2

Table 2. *S marcecens* PYK and *S.marcecens* SLK aptitude in excreting extracellular enzymes

Types of extracellular enzymes	Observation analysis toward both indegenus *S. marcecens*
Protease production	+
Chitinase production	+
Cellulase production	+

Both of the tested *S. marcecens* have antagonist agent characteristics in controlling the blight, they possess extracellular enzyme activity including chitins, protease dan cellulose (table 2).

3.1.5 Inhibitory Capability of Rizo-Bachterium toward Pathogen Fungus

The result of inhibitory tested on S. marcecens toward *Ephelis Oryzae* that caused invitro Udbatta diseases is shown in table 3

Table 3. The inhibitory capability of both *S.* marcescens indegenoes toward *E. Oryzae* in invitro infection

Types of ryzobacterium	Ryzo-bacterium inhibitory capability (%)
A1 *S. marcescen* Slk	58,87 a
A2: *S. marcecens* Pyk	62,2 a

Numbers are followed by small letters; it shows no significant difference in DNMRT 5%

3.2 Discussion

The exploration indicates that *S. marcecens* indigenoes can be found in natural surrounding especially in Payakumbuh and Solok, but it shows no respond in Agam and Tanah Datar district even after re-exploration. It occurs as the result of the destruction of microorganisms which is caused by the application of chemical pesticides on plants. It indicates that the surveyed areas are still using chemical pesticide to bring up the vegetables.

S.marcecens indigenoes that are found in Payakumbuh and Solok districts consist of the straight cell, both of them has negative gram without spore and flagella. The characteristics of their colony are round shape, slick and shiny, and opaque. Its biochemistry characteristics indicate that the bacterium can grow on various carbon sources such as cucrose, arabinose, mannitol, and utilization citric.

The analysis of sensitivity classification toward biologist plate and sequens rRNA indicates that *Serratia* sp that are taken from Payakumbuh is *Serratia marcescens* strain jx1 16S ribosomal RNA gene, partial sequence Length=1372 which is then named as *S. marcecens* PYK. While the indigenous *Serratia* sp. from Solok is *Serratia marcescens* strain DAP35 16S ribosomal RNA gene, partial sequence Length=1461which is then named as *Serratia marcecens* SLK. Both of them are hardly distinguished from their physiology characteristics, but it is possible to identify the difference trough DNA test on their biologist plate. The differences lie in the number of the steamer and the range of DNA fragment migration.

Investigation toward rizo-bachterium physiology characters indicates that both *S. marcecens* indigenoes have chitinase, protease and cellulose activities as they are shown in table 1

Chitinase is basically used to control pathogen fungus and pest, its body cover contains chitin that can be degraded by the enzyme (Wahyuni Suhartono, Khaeruni, Purnomo, Asranudin, 2016) [21], [17]. Nowdays, chitins are used as biologist control on organism with chitin such as fungus and insect. It is used in both pharmaceutical and industrial as fungal biological control by applying microorganism's kinolitik, it can also produce chitinase with lysis of fungal cells capability. [17][18] stated that *S.marcecens* has been used as a pathogen fungus on controller *Sclerotium rolfsii.*[22]

Protease which is also named as peptidase or proteinase is hidrolase enzyme that splits the protein into simple molecules such as short oligopeptides or amino acid by applying hydrolysis

reaction on peptide bond. Protease is needed by the entire organism, as the result of its essential characteristic of protein metabolism.

Cellulase is produced by a microorganism that supports the bioconversion process of organic waste, single-cell protein, fodder, ethanol and others; it also has the ability to change the cellulose to glucose. cellulase can hydrolyze β(1-4) on cellulose to support perfect enzymatic hydrolysis.

Cellulose enzymes can be found in plants, insects, and microorganisms. Even though most of the microorganisms have the capability to degrading the cellulose, but only some of them that can produce a significant amount of cellulase to hydrolyze crystalline cellulose trough invitro [6].

The inhibitory test toward pathogen that cause Udbatta disease indicates that *S. marcecens* indigenoes is classified as bio-control bacterium agent to restrain the spread of Udbatta diseases and to suppress the growth of *S. marcecens* indigenoes which cause the disease by 58,8 % to 62,2%. Various types of rizo bachterium produce secondary metabolites as antibiotics, siderophores, hydrogen cyanide as well as hydrolytic enzymes such as chitinase enzymes, protease, and cellulase. These compounds act as antimicrobial to suppress the growth of pathogens [6].

Some of rizo-bachterium produce hydrolytic enzymes such as chitinase, protease and cellulase to support bacterium antagonism mechanism toward pathogen [23]. The secreted extracellular enzymes by *S.marcecens* are able to suck the pathogen, while the secreted chitinase and cellulase (table 1) can degrade the cell wall of the pathogen to suppress the growth of the pathogen.

Serratia genus is classified as Enterobacteriaceae without spore, it has low pathogenicity when it is spread on insect digestive organs, but its pathogenicity will become higher when it is deployed on hemocoel [24] . It fills attack the hemocoel soon after the infection.

4 Conclusions

Two types of Serratia are found, one in Payakumbuh and one from Solok. Both types of *S. marcecens* indigenoes share a similarity in physiology and biochemistry characteristics. It has a negative gran, straight cell without spore and flagella; the colonies have a round shape and opaque. It can make use of several carbon source including arabinose, sucrose and utilisation citric

Serratia that is taken from Payakumbuh is *Serratia marcescens* indigenoes strain jx1 16S ribosomal RNA gene, partial sequence lengLength=1372, while Solok has Serratia *marcescens* indigenoes strain DAP35 16S ribosomal RNA gene, partial sequence Length=1461

Both types of *S.marcecens* indigenoes that are taken from Payakumbuh and Solok have ability to produce chitinase, cellulase and protease enzymes.

Both types of *S.marcecens* indigenoes that are taken from Payakumbuh and Solok can suppress the growth of *E.Oryzae* fungus that cause Udbatta diseases from 58.8% to 62,2 %, the result is taken from in-vitro test.

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