Potential of endophytic bacteria from corn as biopesticide: a biological control of insect pests

Hasmiandy Hamid, Novri Nelly, My Syahrawati, Zurai Resti, and Jenny Arizona

ABSTRACT

Biocontrol agents can be derived from endophytes or healthy plant tissues. The entomopathogenic bacterial group derived from corn plant tissue has the potential as a biopesticide. The study aimed to obtaine entomopathogenic bacterial isolates, which can kill test insects and be safe for other living organisms. Roots of healthy corn plants collected as a source of isolates. Bacterial isolates were tested for gram test, virulence on *Mirabilis jalapa* flower plants, and entomopathogenic test for *Spodoptera litura*. The results of the study found 29 isolates, which were entomopathogenic with mortality of 35.71 - 100%. Only two isolates showed adverse reactions based on the results of the hypersensitive and hemolysis test namely isolates P1.7 and P2.1. This indicates that there were two isolates from corn root tissue, which were entomopathogenic and safe for living organisms.

Keywords: bacteria endophyte, biological control, biopesticide, entomopathogen.

MS History: 30.03.2019(Received)-25.05.2019(Revised)- 30.05.2019(Accepted).

Citation: Hasmiandy Hamid, Novri Nelly, My Syahrawati, Zurai Resti, and Jenny Arizona. 2019. Potential of endophytic bacteria from corn as biopesticide: a biological control of insects pest. *Journal of Biopesticides,* **12**(1): 40-45.

INTRODUCTION

Farmers use several synthetic pesticides to control pest populations in the field. The use of pesticides continuously gives ecological effect such as presence in the environment and can cause pest rebound and secondary pest outbreaks, and also pest resistance (Zacharia, 2011).

Researches on biocontrol agents to control insect pests are still being pursued. One of the areas is the use of entomopathogenic microorganisms derived from plant tissue is called endophytic microorganisms. Efforts to use entomopathogenic bacterium as an insecticide are still being developed. According to Salaki et al. (2013), the results of testing the power to kill 21 isolates of *B. thuringiensis* against pests *C. binotalis*, *P. xylostella* and *S. litura* which can cause larval mortality of more than 50% from 15 isolates, 20 isolates, and 12 isolates, respectively. *B. thuringiensis* may cause mortality is high enough on the larvae of *C. binotalis*, *P. xylostella*, and *S. litura*. These bacteria can be used as environmentally friendly biopesticide candidates.

Utilization of endophytic bacteria on the corn roots is one of the biological control efforts that are expected to increase the productivity of maize and reduce the population of pests of corn. Information about the diversity of endophytic bacteria from corn root tissue as a potential entomopathogenic has not been widely reported. The study aimed to obtain entomopathogenic bacterial isolates which can kill test insects and be safe for other living things.

MATERIALS AND METHODS

The studies were conducted at Laboratories of Biocontrol. Department of Plant Protection, Faculty of Agriculture, Andalas University. Sampling was done at Nagari Ophir Pasaman Barat. The samples used were corn roots on corn polyculture land with oil palm.

Isolation of endophytic bacteria

The roots of corn plants were cut and washed with running water, then wind dried. One gram

root was taken and was sterilized using 70% alcohol for one minute, and then in NaOCl 2% for 2 minutes. There were rewashed with three times distilled water. Before grinding, 1 cm of corn root was kept on a petri dish containing media NA (as a control). If bacteria grow on the control, it was necessary to identify the bacteria that will grow as endophytic bacteria using a bacterial marker in controls (Munif and Hipi, 2011). The roots crushed with a mortar and added 9 ml of sterile distilled water. A solution of 1 ml was taken and put into a test tube which was filled aquadest 9 ml and homogenized by vortex for 2 minutes. Then made serial dilution up to a strength of 10^{-5}. Further dilution of 1 ml series 10^{-4} and 10^{-5} inserted into a test tube containing the media NA thawed and homogenized using a vortex. Then spread evenly in a petri dish and incubated at room temperature for 2 x 24 hours (2 days). Furthermore, the isolation of bacteria was done with serial dilution method (dilution) and for morphological observation

Entomopathogens test (mortality of larvae)

Endophytic bacteria isolates were used to test the ability as entomopathogenic. Long bean leavesas the larvae feed soaked with bacterial suspension for 10 minutes and inverted using pingset, then wind dried. These leaves were then put in a petri dish containing 15 larvae of *S. litura*. The leaves soaked with distilled water served as the control. The treatment of bacterial suspension was done over two days (two times instead of the feed). On the third day onwards, leaves for feed were washed to remove the other contaminating.

Observations were recorded by counting the number of dead larvae from the first day after the application of Endophytic Bacteria, until the pupa was formed. Mortality was calculated using the formula:

$$M = \frac{n}{N} X\ 100\%$$

Where

M: larval mortality (%);

n: number of larvae die;

N: the number of larvae used

The percentages of mortality obtained were then corrected using Abbot's formula:

$$P = \frac{Po - Pc}{100 - Pc} X\ 100\%$$

where

P: Percentage of test insects was dead after correction (%);

Po: The percentage of test insects was dead at the treatment;

Pc: The percentage of dead insects in the control test

Determination of the time required by entomopathogenic bacteria to kill 50% of the test insects was determined by calculating the LT_{50} value using probit analysis.

Characterization of endophytic bacteria

Characterization of endophytic bacteria was done by gram, hypersensitive, and hemolysis test.

Test gram

Gram test was performed to determine the nature of the bacteria, whether gram positive or gram negative. One drop of 3% KOH solution was placed on a glass slide with a pipette, and then taken one ose-old pure culture of endophytic bacteria 2x24 hours (2 days) and mixed with a solution of 3% KOH. If bacteria was clotting or slimy, then these bacteria were identified as gram-negative, and if the case was not so, then it was identified as gram-positive clumping (Klement *et al.*, 1990).

Hypersensitive Test (HT)

Hypersensitive Test (HT) was performed using methods of Klement *et al.* (1990). This test aimed to determine the nature of the classified pathogenic bacteria. This test uses four O'clock flower leaf (*Mirabilis jalapa*). Bacterial isolates that have been stored in microtube rejuvenated with the scratch method on NA medium and incubated 2x24 hours. Bacterial isolates were grown and scrapped off using a needle ose and added 9 mL aquadest. The bacterial suspension is transferred to the test tube using a micropipette and homogenized with vortex. The suspension is compared to McFarland's turbidity on a scale of eight. The level of turbidity at bacterial population density is 108sel / mL (Klement *et al.*, 1990).Furthermore, the bacterial suspension is infiltrated inter cellularly on the leaf surface tissue to saturate. Infiltrated leaves

are covered with plastic and observed for up to 48 hrs.

Hemolysis test

The hemolysis test was used to identify isolates that have potential pathogens in humans and animal. Isolates were planted on blood agar medium. After incubation for 24 hours at room temperature. The clear zone around the colony indicated that it is positive hemolysis.

RESULTS

Test entomopathogenic (mortality of larvae)

Results of screening of endophytic bacteria isolated from the roots of the corn plant origin from polyculture cultivation of corn-palm which was later shortened to label P (polyculture), obtained 29 isolates of endophytic bacteria and all potentially as entomopathogenic (Table 1).

Treatment of twenty-nine isolates of endophytic bacteria showed a significantly varying effect on mortality of larvae of *S. litura* when analyzed with LSD at 5% significance level. Twenty-nine isolates of endophytic bacteria could influence the mortality of larvae of *S. litura* and all potentially as entomopathogenic. Table 1 shows that the death of larvae of *S. litura* is lowest on the controls after being inserted into ABBOT formula is 0.00%, the highest mortality of larvae found up to 100% in the treatment of P 3.2, P 5.2, P 5.3. The effect on mortality of *S. litura* larvae is presented in **Fig.** 1.

Isolation of Endophytic Bacteria

Twenty-nine isolates of endophytic bacteria were isolated from corn root tissue, and the character of the endophytic bacteria is shown in Table 2. The isolates had morphological and physiological diverse characters. Endophytic bacteria colony color is predominantly white, while others were red, yellow and beige. Colonies from dominant endophytic bacteria were irregular, while others were circular, and filamentous. The further elevation of endophytic bacteria colony was predominantly flat, while others raised, and convex. The widest colony had 0.9 cm diameter. Endophytic bacteria colony periphery was predominantly flat, while others grooved, corrugated and the like yarn. Physiological characteristics of endophytic bacteria showed G^{+}, while G^{--}.

Hypersensitivity (HR) test results obtained 26 negative isolates, and 3 positive isolates. This means that 26 HR isolates are positive; this is safe for plants because it does not cause symptoms of necrosis. There other isolates are negative, which causes symptoms of necrosis (P 1.6, P 2.6, and P 3.6).Hemolysis test results obtained 26 positive isolates and 3 negative isolates (P 1.6, P 1.7, and P 2.1), meaning that of the 29 isolates obtained there were only 3 isolates that were safe for humans and animals. Among the 3 isolates that are safe for humans, one isolate is negative for plants. So of the 29 isolates found only 2 isolates were safe for animals, humans and plants.

Table 1. Mortality of larvae of *S. litura* 14 days after being treated with 29 isolates of endophytic bacteria maize roots

No.	Isolate code	Mortality (%)	Effectiveness (%)	LT50 (day)
1	P 3.2	100 a	75.56	5.311
2	P 5.2	100 a	75.56	6.820
3	P 5.3	100 a	75.56	6.977
4	P 3.5	97.437 ab	75.01	5.974
5	P 1.3	94.103 abc	74.42	5,917
6	P 2.3	93.940 abc	74.42	5,040
7	P 3.6	93.333 abc	74.42	6.788
8	P 3.7	92.307 abc	73.81	5,853
9	P 5.5	91.377 abc	73.81	6.616
10	P 3.3	91.273 abc	73.81	6.408
11	P 3.8	91.073 abc	73.81	6,800
12	P 1.2	90.770 abc	73.81	4.771
13	P 4.1	85.783 abcd	72.51	6.396
14	P 1.7	79.580 bcde	71.06	7.228
15	P 2.1	79.580 bcde	71.06	6.315
16	P 3.4	79.420 bcde	71.06	2,257
17	P 1.5	77.483 cde	70.27	11.011
18	P 1.4	76.713 cde	70.27	7.188
19	P 4.3	72.450 de	69.45	7.867
20	P 5.1	69.580def	68.58	5.502
21	P 1.1	67.320efh	67.65	7.867
22	P 2.6	65.060efgh	66.67	9.917
23	P 2.2	64.757efgh	66.67	4.085
24	P 2.4	64.150	66.67	10.935
25	P 1.6	52.393 fghi	62.07	9.248
26	P 2.5	52.03 fghi	60.72	13,750
27	P 4.2	50.837 ghi	60.72	9.248
28	P 3.1	48.997hi	60.72	17.331
29	P 5.4	35.710 i	52.18	11.181
30	Control	0.000 j	0,00	-
CV = 14.86				Average = 7.606

The numbers followed by the same lowercase letters in the same column by LSD no significant effect on the level of 5%

Table 2. Characteristics of morphological gram, hypersensitive and test hemolysis endophytic bacteria isolated from corn root tissue

No.	Isolate code	Total Population (104)	Morphology colonies					Gram test	Hypersensitivity test (HT)	Hemolysis test
			Color	The colony diameter (cm)	Form	Elevation	Margin			
1	P 1.1	50	Red	0.7	Irregular	Flat	Lobate	-	-	+
2	P 1.2	70	White	0.5	Irregular	Flat	Lobate	-	-	+
3	P 1.3	10	White	0.6	Filamentous	Flat	Entire	-	-	+
4	P 1.4	90	Red	0.3	Irregular	Flat	Entire	-	-	+
5	P 1.5	70	White	0.3	Irregular	Raised	Entire	-	-	+
6	P 1.6	5	Red	0.7	Irregular	Flat	Undulate	-	+	-
7	P 1.7	25	Red	0.4	Irregular	Flat	Lobate	-	-	-
8	P 2.1	10	Red	0.3	Irregular	Raised	Entire	-	-	-
9	P 2.2	10	White	0.5	Irregular	Flat	Lobate	-	-	+
10	P 2.3	10	White	0.8	Irregular	Flat	Lobate	+	-	+
11	P 2.4	1	White	0.7	Irregular	Raised	Lobate	-	-	+
12	P 2.5	2	White	0.5	Circular	Raised	Entire	+	-	+
13	P 2.6	1	White	0.5	Irregular	Flat	Lobate	-	+	+
14	P 3.1	5	White	0.5	Circular	Flat	Rhizoid	+	-	+
15	P 3.2	1	Red	0.3	Irregular	Flat	Entire	-	+	+
16	P 3.3	3	Red	0.6	Irregular	Raised	Entire	+	-	+
17	P 3.4	1	Yellow	0.7	Circular	Raised	Entire	+	-	+
18	P 3.5	1	White	0.7	Circular	Flat	Entire	+	-	+
19	P 3.6	60	White	0.7	Circular	Flat	Undulate	+	-	+
20	P 3.7	10	White	0.3	Circular	Convex	Entire	+	-	+
21	P 3.8	40	White	0.6	Irregular	Flat	Undulate	+	-	+
22	P 4.1	10	Beige	0.7	Irregular	Flat	Undulate	+	-	+
23	P 4.2	10	White	0.5	Circular	Raised	Entire	+	-	+
24	P 4.3	10	White	0.9	Irregular	Flat	Undulate	-	-	+
25	P 5.1	1	White	0.9	Irregular	Flat	Undulate	+	-	+
26	P 5.2	1	White	0.6	Circular	Flat	Entire	+	-	+
27	P 5.3	3	White	0.6	Circular	Raised	Entire	+	-	+
28	P 5.4	10	Yellow	0.4	Circular	Flat	Entire	+	-	+
29	P 5.5	10	Yellow	0.4	Irregular	Flat	Entire	+	-	+

Figure 1. The normal and infected larvae of *S. litura* with endophytic bacteria, A. normal larvae, and B. infected larvae (1. shriveled, 2. blackened).

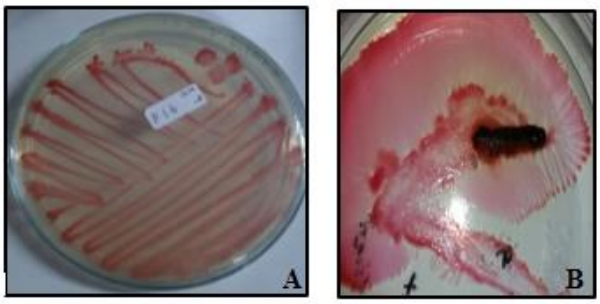

Figure 2. Testing Koch's postulates to prove the death of the larvae according to the infecting bacteria (A. Pure P 1.6 isolate before it was applied to the larvae, B. Koch's postulates for P 1.6 isolate)

DISCUSSION

The feeding activity began to diminish from *S. litura* larvae infected by endophyte movements became slower and less sensitive to the touch. Trizelia (2001) and Tampubolon *et al.* (2013) stated that bacterial symptom attack on insect pests preceded by signs of inactivity, decreased appetite, weakness, the insect had diarrhea and discharge from several parts of the body, eventually, suffocate insects. The dead insects, insect look dark brown or black and insect body becomes dry and shriveled.

Symptoms in infected larvae will release liquid stool (diarrhea). From the mouth comes a yellowish green liquid to dark green (vomiting). Infecting bacteria are proven by the Koch postulate (Figure 2). Test Koch postulates from infected larvae, fluid released by larvae cultured in NA media. The results show bacterial morphology similar to bacteria that infect larvae after incubation for 2x24 hrs.

It can be seen the variation in the value of LT 50 of the root endophytic bacteria of corn against *S. litura* by probit analysis (Table 2). The analysis showed that the average time needed to kill 50% of the test insects was 7.606 days. The infection with bacterial isolates P 3.4 was the best result with a value of LT_{50} of 2,257 days. P 1.7 isolate has a lower LT_{50} value compared to P 3.4 isolate which is 7,228 days. P 3.1 isolate has the highest LT_{50} value when compared with other treatments, which is 17,331 days.

Infected larvae do not survive because of endophytic bacteria also change color and shape. The bodies of dead larvae are dark brown to black, shriveled. Larvae becomes soft and emit a foul odor, then the larvae dry out and shrink with the integument intact. Besides the larva becomes longer in 28-30 days, when compared to controls between 12-14 days. At the time of larval death, the body is dark brown to black, and the shape is curved. Furthermore, the larvae become soft and foul smell, then the larvae dries and shrinks with the integument intact. Besides, the larvae were treated the stadiumwere longer than controls.Vajri (2014) reported the death of larvae of *S. litura* was treated with the bacteria produced crystal protein isolates had been there since the first day of observation. This is presumably because the protein crystals that have been eaten by *S. litura* through direct feed react in the digestive tract. Hadi *et al*. (2009) found that when the crystal protein is eaten by insects that are sensitive, there will be paralysis resulting in the death of the host. The crystals will dissolve bacteria in the digestive tract and then bacteria release toxins that can kill insects. Harjaka et al. (2006) reported that the sensitivity of insects to the order Lepidoptera dekstruksin varied and had higher sensitivity. Protein crystals ingested by the insect will dissolve in the alkaline environment of the intestines of insects. On the target insect, the protein to be activated by the protein-digesting enzymes of insects. Activated proteins that would stick to the protein receptor located on the surface of intestinal epithelial cells. The attachment resulted in the formation of pores or holes in

cells that undergo cell lysis. In the end, insects will experience indigestion and death (Tampubolon *et al.,* 2013).

The results of the study found 29 isolates as entomopathogenic with mortality of 35.71 - 100%. Only two isolates showed negative reactions based on the results of the hypersensitive and hemolysis test, namely P1.7 and P 2.1. This suggests that there were two isolates from corn root tissue, which were entomopathogenic and safe for living organisms, including humans.

ACKNOWLEDGMENTS

We gratitude and appreciation to Rector of Universitas Andalas who funded this study with Research Professor Cluster: No. 04/UN.16.17/ PP.RGB2/ LPPM/ 2018 on behalf of corresponding author (Novri Nelly).

REFERENCES

Hadi, M., Udi, T. and Rully, R., 2009. Biologi Insekta Entomologi, Graha Ilmu: Yogyakarta.

Harjaka, T., Harsojo, A. and Mahnib, E., 2006. Infection of Fungus *Metarrhiziumanisopliae* in Cabbage Leaf Caterpillar *Plutellaxylostella*. *National Seminar Proceeding of Agricultural Research Results*. 206-211

Khairani, G. 2010. Isolation and Testing Capabilities Hormone Producing Endophytic Bacteria IAA (Indole Acetic Acid) From the Roots of Maize (*Zea mays L*). [Thesis]. Medan. FMIPA. The University of North Sumatra.

Klement, Z., Rudolph, K., and Sands, D.C. 1990. Inoculation of Plant Tissue. Methods in Phytobacteriology. Akademiae Kiado: Budapest.

Munif, A. and Hipi, A. 2011. Potential Endophytic Bacteria and Rhizosphere in Improving Growth of Corn. Proceeding of Serealia National Seminar. Maros. **Pp**:1-8

Salaki C. L., Tarore, D., Manengkey, G. 2013. Utilization Prospects of Biopesticides of Entomopathogenic Bacteria from Local Isolates as Biological Control Agent on Vegetable Insect Pests. *Eugenia,* **19**(1): 1-7

Strobel, G. A., Daisy, B., 2003. Bioprospecting for Microbial Endophytes and Their Natural Products. *Microbiology and Molecular Biology Reviews,* **67**(4): 491–502.

Tampubolon, D. Y., Pangestiningsih, Y., Zahara, F. and Manik, F. 2013. Pathogenicity test *Bacillus thuringiensis* And *Metarhizium anisopliae* against Mortality *Spodoptera litura* fabr (Lepidoptera: Noctuidae) In The Laboratory. *Online Journal of Agroecotechnology*, **1**(3): 783-793

El-Tarabily, K. A., Nassar, A. H., Hardy, G. E. St. J. and Sivasithamparam, K.,2003. A fish emulsion as a food base for Rhizobacteria Promoting The growth of radish (*Raphanus sativus*L. var. *sativus*) in a Sandy Soil. *Plant andSoil*, **252**, 397–411

Vajri, I. 2014. Producing Bacteria toxicity Some Crystal Protein Isolates From Rhizosphere Several Types of Plant Pests *Spodoptera litura* F. (Lepidoptera: Noctuidae). [Skripsi]. Padang. Faculty of Agriculture, University of Andalas.

Zacharia, J. T. 2011. Ecological Effects of Pesticides. In: Pesticides in the Modern World - Risks and Benefits. Stoytcheva M. (Ed). InTech: Rijeka. **Pp** 129-142

Hasmiandy Hamid, Novri Nelly*, My Syahrawati, Zurai Resti and Jenny Arizona

Department Plant Protection, Agriculture Faculty, Universitas Andalas

Kampus Unand Limau Manis Padang. West Sumatera, Indonesia. Post Code 25163

*Correspondence author

Phone: +62751 72701 Faks. +62751 72702.

E-mail: novrinelly@agr.unand.ac.id