Genetic Diversity of Indonesian Bacterial Leaf Blight Isolate (Xanthomonas oryzae pv. oryzae) Core Collection based on the VNTR and avrXa7 Molecular Markers

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Genetic Diversity of Indonesian Bacterial Leaf Blight Isolate (Xanthomonas oryzae pv. oryzae) Core Collection based on the VNTR and avrXa7 Molecular Markers

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Abstract

Bacterial leaf blight (BLB) is one of the major diseases in rice caused by Xanthomonas oryzae pv. oryzae. This study aimed to identify and analyze the genetic diversity of 18 BLB isolates that consist of 7 races and 11 haplotypes from various locations in Indonesia. The genetic diversity analysis was conducted on the basis of the VNTR (Variable Number of Tandem Repeat) markers and the avrXa7 gene marker. The banding pattern of the amplification product was made into binary data as input for the construction of a dendogram. Based on the dendogram, three X. oryzae pv. oryzae genotype groups with different virulence levels were formed. The VII (IXO80_021) race of X. oryzae pv. oryzae genotype group I and the VIII-A (IXO 80_024) race of genotype group II were avirulent, whereas the races and haplotypes of genotype group III were virulent.

Keywords: bacterial leaf blight, molecular marker, Xanthomonas oryzae pv. oryzae

Introduction

Bacterial leaf blight (BLB) is one of the major diseases in rice caused by Xanthomonas oryzae pv. oryzae [1]. The disease can cause yield losses up to 80% of total rice production [2,3]. According to Naveed et al. [4], the BLB disease causes 90% reduction in grain weight. The BLB disease has a widespread distribution and is primarily destructive in the lowland rice area [5]. The BLB incursion reaches 14,178 ha [6] of the 7.4 million ha of rice areas in Indonesia [7]. Currently, the identification of X. oryzae pv. oryzae is conducted on the basis of the virulence level in the different varieties of rice. Therefore, many races or pathotypes have been determined in these pathogenic bacteria.

A total of 12 pathotypes of X. oryzae pv. oryzae have been identified in Indonesia [8]. Three of them, namely, pathotype groups III, IV, and VIII, are dominant in several rice production areas in Java. Pathotype groups I and II, initially found in Japan, were also found in Indonesia in 1991–1992. According to Yamamoto et al. [9], X. oryzae pv. oryzae pathotype group III, which has a low virulence level, is distributed in South Sulawesi, South Kalimantan, West Java, Central Java, East Java, Bali, and North Sumatera. Conversely, X. oryzae pv.
oryzae pathotype group IV, which has a high virulence level, is spread in South Sulawesi, West Java, Central Java, East Java, Bali, and North Sumatera [10]. Pathotype group V is found only in Bali, while pathotype groups VI and VII are found in West Java [11]. Pathotype group VIII has a medium virulence level and is found in South Kalimantan, West Java, and North Sumatera [10]. Some pathotype groups have shown to have high pathogenicity and virulence level in different rice varieties. Therefore, the development of a plant disease management strategy for X. oryzae pv. oryzae is required to reduce yield losses and to prevent the development of the BLB disease epidemic.

The identification of X. oryzae pv. oryzae pathogen isolates is conducted by utilizing several molecular markers, including the markers from the virulence gene [13] and multiple loci variable number of tandem repeat (VNTR) [14]. X. oryzae pv. oryzae is a plant pathogenic bacterium that belongs to phylum Proteobacteria and is characterized by having the secretion system of the protein virulence effector (PVE) type III. Generally, the PVE of Xanthomonas spp. is coded by the AvrBs3/PthA gene group. Several molecular markers of this gene have been developed and used in the BLB pathogen virulence analysis [15]. The pathotype analysis using the VNTR marker has also been utilized in pathotype analysis as part of an epidemiological observation on the pathogen [16]. By utilizing the VNTR marker, several loci associated with the temporary and spatial diversity of the pathogen population can be identified and characterized [14]. In this study, the genetic diversity of X. oryzae pv. oryzae core collection isolated from various locations in Indonesia was determined using the VNTR markers and the avrxa7 gene marker.

Materials and Methods

Bacterial leaf blight (BLB) isolate. A total 18 BLB isolates consisting of 7 representative isolates from the race or pathotype group and 11 representative isolates from the haplotype group (Table 1) were used in this study. These isolates were included in the BLB core collection of the Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development, Ministry of Agriculture, Bogor. These BLB collections belong to the 1974–1994 collection isolated from several BLB disease endemic locations in Indonesia. All these isolates were grown in the Wakimoto plus Ferrous sulfate or WF medium [17] consisting of 20 g sucrose, 5 g peptone, 0.5 g Ca(NO\textsubscript{3})\textsubscript{2}.4H\textsubscript{2}O, 1.8 g Na\textsubscript{2}HPO\textsubscript{4}.7H\textsubscript{2}O, 0.05 g FeSO\textsubscript{4}.7H\textsubscript{2}O, 18 g bacto agar, and 1 L dH\textsubscript{2}O.

| Race/Haplotype | ID Number   | Isolate Year | Origin Cultivar | Location       |
|----------------|-------------|--------------|-----------------|----------------|
| I              | IXO 92_002  | 1992         | Line            | Muara, Bogor, West Java |
| III-A          | IXO 94_013  | 1994         | Way Seputihi    | Jatisari, Cikampek, West Java |
| III-B          | IXO 94_003  | 1994         | Way Seputihi    | Jatisari, Cikampek, West Java |
| IV B           | IXO 80_004  | 1980         | Local           | Cianjur, West Java |
| VII            | IXO 80_021  | 1980         | Local           | Pusakanegara, Subang, West Java |
| VIII-A         | IXO 80_024  | 1980         | Siyam Halus    | Banjarmasin, Kalimantan |
| VIII-B         | IXO 79_008  | 1979         | IR36            | Pusakanegara, Subang, West Java |
| AVRxa-10-C     | IXO 93_103  | 1993         | Pandanwangi     | Cikondang, Cianjur, West Java |
| AVRxa-10-C     | IXO 93_129  | 1993         | IR-64           | Buronalit, Gianyar, Bali |
| AVRxa-10-C     | IXO 93_229  | 1993         | Cisadane        | Mertoyudan, Magelang, Central Java |
| AVRxa-10-H     | IXO 92_048  | 1992         | IR64            | Jatisari, Subang, West Java |
| AVRxa-10-V     | IXO 93_230  | 1993         | Cisadane        | Harjoibinganung, Sleman, Yogyakarta |
| AVRxa-10-Q     | IXO 74_037_2| 1974         | Padi Tahun (Black) | Benong, Badung, Bali |
| AVRxa-10-A     | IXO 93_066_1| 1993         | IR48            | Sukamandi, Subang, West Java |
| TNX-A.002      | IXO 76_011  | 1976         | KetaN, Local    | Kota Batu, Bogor, West Java |
| TNX-A.001      | IXO 92_093  | 1992         | IR64            | Bojong, Cianjur, West Java |
| TNX-A.003      | IXO 94_035  | 1994         | Way Seputihi    | Jatisari, Cikampek, West Java |
| TNX-A.001      | IXO92_046   | 1992         | IR64            | Jatisari, Subang, West Java |

Note : number 1-7 : races group and 8-18 : haplotypes group.
Table 2. The VNTR and avrxa7 Gene Markers Used in this Study

| VNTR loci | Tandem Repeat Sequence | Primer (5'-3') |
|-----------|------------------------|---------------|
| B01       | TTCCCAA                | GCACAGCAGCCACGGCAA/GCCTGGACGGATGCGAC | |
| B02       | CGCACAG                | AGGCAGGGTTTCACTCGTG/AAACAGGCGTGAC | |
| B03       | TCCCTCGGAA             | GCCCTGTCGTAACGGAC/CCGACACGGGTCGTCG | |
| B04       | CGATGCTC               | CTGTCGCGCAAGATCG/TCGCTCAGACGCGACG | |
| B05       | CGATAC                 | GCAGACGAGATGGGCTTG/CCGACGAGGCGAGG | |
| B06       | AATCCGGG               | GAAATGCGGAAAGCGTCC/TCGGTACGTTCCGAC | |
| B15       | GCAAGT                 | CGCAGGCGTCCGAGCC/TGACGTCGTCGTCG | |
| B16       | GAGATTT                | GCTGACGCGACGAGCC/TGACGTCGTCGTCG | |
| B17       | CTGCTG                 | AGGCAGGCGGACACGGCG/AGGGCGGAGGCGAGG | |
| B18       | CGATTTC                | GCAAGTCGCGGACACGGCG/AGGGCGGAGGCGAGG | |
| B19       | TGCTGTTGC              | GAACACAGGCGGCGGTGCA/GCAGACGCGTTCCG | |
| B20       | TGCTGATT               | GTGTCGCGACGACACGGCG/AGGGCGGAGGCGAGG | |
| B21       | TGATCGGCC              | ACCGAGGCGAATCGCGG/AGGGCGGAGGCGAGG | |
| G01       | GGCGGT                 | CGCAGATCTCGGCAAG/GGCGGACACGGCGAGG | |
| G04       | CTCTCT                 | ACAGGACAGGATCGGCTG/TCGCTGACGTCG | |
| G05       | CGGCCT                 | CAGCAGGCGAAGGAGGCG/AGGGCGGAGGCGAGG | |
| G09       | CGCGAAG                 | GCAAGGCGATCGGCTG/TCGCTGACGTCG | |
| G11       | CCGCCT                 | AGCCCCAACAAATCGGC/GGCGGAGGCGAGGCGAGG | |
| M01       | CGGGCTG                | CGCAGATCTCGGCAAG/GGCGGACACGGCGAGG | |
| M02       | GGGATTTC                | CAGGAGGCAAAGGGCTGAG/GCGATCGGTTGC | |

*Pth* Xo4 (AvrXa7-1)  
*PthXo5 (AvrXa7-3)*  
Avr-a7Sac50 (AvrXa7-4)

DNA extraction. About 2 mL of X. oryzae pv. oryzae from the NB medium was precipitated in Eppendorf tubes and centrifuged at 10,000 rpm for 10 min. The supernatant was discarded, and 250 µL Tris-EDTA (TE) buffer was added to the precipitates composed of bacterial cells and further vortexed until homogeneous. About 50 µL SDS 10% and 5 µL proteinase-K were added. The single colony was further grown in the nutrient broth (NB) medium and shaken in an incubator overnight at 100 rpm–200 rpm for DNA extraction materials.

**Rejuvenation and preparation of isolates.** About 10 µL of the stock isolate preserved in the skimmed milk medium 10% (b/v), which contains 0.05% L-glutamic acid at 0 °C [16], was taken out and sinusuously streaked in the WF petri dish agar medium by using a sterile skewer and incubated at 28-30 °C for two days. The colonies’ characteristics are yellowish and slimy (Figure 1). The single colony was further grown in the nutrient broth (NB) medium and shaken in an incubator overnight at 100 rpm–200 rpm for DNA extraction materials.
added to the homogenous solution, which was then incubated in a water bath at 37 °C for 1 h. About 65 µL NaCl 5M and 80 µL CTAB were further added, followed by dispensing by vortex. The solution was incubated in a water bath at 65 °C for 20 min. After incubation, the solution was diluted with the same volume of chloroform: isomilalcohol (24:1) solution and gently shaken, followed by double centrifugation. The first centrifugation was conducted at 15,000 rpm for 5 min at room temperature and the second centrifugation at 8,000 rpm for 15 min. The cell suspension was gently poured into new Eppendorf tubes, added with cold isopropanol, and then inverted slowly. The whitish DNA was stored in a freezer (-20 °C) for 30 min. The DNA was purified by cold ethanol 70% and then centrifuged for 5 min at 14,000 rpm. The ethanol was discarded, and the DNA pellet was dissolved in 25 µL–50 µL TE [18].

**DNA quality and quantity test.** The quality of bacterial DNA was tested by 1% gel electrophoresis added with GelRed (GelRed™ Biotium). The TAE buffer 0.5x was used as a running buffer. About 2 µL of the DNA pathogen solution from each sample was mixed with 2 µL loading solution or Blue Juice, 2 µL DNA marker (bp), and 3 µL loading solution, and the solution was further homogenized with a micropipette. Each mixture was poured into the agarose gel wells. The electrophoresis apparatus was connected to the remaining electrical voltage at 90 v until the Blue Juice color travels to as far as 85% of the agarose length. The electrophoresis product was examined under ultraviolet (UV) ChemiDoc gel scanning from BioRad. The quantity of DNA was measured by a spectrophotometer at 260 nm and 280 nm based on the Sambrook method [19]. Concentration and purity of the DNA were calculated as follows: DNA concentration = OD260 x 50 µg/mL x the dilution factor. This equation is based on the prediction that OD260= 1, and the DNA concentration is 50 µg/mL. The DNA purity = OD280/OD260. Dilution of the DNA was conducted to reach a 10 ng/µL concentration.

**Polymerase chain reaction (PCR) amplification.** The PCR amplification reaction was conducted in a final volume of 10 µL, and it contained 5 µL KAPA kits (buffer solution, dNTP, and Taq polymerase enzyme), 3 µL DNA pathogen 10 ng/µL, 1 µL primer (forward + reverse) 10 mM, and 1 µL dH2O. All reactions were performed for 35 cycles. Each cycle involved 30 s denaturation at 94 °C, 30 s annealing at 50-67 °C (depending on the primer pairs), and 60 s elongation at 72 °C, with the first step of activation at 5 min at 94 °C and the last extension step at 10 min at 72 °C. The PCR product was separated on 1.2% agarose gel with GelRed and visualized under UV light. At least 30 ng/µL DNA was required for the PCR amplification of the VNTR locus from the panel of 18 *X. oryzae* pv. *oryzae* races and haplotypes [14]. The DNA separation of the PCR product was conducted in an electrophoresis machine using 1.2% agarose gel immersed in 2 µL GelRed at an electrical voltage of 90 v for 45 min and visualized using a UV transilluminator ChemiDoc gel scanning (Bio Rad). The DNA bands were scored for the presence and absence of DNA, and the profile was further translated into binary data.

**Data analysis.** Each SSR band in the gel that represents the DNA fragment from each race genotype or haplotype of the bacteria was scored based on the marker sized used (100 bp). The scores were analyzed using the TASEL program.

**Results and Discussion.**

**DNA extraction of *X. oryzae* pv. *Oryzae* isolates.** Extracted DNA from all *X. oryzae* pv. *oryzae* isolates in this study was almost uniform and ranges from 100 ng/µL to 150 ng/µL. Not all genomic DNA was in good quality (Figure 2). The genomic DNA with good quality was clean, not degraded, and not contaminated. Contamination by Chisam and other organic materials could be observed from the appearance of the background that was smeared along the movement path of genomic DNA bands. Purity of the extracted DNA samples shows the purity value that ranges from 1.8 to 2.0. This purity level is the standard for DNA quality for molecular analysis [19].

**Genotype profile of the BLB isolates based on the VNTR markers.** After screening with 20 VNTR primers and 3 primers that were compatible with the AvrXa7 gene, the result obtained did not vary. Figure 3 shows that *X. oryzae* pv. *oryzae* races and haplotypes amplified by B02 primer produced polymorphic bands with sizes ranging from 150 bp to 950 bp. This result showed that several races and haplotypes used in this study have high virulence level or endemic in a particular area. As an example race IVB (IXO80-004) and race VIIIIB (IXO79-008) were outbreak in West Java with dominant virulence on local rice host. While haplotype AvrXa-10- C (IXO93-103) and haplotype AvrXa-10-V (IXO93-230) were dominant virulence on local and elite rice varieties, particularly in Central Java and Bali. So, each race or haplotype has a specific virulence to the host and location endemic.

The VNTR marker is the molecular marker that denotes the tandem repeated sequence variation in various pathogenic bacteria, including *X. oryzae* pv. *oryzae*. This marker is considered effective in detecting the genetic variation of bacteria population associated with the important factor in the pathogen epidemiology, which includes temporal and spatial geographical factors [14]. The genotypic diversity analysis of the BLB isolates in this study shows several variations among isolates collected in different years, host origins, and locations.
Some of them have similar genotype profiles. This finding indicates that certain BLB genotypes have high adaptability to a particular season, host cultivars, and different locations. Figure 3 shows that the highest genotype profile variation is exhibited using the B02 primer. Less genetic variations were detected using three other primers, namely, G01, B06, and B20. The specific variation was shown by sample number 4 (ID: IXO80-004). This isolate produced the amplicon fragments with sizes of 500, 450, and 400 bp using primer B02 (Figure 3a). The genetic profile that is the same as sample 4 is sample 9. This specific profile was detected in sample number 8 (ID: IXO79-008), which had an amplicon fragment of 800 bp in the B02 primer. The polymorphic sample was also found in the profile generated from the B06 primer. Sample number 9 (IXO93-103) was specific to the amplicon fragment of 250 bp. The genetic specific variations were seen in sample numbers 13 (ID: IXO93-230) and 14 (IXO74-037-2) in the profile generated from the B02, G01, and B20 primers.

The genetic diversity of the BLB isolate based on the temporal and spatial geographical factors was shown as a genetic profile based on the B02 marker (Figure 4). Figure 4 shows that three different genotype profiles, namely, types A, B, and C, were detected. Among them, the isolates belonging to type B were found from different years and locations. Genotype types A and C have a narrow distribution based on the time (year/season) and/or location of the collection.

**Genotype profile of the BLB isolates based on the avrXa7 gene marker.** The genotype profile of the BLB isolates based on the avrXa7 gene marker (AvrXa7_1 primer) showed a variation with band size ranging from ±150 bp to ~750 bp; the AvrXa7_3 primer had a band size of ±150 bp; and the AvrXa7_4 primer had a band size of ±250 bp. This result shows that several BLB races and haplotypes possess the PthXo4, PthXoS, and AvrXa7-sacB50 genes that belong to the AvrBs3/PthA gene family [15].

**Genotype diversity of the BLB isolates based on the VNTR markers and the avrXa7 gene marker.** The dendogram of Figure 6 shows that several isolates have specific relationships with specific isolates. This finding is probably due to the genetic variation caused by mutation, which often occurs among X. oryzae pv. oryzae isolates. Three groups were detected from the dendogram (Figure 6). Genotype group I contains a single isolate (race VII, IXO80_021) from the Pusaka Negara Subang isolate, which has the PthXo4 gene of Genotype group II also contains a single race VIII-A (IXO 80_024) from the Kalimantan isolate, which has

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![Figure 2. Concentration of the X. oryzae pv. oryzae Indicated by Bands: 1. Lambda DNA 25 ng, 2. Lambda DNA 50 ng, 3. Lambda DNA 100 ng, 4. Lambda DNA 150 ng, 5. IXO 76-011, 6. IXO 94-013, 7. IXO 94-003, 8. IXO 80-004, 9. IXO 92-046, 10. IXO 80-021, 11. IXO 80-024, and 12. IXO 79-008](image)

![Figure 3. Genotype Profile of 18 X. oryzae pv. oryzae Isolates from the VNTR Markers. A. Amplification by B02 Primer, B. Primer G01, C. Primer B06, D. Primer B20. 1= IXO76_011, 2= IXO 94_013, 3= IXO 94_003, 4= IXO 80_004, 5= IXO92_046, 6= IXO 80_021, 7= IXO 80_024, 8= IXO79_008, 9= IXO 93_103, 10= XO 93_129, 11= IXO 93_229, 12= IXO92_048, 13= IXO 93_230, 14= IXO 74_037_2, 15= IXO 93_066_1,16= IXO 92_002, 17= IXO 92_093, and 18= IXO 94_035](image)
the AvrBs3/PthA gene family. The PthXo4 gene has a
dependent elicitor activity because it is associated with
the loss of the PVE production activity [15].

the PthXoS gene. The AvrBs3/PthA gene of genotype
groups I and II is compatible with the avrXa7 gene. The
PthXo4 and PthXoS genes have a TGA base variation,
which is a signal for the stop codon that causes the
absence of the PVE transcriptional activity, and
therefore the pathogen becomes avirulent [15]. In this
study, the pathogens belonging to genotype groups I and
II were avirulent, and thus these races could not be used
again to screen for plant resistance. The avirulent
reaction from these races could be caused by the time of
preservation, as the isolate was collected in 1980.
Therefore, the virulence activity could have been
decreased. The loss of virulence activity could also have
been caused by gene mutation.

In contrast to groups I and II, genotype group III, which
has the AvrXa7sacB50 gene, contains the following
various races and haplotypes: race I (IXO 92_002, race
IIA (IXO 94_013), race III-B (IXO 94_003), race IV-B
(IXO 80_004), race VIII-B (IXO 79_008), haplotype
IXO 93_103, haplotype IXO 93_129, haplotype IXO
93_229, haplotype IXO 92_048, haplotype IXO 93_230,
 haplotype IXO 74_037, haplotype IXO 93_066_1,
haplotype IXO 76-011, haplotype IXO 92_093, and
haplotype IXO 94_035. The AvrXa7sacB50 gene, which
is a specific sequence of the Xa7 gene, has the GCG
base at position 39 or the 13th amino acid of the
transcriptional domain of this gene that encodes the
amino acid of alanine. As the stop codon in this gene
has no stop signal, the transcription activity of PVE by
the pathogen remains active [20]. The AvrXa7sacB50
gene has an NLS signal that functions in the process of
protein virulent targeting into the host cell nucleus [21].
Genotype group III has a race group with a strong
virulence activity. This virulence activity is shown in
the pattern of race distribution and spread, which
includes race I, race III-A, race III-B, race IV-B, race
VIII–B, and all haplotypes used in this study. The races in group III are endemic to the X. oryzae pv. oryzae isolates from the BLB population in Kota Batu (Bogor, West Java), Jatisari (Cikampek), Cianjur (West Java), and Pusakanegara (Subang). This study revealed the possibility of a relationship between the virulence of the X. oryzae pv. oryzae pathotype and that of the X. oryzae pv. oryzae genotype.

Conclusion

The diversity of the X. oryzae pv. oryzae isolate core collection using the VNTR and avrXa7 gene markers produced three X. oryzae pv. oryzae genotype groups with different virulence levels. The avirulent X. oryzae pv. oryzae belongs to genotype group I (race VII, IXO80_021) and genotype group II (race VIII-A, IXO80_024), while the majority of virulent isolates belong to genotype group III.

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References

[1] Swing, J.M., Van den Mouten, Vantening, L., Hoste, B., Gillis, M., Mew, T.W., Kerters, K. 1990. Reclassification of the causal agents of bacterial blight (Xanthomonas Campestris pv. oryzae) and bacterial leaf streak (Xanthomonas Campestris pv. oryzicola) of rice as pathovar of Xanthomonas oryzae sp. Rev. inf. J. Syst. Bacterial, 40:309-311.
[2] Singh, G.P., Srivastaba, M.K., Singh, R., Singh, R.M. 1977. Variation in quantitative and qualitative losses caused by bacterial blight in different rice varieties. Indian Phytopathol, 30:180-185.
[3] Ou, S.H. 1985. Rice Disease. 2nd ed. Commonwealth Mycological Institute, London, p. 380.
[4] Naveed, S.A., Babar, M., Arif, A., Zafar, Y., Sabar, M., Ali, I., Chragh, M., Arif, M. 2010. Detection of bacterial blight resistant gene xa5 using lingked marker approaches. Afr. J. Biotechnol, 9(24):3549-3554.
[5] Khan, M.G. 1996. A brief note on rice research institute Kala Shah Kaku. In: Report of the monitoring visit on fine grain aromatic rice in India, Iran, Pakistan and Thailand, International Rice Research Institute, Manila, Philippines, pp.96-101.
[6] Direktorat Perlindungan Tanaman Pangan, Prakiraan Serangan BLB Pada Padi di Indonesia 2012, http://tanamanpangan.deptan.go.id/doc_upload/padi_blb.pdf.
[7] Sukarman, Suharta, N. Analisis Sumber Daya Lahan Menuju Ketahanan Pangan Berkelanjutan, Badan Penelitian dan Pengembangan Pertanian, Jakarta, 2010, p.111.
[8] Kadir,S.T., Hifni, H.R., Suwarsa. 1999. Analisis struktur populasi Xanthomonas oryzae pv. Oryzae untuk menunjang penyaringan varietas padi terhadap HDB. In: Prosiding simposium V Perhimpunan Ilmu Pemuliaan (PERIPI), Komisariat daerah Jawa Timur, Univ. Brawijaya Malang, pp.48-53.
[9] Yamamoto, T., Hifni, H.R., Machmud, M., Nishzawa, T., Tantera, D.M. 1977. Genetic diversity within population of Xanthomonas oryzae pv. oryzae in India. Phytopathol. 87:760-765.
[10] Sudir, Yuliani, D., Faizal, A., Yusuf, A. 2012. Pemetaan patotipe Xanthomonas oryzae pv. oryzae, penyebab penyakit hawar daun bakteri padi di sentra produksi padi di Sulawesi Selatan dan Sumatera Utara, Laporan Hasil Penelitian 2012, Balai Besar Peneltian Tanaman Padi Sukamandi, p.53.
[11] Horino, O., Hifni, H.R. 1978. Resistance of some rice varieties to bacterial leaf blight and a newpatogenic group of causal bacterium Xanthomonas oryzae, Inst. Agrie Bogor, p.22.
[12] Chen, H. Wang, S., Zhang, Q. 2002. New gene for bacterial blight resistance in rice located on chromosome 12 identified from Minghui 63, an elite restorer line. Phytopathol. 92:750-754.
[13] Bai, J., Choi, S.-H., Ponciano, G., Leung, H., Leach, J.E. 2000. Xanthomonas oryzae pv. oryzae avirulence genes contribute differently and specifically to pathogen aggressiveness. Mol Plant-Microbe Interact. 13 (12):1322-1329.
[14] Zhao, S., Poulin, L., Rodriguez-R, L.M., Serna, N.F., Liu, S.-Y, Wonni, I., Szurek, B., Verdier, V., Leach, J.E., He, Y.-Q., Feng, J.-X., Koebnik, R. 2012. Development of a variable number of tandem repeats typing scheme for the bacterial rice pathogen Xanthomonas oryzae pv. Oryzicola. Phytopathology, 102(10):948-956. doi: 10.1094/PHYTO-04-12-0078-R.
[15] Utami, D.W., Kadir, T.S., Yuriah, S. 2011. Aktor Virulensi AvrBs3/PthA pada Ras III, Ras IV, Ras VIII, dan IXO93-068 Patogen Hawar Daun Bakteri (Xanthomonas oryzae pv. Oryzicola). J. AgroBiogen, 7 (1):1-8.
[16] van Belkum, A., Schere, S., van Leeuwen, W., Willemsen, D., van Alphen, L., Verbrugh, H. 1997. Variable number of tandem repeats in clinical strains of Haemophilus influenza. Infect. Immun. 65 (12):5017-5027.
[17] George Luz, M.C. 1993. ARBN Phase I Research Training and Planning Course, International Rice Research Institute, Manila, Philippines, p.53.
[18] Onasanya, A., Mignouna, HD., Thottappilly, G. 2003. Genetic fingerprinting and phylogenetic
diversity of isolates of Staphylococcus aureus from Nigeria. Afri. J. Biotechnol. 2(8):246-250.
[19] Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. Molecular cloning: A laboratory manual, CHSL Press, New York
[20] Yang, B., Zhu, W., Johnson, L.B., White, F.F. The virulence factor AvrXa7 of *Xanthomonas oryzae* pv. *oryzae* is a type III secretion pathway-dependent nuclear-localized double-stranded DNA-binding protein. Proc Natl Acad Sci. 97(17):9807-9812.
[21] White, F.F., Yang, B. 2009. Host and pathogen factors controlling the rice *Xanthomonas oryzae* interaction. J. Plant Physiologi, 150:1677-1686.