Journal of Applied & Environmental Microbiology, 2016, Vol. 4, No. 3, 63-69
Available online at http://pubs.sciepub.com/jaem/4/3/3

DOI:10.12691/jaem-4-3-3

Xanthomonas oryzae pv. *oryzae*, Biochemical Tests, Rice (*Oryza sativa*), Bacterial Leaf Blight (BLB) Disease, Sekinchan

N. Q. Jonit[1], Y.C. Low[2], G.H.Tan[1,*]

[1]Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia
[2]CABI South East Asia Regional Centre, Building A19, MARDI, 43400 Serdang, Selangor, Malaysia
*Corresponding author: geok_hun@upm.edu.my, tangeokhun@gmail.com

Abstract Bacterial leaf blight (BLB) disease caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most devastating diseases in rice which limits the annual rice production in both tropical and temperate regions of the world. The present study was conducted to isolate, characterize and identify the *Xanthomonas oryzae* pv. *oryzae* obtained from infected rice foliar samples. Plant samples were collected from one of the major agro-ecological rice zones in Sekinchan, Peninsular Malaysia. The infected leaf samples were plated on nutrient agar and gave light yellow, circular, smooth, convex and viscous bacterial colonies. There were 15 isolates obtained and subjected to different biochemical tests. KOH test was conducted to re-confirm gram staining of all isolates. The egg yolk reaction gave negative for the eleven isolates (Xoo-1, Xoo-4, Xoo-11, Xoo-12, Xoo-13, Xoo-14, Xoo-15, Xoo-16, Xoo-17, Xoo-18 and Xoo-20) and positive for four isolates (Xoo-9, Xoo-10, Xoo-19 and XOR). Results of biochemical tests like starch hydrolysis, anaerobic nature and acid production from carbohydrates varied among the isolates. In the starch hydrolysis, nine isolates showed positive reaction. In anaerobic and acid production from carbohydrate test, nine and five isolates showed positive reaction respectively. It is about 21% isolates were similar in terms of their reactions to these tests. Based on biochemical responses it was established a genetic variability was detected in *Xathamonas oryzae* pv. *oryzae* isolates. Some of the isolates diverse from the normal pattern and had overlapping results. The phylogenetic trees showed Xoo-9 and XOR are closely related to *Micrococcus aloeverae* with 100% and *Xanthomonas sacchari* with 99% of similarity respectively. The XOR isolate showed similar characteristic to X. *oryzae* pv. *oryzae*. However, the DNA identification did not match 100% similarity to target pathogen, even though they both are from the same origin. Further study need to be carried out for comparative and functional genomics to improve our understanding of their modes of adaptation to different ecological niches and the genes that determine their pathogenicity.

Keywords: *Xanthomonas oryzae pv. oryzae, biochemical tests, rice (Oryza sativa), bacterial leaf blight (BLB) disease, Sekinchan*

Cite This Article: N. Q. Jonit, Y.C. Low, and G.H.Tan, "*Xanthomonas oryzae* pv. *oryzae*, Biochemical Tests, Rice (*Oryza sativa*), Bacterial Leaf Blight (BLB) Disease, Sekinchan." *Journal of Applied & Environmental Microbiology*, vol. 4, no. 3 (2016): 63-69. doi: 10.12691/jaem-4-3-3.

1. Introduction

Rice is grown in tropical and subtropical regions of the world and it is a staple food for 2.7 billion people worldwide including Malaysia [13,18]. It is susceptible to a number of diseases among which the bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* [22] has been an important constraint to rice production in Asia [11]. Bacterial blight disease (BLB) was first reported in India in 1951. In Philippines, the present yield losses of susceptible rice crops is about 22.5% in wet season and 7.2% in dry seasons; while in resistant rice crops is about 9.5-1.8%, respectively [5,20]. The incidence of disease was observed in the rice fields of Peninsular Malaysia in the early 80s, and estimated loss of about RM 50 million during the period of 1982 to 1994 [17]. The recent outbreak in Feb 2014 was reported from Padang Besar, Perlis about 60,000 metric ton [23].

X. oryzae pv. *oryzae*, belongs to c-subdivision of the Proteobacteria. It is a major pathogen of rice (*Oryza sativa* L.) causing bacterial leaf blight (BLB) disease [6]. The bacterium can infect rice from seedling stage to mature plant and the disease is manifested by either leaf blight or kresek symptoms [1,14]. During the leaf blight phase, causal organism enters the plant through wounds or through water pores located on the margins of upper part of the leaves, producing lesions, which are water soaked, yellow with irregular, wavy margins and progresses down the leaves. The lesion usually starts from the leaf margin near its tip. Bacterial ooze, which consists of small, yellowish, spherical masses, may sometimes be seen on the margins or veins of the freshly infected leaf under moist conditions. On the other hand, the kresek phase is a systemic phase during which acute wilting of the

seedlings take place. This symptom usually appears one or two weeks after transplanting. Leaves turn grayish green, wither suddenly and roll upwards. The kresek phase was first reported from Indonesia and was later reported to occur in most of the rice growing areas in the tropics [15]. For developing a successful integrated disease management package the first step is to know the characteristics of the pathogen. In the present study some efforts have been made on biochemical characterization and molecular identification of *X. oryzae* pv. *oryzae*.

2. Material and Methods

2.1. Sample Collection

A comprehensive survey of various agro-ecological zones in Selangor was conducted for the collection of leaf samples of rice suspected to be infected with bacterial leaf blight. The samples were used for isolation and characterization of bacterium.

2.2. Isolation

Ten samples of rice at panicle initiation stage, showing typical bacterial blight symptoms were collected. The diseased leaves were cleaned with tap water and cut about 2 to 4 cm and sterilized with 1 % sodium hypochloride solution for 0, 30, 60 and 90 seconds respectively, then washed in sterilized distilled water. These pieces were put into the test tube containing 1 to 2 ml of sterilized distilled water and grinded into smaller pieces to allow the bacteria to ooze out from the leaf tissue. One loop with bacterial suspension was streak onto nutrient agar (NA), nutrient agar yeast extract (NYA) and peptone sucrose agar (PSA) medium. The plates were incubated in room temperature (28-30 °C) for 3 to 4 days. The single yellow, round and smooth margin, non flat, mucous colonies were selected and transferred into slant nutrient medium as pure culture. The single colony was selected as a representative strain for this study. These strains were maintained at 4 °C up to 1 month for further evaluation.

2.3. Gram Staining

Gram staining procedure was performed as described by [7]. Bacteria were heat fixed on a glass slide treated with (0.5%) crystal violet for 30 seconds then washed with tape water. Then treated with Lugol's solution for 1 min, washed again and decolorized with (95%) ethanol for 30 seconds, washed again and counter-stained with safranin for 1 min. Magnifications of X-10, X-40 and X-100 were used for microscopic observation. Gram negative bacteria stained red whereas Gram positive bacteria retained the color of crystal violet.

2.4. Biochemical Characterization of *Xanthomonas oryzae pv. oryzae*

To identify biochemical characteristics of *X. oryzae* pv. *oryzae*, various tests were applied including potassium hydroxide test (3% KOH), catalase, starch hydrolysis test, egg yolk reaction, acid production from carbohydrates and anaerobic growth test.

(i) 3% KOH (Potassium hydroxide) test

The bacterial culture taken was vigorously stirred in drop of 3% KOH solution. The thread-like slime formation will be indicated by the presence of Gram negative bacterium [21].

(ii) Catalase test

One colony from pure culture was taken and put on the slide. One drop of 3% of hydrogen peroxide was put onto the colony. The production of bubble gives positive results.

(iii) Starch hydrolysis test

For each hydrolysis test, 2 g nutrient agar (NA) was added to 80 ml of water and dissolved by successive heating and stirring similarly 2 g starch was then thoroughly dissolved in 10 ml distilled water separately and added to hot molten agar with through stirring. The plates were then inoculated with individual isolate aseptically, labeled and sealed to avoid chances of contamination. These plates were then incubated in upside down position at 28 °C for 7 days. After scraping bacterial growth to each plate Lugol's iodine was added which was prepared by mixing 1 g iodine and 2 g potassium iodide in 300 ml distilled water, stirred for until dissolved completely. The appearance of blue cleared zones around the colonies was indicative of presence or absence of starch hydrolysis as described by [2].

(iv) Egg yolk hydrolysis

Egg yolk emulsion was prepared from a fresh egg, which was washed well in soap solution, rinsed and surface sterilized with 70% ethanol for 5 minutes. The egg was then flamed, broken aseptically, yolk separated into a sterile graduated cylinder and diluted to 40% v/v with sterile water. An aliquot of 10 ml of the egg yolk was incorporated into 100 ml molten nutrient agar (cooled to 55 °C) prior to pouring into the plates. The medium was spot cultured and incubated for three days at 28 °C. Production of turbid zone of free fats around the colonies was considered positive [12].

(v) Acid production from carbohydrates

10 ml of medium [$NH_4H_2PO_4$ (0.5 g), K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (5 g), yeast extract (1 g), agar (12 g) water (1 L), bromocresol purple (1.5% alcohol solution 0.7 ml)] was prepared. A 10% (w/v) aqueous solution of glucose was filter-sterilized through millipore injection and added to the molten base to give a final concentration of 1%. Each isolate was then transferred aseptically into a tube, incubated at 28 °C and checked for acid production from carbohydrates after two, four and six days. Production of yellow color indicated production of acid [4].

(vi) Anaerobic growth test

Basal medium [Peptone: 2 g; NaCl: 5 g; Agar: 0.3 g; KH_2PO_4: 0.3 g; bromothymol blue: (3 g) in 1 % aqueous solution, 5 ml] was prepared. An amount 0.5 ml 10% glucose suspension was added to each tube aseptically. For each isolate two test tubes were inoculated. One of the tubes was seal with paraffin, and incubated at 28 °C. An anaerobic growth was noticed if color change occurred from blue to yellow [9].

2.5. Identification of Potential Bacteria Using 16S rRNA Analysis

(i) DNA extraction

A loopful of *Xoo* isolate was picked up and suspended in TE buffer [100 mM Tris (pH 8.0), 30 mM EDTA (pH

8.0), 360 µl]. Lsozyme (50 mg/ml, 20 µl) was added into the tube and incubated at 37°C for 30 min. SDS [10% (w/v), 40 µl] was added, and the tube was inverted for 5-6 times, then incubated at 55 °C for 10 min. Phenol: chloroform: isoamyalcohol [25:24:1, 400 µl] was added and mixed well. The mixture was spun at 13,000 rpm rpm for 15 min. The upper solution was transferred to new microtube. The same procedure was repeated if the solution was not clear. 3 M Sodium acetate (1/10 volume) and 2 volume of cold ethanol was then added. DNA was then pooled by glass rod and dried for 5-10 min until DNA became clear. Finally, the DNA was dissolved in 100 - 200 µl of sterilized distilled water and stored at -20°C.

(ii) Polymerase Chain Reaction Analysis

Polymerase Chain Reaction (PCR) of the 16S rRNA for *Xoo* isolates were conducted and the PCR reactions were carried out as followed: sterile distilled water (59.5 µl); 10X PCR buffer (Promega, USA, 10.0 µl); 25 mM $MgCl_2$ (Promega, USA, 8.0 µl); 2 mM dNTP mix (Promega, USA, 10.0 µl); universal primers (Forward: 5' GAG TTT GAT CCT GC TCA G 3'; Reverse: 5' GTT ACC TTG TTA CGA CTT 3'] Invitrogen, USA, 10 pmol/µl, 4.0 µl); Taq Polymerase (5U/µl; 0.5 µl) and genomic DNA as template (4 µl). The PCR tubes was then put into thermal cycler and preheat at 94 °C for 3 min, followed by 25 cycles of denaturing at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 2 min and final elongation at 72 °C for 3 min. The reactions were kept at 4 °C until loaded onto the gel. The PCR product was purified, sequenced and compared with the 16S rRNA sequence of bacteria from the NCBI Gene Bank nucleotide sequence database (http://www.ncbi.nih.gov). The sequence and identity of each isolate was then double confirmed by constructing the Phylogenetic Tree using BioEdit (CA) and MEGA 4.0.2 software (UK).

3. Result and Discussion

3.1. Isolation and Gram Staining

The infected leaf samples with bacterial leaf blight were collected from rice granary areas at Sekinchan, Peninsular Malaysia. The causal bacterium was isolated from green leaves with yellow BLB lesion and not from torn and rotten tissue that are usually overgrown by microorganism. The bacterial exudates from fresh lesion are better isolation material as compared to infected tissue because of less contamination [10]. Similarly, the recovery of *X. oryzae* pv. *oryzae* colonies from infected leaves sample is easy rather than infected seeds, due to presence of other bacteria and fungi in high population in seeds [3]. Nutrient agar yeast extract medium (NYA), peptone sucrose agar (PSA), and nutrient agar (NA) were used for isolation and screening of the pathogens. After 48-72 hours of incubation at 28 °C, the infected leaf samples were then plated on nutrient agar (NA) and light yellow, circular dome shaped colonies were formed (Figure 1). The yellow color and mucoid colonies is cultural characteristics of Xanthomonads and was due to the production of extracellular polysaccharides slime (EPS in media containing sugar). The samples also gave a light yellow, mucoid, round and smooth bacterial colonies (1 to 2 mm in diameter) when streaked on nutrient agar yeast extract medium (NYA) and it was similar on peptone sucrose agar (PSA) which produces yellow water-soluble pigment, pale yellow colonies, mucoid and shiny. In present study, there were 15 isolates recovered from ten rice plants samples. For Gram staining study, all isolates were stained as Gram negative with rod shape (Figure 2), and showed positive reaction in 3% KOH test by forming the thread-like slime.

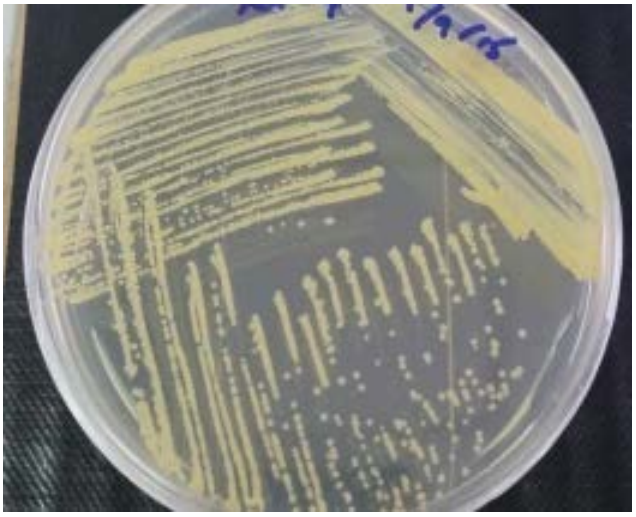

Figure 1. Isolation of Xoo bacterium on Nutrient agar (NA) medium

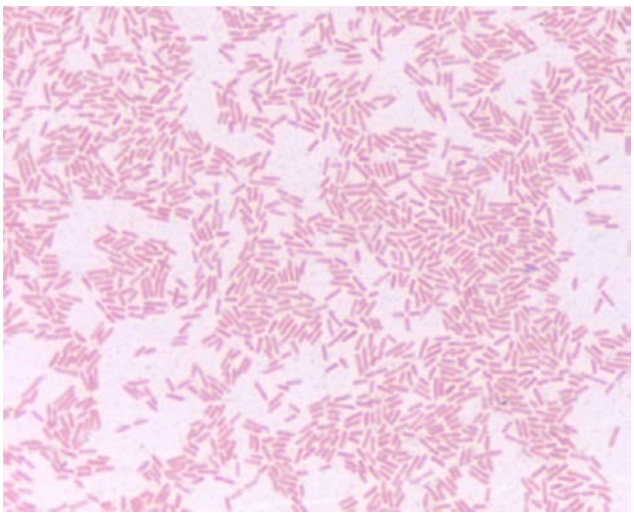

Figure 2. Gram negative of Xoo bacterium shows red stain, rod-shaped bacteria under X-100 magnifications

3.2. Biochemical Tests

In biochemical tests, most of the isolates showed positive reaction against catalase test. However, there were two isolates which showed negative reaction against catalase [Xoo-19 and Xoo-20 (Table 1)]. So these two isolates will be excluded from be the potential candidate for *X. oryzae pv. oryzae*. Beside catalase, starch hydrolysis also used to identify the bacterial species belongs to Xanthomonas by having the property of amylase production and ability to produce amylase to breaks down the starch into maltose [19]. The presence of starch can be indicated by the development of blue color. Therefore, the absence of a blue color indicates starch hydrolysis (Figure 3). Both positive and negative isolates for starch hydrolysis have been reported from this pathogen. According to

previous study, the pathogen can hydrolyze starch after a week's incubation [22]. However, Guvera and Marsella (1999) did not find this feature in their isolates [8]. Both the cases of starch hydrolysis were observed in this study. The color changes of inoculated media in the tubes from purple to yellow after two, four and six days of incubation, indicated positive reaction (Table 1). The egg yolk hydrolysis can be one of indicator to determine the characteristic of *X. oryzae* species. This test is based on the observation that the enzyme lecithinase can break down the phospholipid emulsion of egg yolk, liberating a turbid zone of free fats around the colonies. The egg emulsion in the agar provides the lecithin to be degraded by the enzyme. A given isolates that contain lecithinase break down the egg yolk and cause clear zones around the colonies [16]. Sometimes an opaque zone of precipitation may form due to lipase activity around that clear zone. However, out of 15 Xoo isolates, only four isolates (Xoo-9, Xoo-10, XOR and Xoo-19) managed to hydrolyze the egg yolk (Table 1), the rest unable to degrade the protein due to an absence of the enzymes. In anaerobic growth study, nine isolates gave positive anaerobic activity, whereas the other six isolates gave negative results for anaerobic activity (Table 1). According to previous study, this test was indicated the true aerobic nature of the bacterium when there were negative to anaerobic growth test, that mean they are aerobic bacteria. However, based on current study, there are some genetic variability was detected in *X. oryzae pv. oryzae*. It is about 21% of isolates were similar while others were variable. Thus, this study needs to be confirmed the genus and species using 16S rRNA method.

Table 1. Biochemical characterization of various isolates of *Xanthomonas oryzae* pv. *oryzae* collected from Sekinchan, Peninsular Malaysia during 2015

No	Isolates	Gram reaction	Catalase test	3% KOH test	Starch hydrolysis	Anaerobic growth test	Egg yolk hydrolysis	Acid from carbohydrates
1	Xoo-1	-	+	+	+	-	-	-
2	Xoo-4	-	+	+	-	+	-	+
3	Xoo-9	-	+	+	+	-	+	-
4	Xoo-10	-	+	+	-	-	+	-
5	Xoo-11	-	+	+	-	+	-	-
6	Xoo-12	-	+	+	+	-	-	-
7	Xoo-13	-	+	+	+	+	-	-
8	Xoo-14	-	+	+	-	+	-	-
9	Xoo-15	-	+	+	+	+	-	-
10	Xoo-16	-	+	+	+	+	-	+
11	Xoo-17	-	+	+	-	+	-	+
12	Xoo-18	-	+	+	+	+	-	+
13	Xoo-19	-	-	+	-	-	+	-
14	Xoo-20	-	-	+	+	-	-	-
15	XOR	-	+	+	+	+	+	+

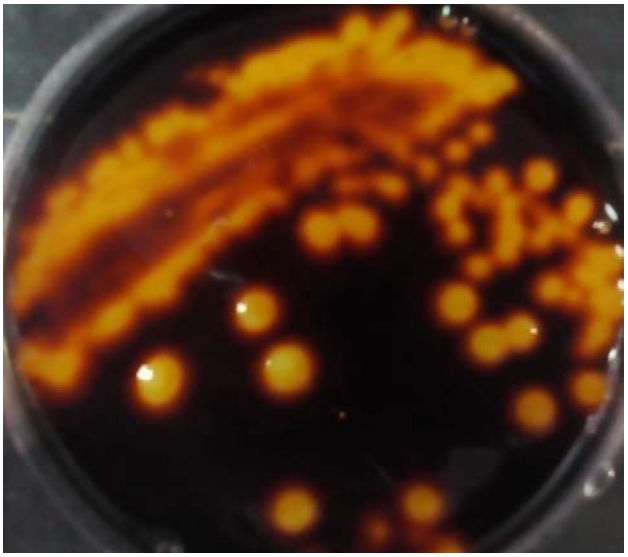

Figure 3. Starch hydrolysis as indicated by a non-blue halo surrounding the growth indicates positive result. However, both positive and negative isolates for Starch hydrolysis have been reported for this pathogen

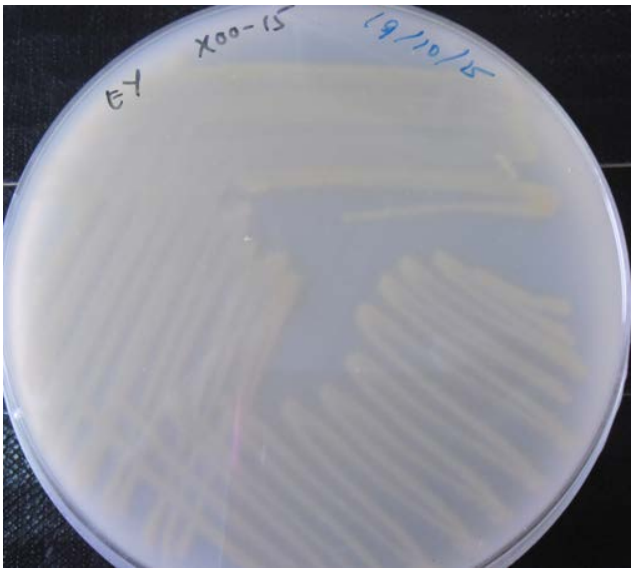

Figure 4. No production of turbid zone (negative results) around the colonies when streaked on egg yolk emulsion medium indicates positive for this pathogen

3.3. Identification of Potential Bacteria Using 16S rRNA Analysis

All 15 isolates were extensively characterized and identified using standard procedures as mentioned previously. However, some of the biochemical tests gave overlapping results regarding the identity of the causal organism of bacterial blight. For these reasons, PCR was performed for two most identical isolates (Xoo-9 and XOR) which showed the major characteristic of *X. oryzae* based on biochemical tests. The conserve regions of Xoo-9 and XOR were amplified by PCR and showed in Figure 5. Both have the expected size around 1500 bp. Based on 16S rRNA sequence analysis, the isolate Xoo-9 was identified as *Micrococcus aloeverae* and XOR as *Xanthomonas sacchari*. These sequences were further confirmed by constructing the phylogenetic tree to correlate with the family tree of both species. The known genus sequence of Micrococcus and *Xanthomonas* were downloaded from the Gene Bank and computed using BioEdit (CA) and Phylogenetic Tree using Neighbor Joining (Unrooted Tree) by NCBI Blast Tree Method. The phylogenetic trees (Figure 6 and Figure 7) showed that the new sequence of Xoo-9 and XOR are closely related to *Micrococcus aloeverae* with 100% and *Xanthomonas sacchari* with 99% of similarity respectively. The XOR isolate showed similar characteristic to X. *oryzae* pv. *oryzae*. However, the DNA identification did not match 100% similarity to target pathogen, even though they both are from the same origin. This may be due to the nature and evolution of interactions with their hosts and with the wider environment. Further study need to be carried out for comparative and functional genomics to improve our understanding of their modes of adaptation to different ecological niches and the genes that determine their pathogenicity.

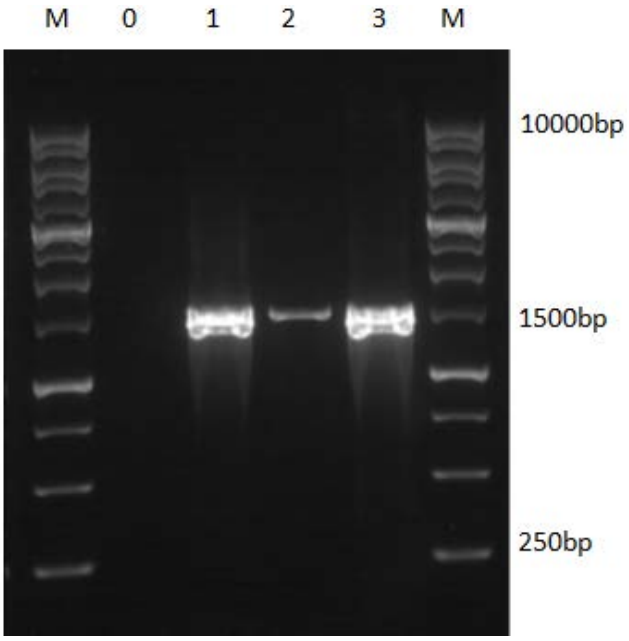

Figure 5. Gel Electrophoreses, PCR Amplified ~1500 bp bands from Xoo isolates (with universal primers), isolate 2 (Xoo-9) and 3 (XOR) confirming Xoo-specific bands. Where, 0 is known as negative control (PCR no-template control; water to replace DNA template), 1 is known as positive control (DNA extracted from *E.coli* is used as template) and M representing Ikb DNA ladder used as marker for comparison

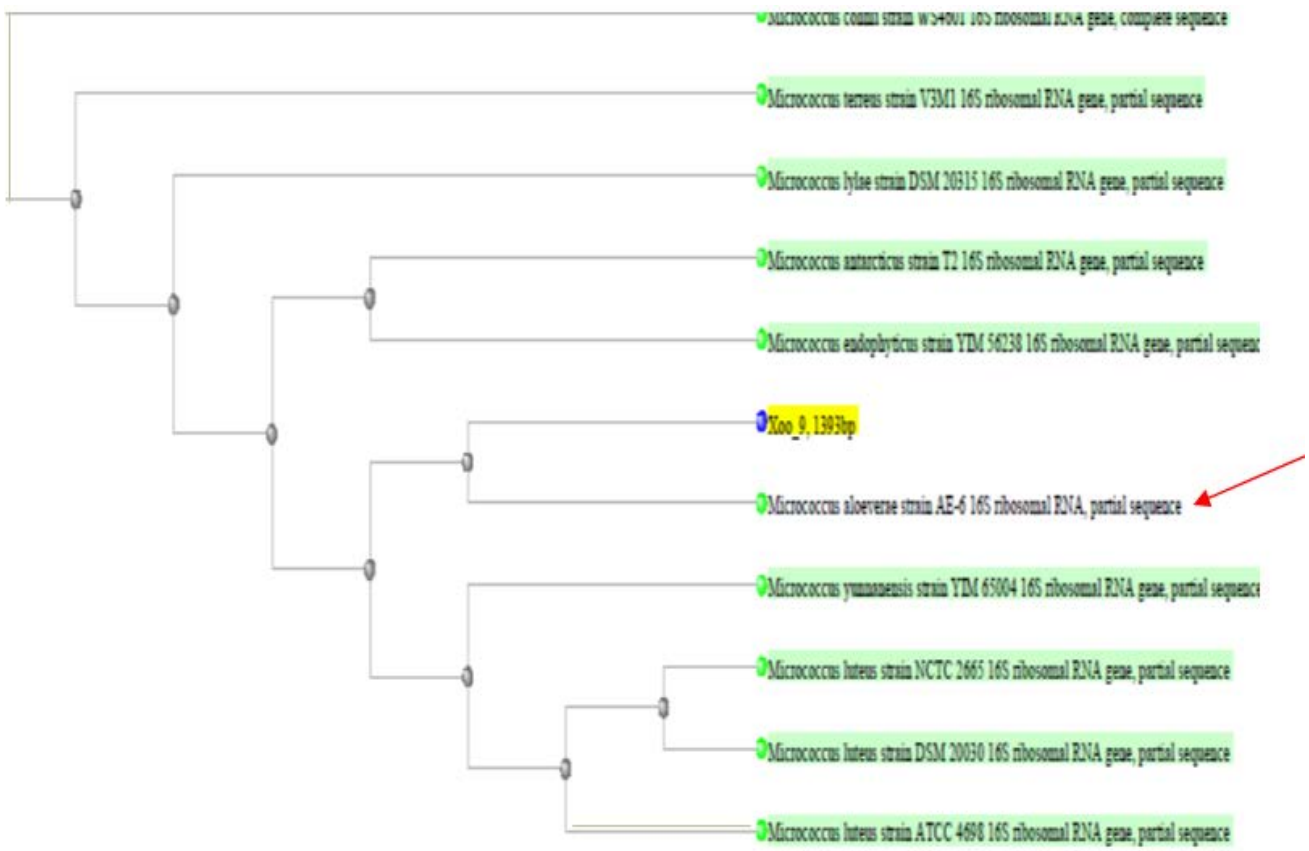

Figure 6. Phylogenetic tree of *Micrococcus* genus. Xoo-9 is closely related to *Micrococcus aleoverae* (arrow showed) with 100% of similarity

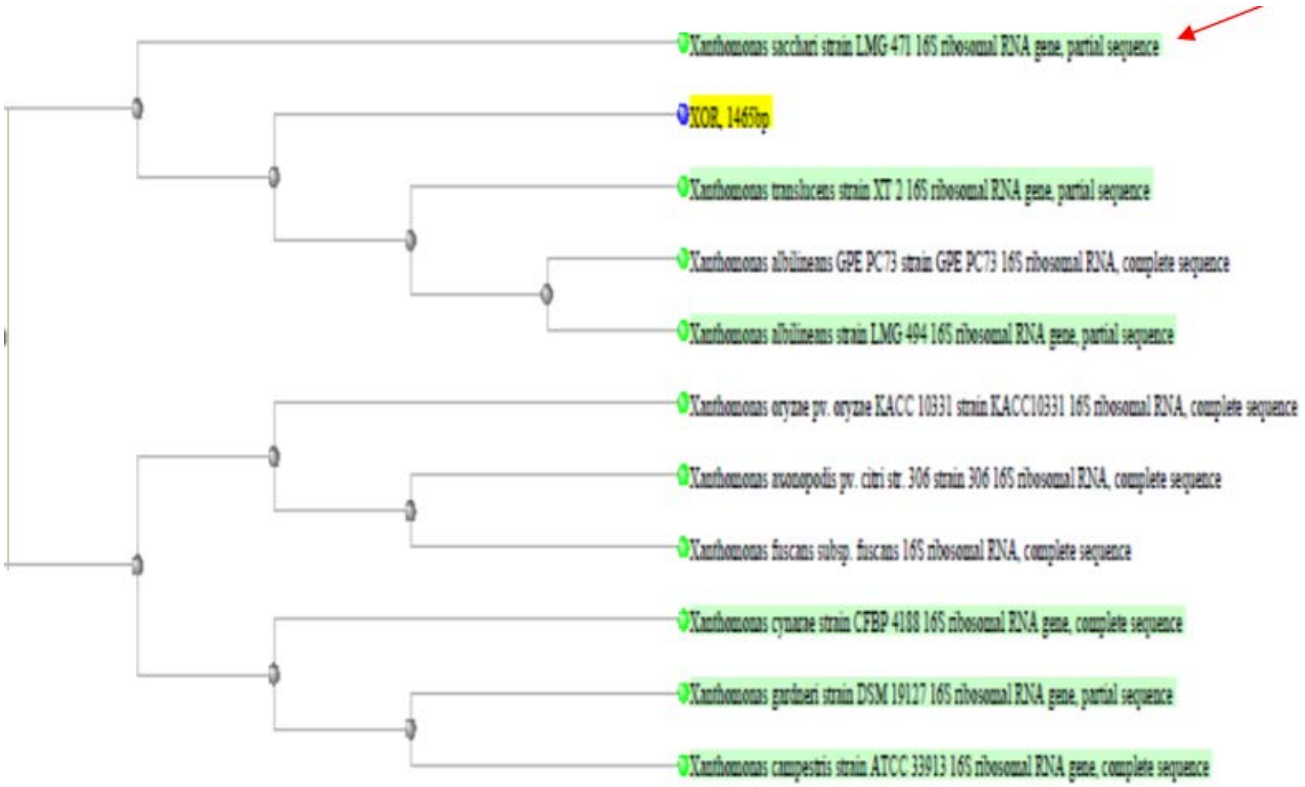

Figure 7. Phylogenetic tree of *Xanthomonas* genus. XOR is closely related to *Xanthomonas sacchari* (arrow showed) with 99% of similarity

4. Conclusion

Xanthomonas isolates have been isolated and characterized based on certain biochemical tests. However, some of the *Xanthomonas* isolates established a genetic variability among them. Identification at the genetic level was determined and found out that the DNA identification did not match 100% similarity to target pathogen, even though they both are from the same origin. Further study need to be carried out to confirm the mutation and adaptation at the ecological effect.

Acknowledgements

We wish to acknowledge the Ministry of Education Malaysia (MOE) for the Fundamental Research Grant Scheme (FRGS) under project no: FRGS/2/2014/STWN03/UPM/02/10 and Universiti Putra Malaysia for funding this research project and technical support.

References

[1] Akhtar, M.A., Rafi, A. and Hameed, A. 2008. Comparison of methods of inoculation of *Xanthomonas oryzae* pv. *oryzae* in rice cultivars. *Pak. J. Bot.*, 40(5): 2171-2175.

[2] Cowan, S.T. 1974. Manual for the identification of medical bacteria. Cambridge University Press. Great Britian. pp.238.

[3] Di, M., Yc, U., Schaad, N.W. and Roth, D. A. 1991. Selective recovery of *Xanthomonas* sp from rice seed. *Phytopathology*, 81: 1358-1363.

[4] Dye, D.W. 1968. A taxonomic study of the genus *Erwinia* 1. The "amylovora" group. New Zealand. *J. of Sci*. 11: 590-607.

[5] Exconde, O.R. 1973. Yield losses due to bacterial leaf blight of rice. *Philippines Agriculture.* 57: 128-140.

[6] Feng, J.X., Song, Z.Z., Duan, C.J., Zhao, S., Wu, Y.Q., Wang, C., Dow, J.M. and Tang, J.L. 2009. The xrvA gene of *Xanthomonas oryzae pv.oryzae*, encoding an H-NS-like protein, regulates virulence in rice. Microbiology 155: 3033-3044.

[7] Gerhardt, P. 1981. *Manual of methods of General Bacteriology*. American Society of Microbiology Washington, D.C.

[8] Guvera, Y. and Marsella. A. 1999. Rice bacterial blight in Venezuela. *Agri. Trop. Maracay* 49: 505-516.

[9] Hugh, R. and Leifson, E. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates of various Gram-bacteria. *J. of Bact*. 66: 24-26.

[10] Isaka, M. 1970. A new detection method for the pathogen of bacterial leaf blight of rice (*Xanthomonas oryzae*) by means of bacterial exudation. *Annals of Phytopathological Society of Japan*, 36: 313-318.

[11] Lozano, C.J. 1981. Proc. 5 Int, Conf. On Plant Pathogenic Bacteria. Centra Int'l. de Agricultura Tropical (C1AT) in cooperation with Agric. Editor's office. College of Agric., Univ. of Missouri-Colombia. pp.618.

[12] McClung, L.S. and Toabe, R. 1947. The Egg Yolk Plate Reaction for the Presumptive Diagnosis of *Clostridium sporogenes* and Certain Species of the Gangrene and Botulinum Groups. *J. Bacteriol*. 53: 139-147.

[13] Muneer, N., Rafi, A. and Akhtar, M.A. 2007. Isolation and characterization of Xanthomonas oryzae pv. oryzae isolates from North West Frontier Province (NWFP) Pakistan. Sarhad. J. Agri. 23: 743-751.

[14] Ou. S.H.. 1985. *Rice Diseases*. 2nd ed. Common Wealth. Mycological Institute. Kew, Surrey. England. pp. 61-96.

[15] Reitsma, J. and Schure, P.S.J. 1950. "Kresek", a bacterial blight disease of rice. *Contr., Gen. Agric. Res. St., Bogor* 117: 1-17.

[16] Rukhsana, J., Tehreema, I. and Huma, B. 2012. Isolation, characterization, preservation and pathogenicity test of *Xanthomonas oryzae* pv. *oryzae* causing BLB disease in rice. Pak. J. Bot. 44: 261-265.

[17] Saad, A. and Habibuddin, H. 2010. Pathotypes and virulence of *Xanthomonas oryzae* causing bacterial blight disease of rice in Peninsular Malaysia.*J. Trop. Agric. and Fd. Sc*. 38(2): 257– 266.

[18] Salim, M., Akram M., Akhtar, M.H. and Ashraf, M. 2003. Rice - a production hand book. Pak. Agric. Res. Council, Islamabad. pp.70.

[19] Samanta, T.T., Samanta, P and Das, A. 2014. Isolation and characterization of *Xanthomonas oryzae* isolates from different regions of Midnapore district of West Bengal and their ecofriendly

management by some medicinal plant extracts. *Int. J. of Phytomedicine*. 6: 29-42.

[20] Savary, S., Willocquet, L., Elazegui, F.A., Teng, P.S., Du, P.V., Zhu, D., Tang, Q., Huang, S., Lin, X., Singh, H.M. and Srivastsava, R.K, 2000. Rice pest constraints in tropical Asia: characterisation of injury profiles in relation to production situations. *Plant Disease*. 84: 341-356.

[21] Schaad, N.W. 1980. Laboratory guide for identification of plant pathogenic bacteria. Deptt. Plant pathology Univ. of Georgia. pp. 28.

[22] Swings, J., Van Den Mooter, M., Vauterin, L., Hoste, B., Gillis, M. and Mew, TW. 1990. Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* pv. *oryzae)* and bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) of rice as pathovars of *Xanthomonas oryzae* (ex Isiyama 1922) sp. nov., nom. rev. *International Journal of Systematic Bacteriology*. 40: 301-311.

[23] Utusan, 2014. Perlis sasar lapan metrik tan padi sehektar http://ww1.utusan.com.my/utusan/Utara/20140222/wu_02/Perlis-sasar-lapan-metrik-tan-padi-sehektar. Retrieved on 19th Dec 2014.

[24] Bibi, A., Rafi., A., Junaid, M. and Ahmad, M. 2013. Studies on the characterization and pcr identification of the pathogen of the bacterial blackleg/soft rot of potato. *Sarhad J. Agric*. 29(2): 225-234.