JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 48, No. 1 (2008)

ISOLATION AND IDENTIFICATION OF *XATHOMONAS ORYZAE* PV. *ORYZAE* THE CAUSAL AGENT OF BACTERIAL BLIGHT OF RICE IN IRAN

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Received: September 19, 2007
Accepted: February 25, 2008

Abstract: Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the important bacterial diseases on rice. This disease causes typical symptoms on infected rice such as leaf blight which appears on leaves of young plants, after planting out, as pale-green to grey-green water-soaked streaks near the leaf tip and margins. In this research, during the period from 2004 to 2005, samples of infected plant were collected from different areas of Guilan province (Rasht, Lahijan, Foman, Anzaly, Talesh, Roudsarm Roudbar and Astara), to identify the causal agent of disease. For isolation of bacteria, infected tissue of leaves, stems and roots were crushed in pepton water then 100 µl of homogenate were cultured on nutrient agar (NA) and yeast dextrose carbonate (YDC) containing cyclohexamid antibiotic (50 µg/ml). Isolates of bacteria rod-shaped, gram negative bacteria and aerobic bacterium were obtained. The former isolates produced levan on media including sucrose. All isolates induced hypersensitive reaction (HR) on tobacco and geranium leaves. All of the isolated bacteria were oxidase, nitrate, urease, Tween 80 hydrolysis and indole negative and could not produce rot on potato tuber slices, produced H_2S and grew in 36°C. The isolates could use citrate, L-lysin and cystein. The isolates produced acid from arabinose, galactose, myo-inositol, fruoctose, trehalose and mannose and hydrolyzed gelatin. Based on morphological, physiological, biochemical tests, PCR method with specific primers and pathogenicity properties, the predominant pathogenic type was identified as *X. oryzae* pv. *oryzae.* To the best of our knowledge, this is the first record of bacterial blight of rice in Iran.

Key words: *Xanthomonas oryzae* pv. *oryzae*, rice, bacterial blight, PCR, Guilan

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INTRODUCTION

Rice (*Oryza sativa*) is one of the most important crops in the world (Akhtar *et al.* 2003). It is the second major cereal crop of Iran after wheat. Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Swings *et al.* 1990) is one of the most destructive diseases of rice in Asia (Mew 1987). The disease became prominent in the 1960s, when new high yielding cultivars were first developed and introduced (Mew 1987). Yield loss ranging up to 26% has been reported on susceptible rice cultivars (Adhikari *et al.* 1994). It is particularly destructive in Asian countries. Rice is a major target for crop improvement, and several strategies to improve disease resistance in rice through genetic engineering have been proposed (Toenniessen 1991). Host-plant resistance is an important component of an integrated management program for this disease. The disease occurs at all stages of the rice crop and shows either streaks or leaf blight symptoms. If plant produces panicles, under zero tillage symptoms are essential (Akhtar *et al.* 2003). The bacterium enters through of hydathodes and wounds on the roots or leaves. Once inside the vascular system, the bacterium multiplies and moves in both directions. Spread takes place during wind and rain, but primarily in flood and irrigation water (Dath and Devadath 1983). Seed transmission is generally thought to occur to a certain extent (Hsieh *et al.* 1974), but Murty and Devadath (1984) had difficulty in demonstrating this on experimentally infected seeds, this did not give rise to infected seedlings but did introduce the bacterium into the soil. Many different races (or pathotypes) of the bacterium exist, distinguished by their behavior on differential cultivars (Mew 1987). New races appear readily and the bacterium is very variable in virulence. *X. oryzae* pv. *oryzae* population structure can be influenced by environmental changes such as seasonal variations and the presence of plant disease resistance genes in the planted rice cultivars (Mew *et al.* 1992; Adhikari and Mew 1994; Ardales *et al.* 1996). Molecular techniques such as restriction fragment length polymorphism (RFLP) analyses have also been successfully used to detect genetic variability within populations of *X. oryzae* pv. *oryzae* in the Philippines and other Asian countries (Leach *et al.* 1992; Nelson *et al.* 1994; Adhikari *et al.* 1995; Ardales *et al.* 1996; Yashitola *et al.* 1997). Host resistance is the only effective control method for the disease; therefore, breeding for resistance has been important in many countries (Ogawa *et al.* 1991; Mew *et al.* 1993). More than 21 bacterial blight resistance genes have been identified and characterized (22–24). Bacterization of seeds with fluorescent pseudomonads has been tried as a biological control method (Anuratha and Gnanamanickam 1987). The objectives of the present research was the isolation of causal agent of bacterial blight on rice in the Guilan province and identification of isolates by biochemical and nutritional methods, pathogenicity tests and PCR technique.

MATERIALS AND METHODS

Bacterial isolation from leaf blight

Rice samples were collected from fields of rice in Rasht, Lahijan, Foman, Anzaly, Talesh, Roudsarm Roudbar and Astara during 2004–2005. Isolations were made from infected leaves. From each field, four replications of 30 rice disease seedling were collected at random. Individual leaves were ground in 3 ml of sterile distilled water with a homogenizer (Pro200, Pro Scientific Inc., Monroe, CT, USA) and 100 μl of ho-

mogenate was streaked on luria pepton glucose agar (LPGA) medium, containing of cyclohexamid antibiotic (50 μg/ml). At least 30 samples were tested from each field. From each infected leaf sample, three single colonies were isolated and one isolate/field was selected as a representative for this study. For long-term storage, the purified isolates were grown in peptone sucrose and frozen at –80°C in 20% glycerol. The isolates were revived on LPGA medium for pathogenicity tests.

Pathogenicity tests on rice

Seeds of rice cultivar Khazar were sown in plastic boxes, and 3 weeks later, seedlings were transplanted to 30 cm diameter plastic pots. Rice plants were grown under greenhouse conditions for 3 months. For inoculations bacterial suspensions were prepared in 10 ml of sterile distilled water at 10^9 CFU/ml. To test the pathogenicity of the isolates, plants with fully expanded leaves were inoculated by the leaf-clipping method (Kauffman *et al.* 1973). The instrument used for inoculation of the rice plant with the bacteria were scissors. Before using the scissors they were sterilized using 70% ethanol. The scissors were dipped in the bacterial suspension and used to cut the rice plant. Lesions on leaves were observed 14 days after inoculation (Backer 2002). Individual leaves were ground in 3 ml of sterile distilled water. The suspensions were then appropriately diluted and 50-μl aliquots and spotted on duplicate LPGA plates. Control leaves were treated with sterile distilled water.

Biochemical, biological and physiological tests

Isolates were characterized on the basis of the following tests: Gram test in 3% KOH (Sulsow *et al.* 1982), oxidative/fermentative test (Hugh and Leifson 1953), production of fluorescent pigment on King's B medium (Sulsow *et al.* 1982), hypersensitive reaction (HR) in tobacco and geranium leaves (Lelliot *et al.* 1987), oxidase test, levan formation, catalase, urease, gelatin liquefaction, litmus milk, salt tolerance (5% and 7%) and gas formation from glucose. In addition, tests were performed for arginine dihydrolase, hydrogen sulfide production from peptone, reduction substance from sucrose, tyrosinase casein hydrolase, nitrate reduction, indole production, 2-ketogluconate oxidation lecitinase, starch hydrolysis, phenylalanine deaminase, aesculin and Tween 80 hydrolysis and optimal growth temperature (Schaad *et al.* 2001). The presence of DNAse was tested on DNA agar (Diagonistic Pasteur, France). Carbohydrate utilization using Ayer basal medium was carried out and the results were recorded daily up to 2–8 days (Hildebrand 1988). For each test defined in this study, a representative isolate has been deposited in the Collection Française de Bactéries Phytopathogèns (CFBP) culture collection. This reference isolate was considered as a typical isolate of *X. oryzae* pv. *oryzae.*

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS–PAGE) of total proteins

Electrophoresis of soluble proteins was carried out in a discontinuous SDS polyacrylamid gel according to the method of Laemmli (1970) with some modifications as described by Rahimian (1995). For each culture, a loopful of 24 h growth from NA plate was suspended in 50 ml King's B broth and incubated in a rotating incubator for 16 h (at 27°C, 150 rpm). The samples were then transferred into eppendorf tubes and centrifuged for 5 minutes at 10000 × g. The collected cells were washed three times

with sterile distilled water. The washed cells were stirred after the addition of 25 µl SDS sample buffer (0.06 M Tris, 2.5% Glycerol, 0.5% SDS, 1.25% β-mercaptoethanol and 0.001% bromophenol blue) and the proteins were denatured in boiling water for 5 minutes. The supernatant was then centrifuged again for 5 minutes at 10000 × g, collected in an eppendorf tube and kept at –50°C until electrophoresis was carried out. Fifty µl of soluble proteins was loaded in each well in a 13 × 17 cm polyacrylamide slab with 0.75 mm thickness. Proteins were fractionated in 10% resolving gel at a constant current of 20 mAmps for 4 h. The gel was stained in methanol, water and acetic acid (5:5:1) containing 0.5% coomassie brilliant blue G250 overnight and destained in the same solution without dye. The gel was kept in 7% acetic acid.

DNA analysis and PCR conditions

Bacterials cells, which were grown on LPGA for 24 h, were resuspended in sterile distilled water. The cell suspensions (approximately 1×10^7 CFU/ml) were boiled for 10 min and were used for PCR assaying (Manceau and Horvais 1997).

The 20 oligonucleotid XOF, 5'-ATGCCGATCACCATGCCGAT, and XOR 5'-TGGCCTTGTCGTACGAGCTC-3' were designed and tested for *X. oryzae* pv. *oryzae* (Lee *et al.* 2004). PCR amplifications were carried out with a PTC-225TM thermocycler (Mastercycler gradient, Germany). PCR reaction was performed in a 100 µl PCR mixture, 10 µl volume of boiled bacterial cells was added to 90 µl of PCR mixture contained, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 2 mM $MgCl_2$, 0.2 mM of each dNTP, and 2 uint of *Taq polymerase* (CinnaGene, Inc. Iran). Samples were amplified through 1 cycle of 94°C (5 min), followed by 25 cycles of 94°C (15 s), 62°C (15 s), and 72°C (30 s) and then 1 cycle of 72°C for 7 min in a thermal cycler (Mastercycler gradient, Germany) programmed.(Lee *et al.* 2004). Amplified DNA fragments were examined by horizantal electrophoresis in 2% agarose gel in TBE buffer (Martins *et al.* 2005) with 10 µl aliquots of PCR products. Gel was stained with ethidium bromide and photographed under UV light (312 nm).

RESULTS

Biochemical, biological and physiological tests

All 18 isolates were gram, oxidase, catalase, pectinase, arginine dihydrolase negative. All isolates produced HR on tobacco and geranium, leaf blight on rice and capable of hydrolyzing gelatin. All isolates produced acid from lactose, mannitol. The isolates of *X. oryzae* pv. *oryzae* were tested for presence of DNAse on DNA agar (Diagonistic Pasteur, France). Results from the phenotypic tests are presented in Table 1.

Pathogenicity test

All isolates of *X. oryzae* pv. *oryzae* caused leaf blight on the surface of rice leaves two weeksafter inoculation. Bacterial leaf blight appears on leaves of young plants, as pale-green to grey-green water soaked streaks near the leaf tip and margins. These lesions coalesce and become yellowish-white with wavy edges. Leaf sheaths and culms were attacked. These symptoms did not occur in the control plants.

Table 1. Phenotypic characteristics of *Xanthomonas oryzae* pv. *oryzae* strains tested

Charactristics	18 Iranian isolates	Reference isolate CFBP 2532
Gram reaction	–	–
Oxidative/Fermentative	–	–
Fluorescent pigment	–	–
HR on tobacco and geranium	+	+
Leaf blight on rice	+	+
Pectinase	–	–
Arginine dihydrolase	–	–
Levan formation	+	–
Catalase	–	+
Tween 80 hydrolysis	–	+
Oxidase	–	–
Starch hydrolysis	–	–
Gelatin hydrolysis	+	+
Aesculin hydrolysis	±	+
DNAse activity	±	+
Indole formation	–	–
H_2S from cysteine	–	–
Casein hydrolysis	±	+
Urease	±	–
MR/VP	–	–
Utilization of:		
L-lysine	–	–
Citrate	+	+
lecithinase	–	±
Growth in 5% NaCl	+	
Acid from:		
L-Arabinose	–	–
Inositol	–	–
Mannitol	+	±
Xylose	+	±
Trehalose	–	–
Maltose	+	
L-tartrate	–	–
Galactose	+	±
D-Sorbitol	±	±
Sucrose	–	–

– Negative reaction or no growth; + Positive reaction or growth; ± some isolates positive

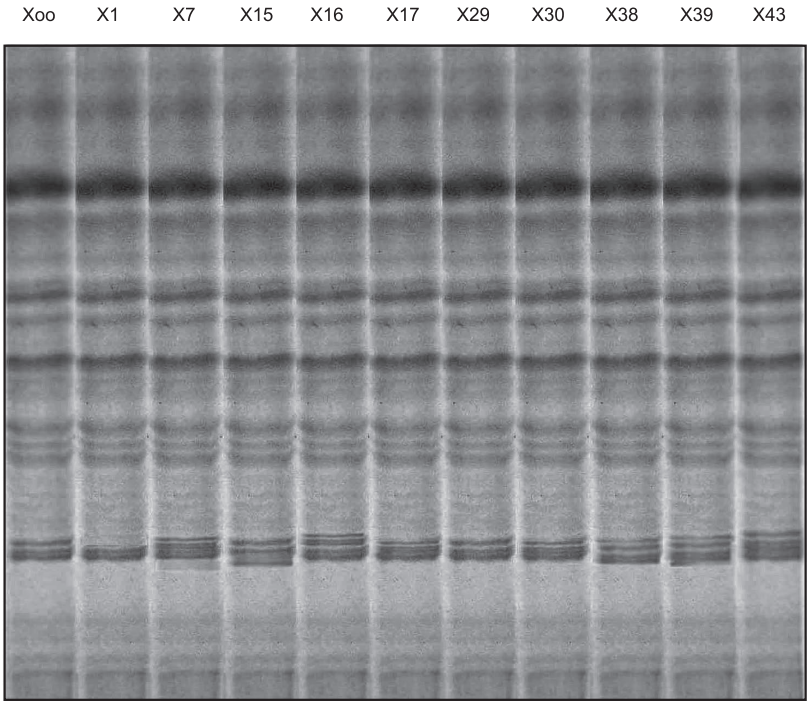

Fig. 1. Protein patterns of *X. oryzae* pv. *oryzae* isolates in comparison to the reference strain; Xoo: reference strain of *X. oryzae* pv. *oryzae* (CFBP 2532); X1, X7, X15, X16, X17, X29, X30, X38, X39 and X43: isolates from rice fields of Guilan province

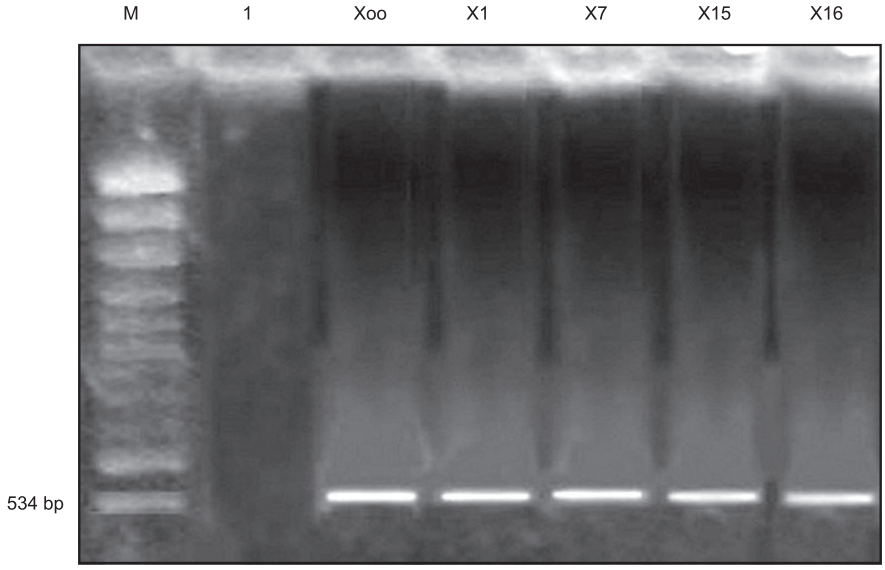

Fig. 2. Agarose gel electrophoresis of products from polymerase chain reaction (PCR) performed on DNA of *Xanthomonas oryzae* pv. *oryzaes* with sepcefic primers of Xof and Xor, M, 100 bp DNA marker; lane 1 is negative control (distilled water); lane Xoo is positive control (CFBP.2532) showing the amplification approximately 534 bp; X1, X7, X15 and X16 isolates from rice fields in Guilan privince

Protein profile

Total protein pattern of isolates were compared to reference isolate. Protein bands of isolates were nearly similar to protein bands of reference isolate (Fig. 1).

Detection of *X. oryzae* pv. *oryzae* by direct PCR

All isolates of *X. oryzae* pv. *oryzae* were identified by specific primers XOF and XOR. On agarose gel electrophoresis 2%, isolates produced a band of 534 bp (expected size). The bands of isolates were similar to those of the reference isolate reference CFBP 2532 (Fig. 2). Based on the phenotypic characters differentiation, pathogenicity and PCR tests, all 18 isolates were classified as *X.oryzae* pv. *oryzae*.

DISCUSSION

Based on morphological, phenotypical, nutritional characteristic, total protein pattern, pathogenicity tests and PCR using specific primers, we identified causal agent of bacterial blight of rice as *X. oryzae* pv. *oryzae*. All isolates of *X. oryzae* pv. *oryzae* produced blight on rice. No significant differences were observed in the degree of blight on inoculated plants. These results suggest that isolates obtained from different fields do not differ in their degree of virulence. Ochiai *et al.* (2000) demonstrated that RFLP analysis with the repetitive DNA element revealed a high level of polymorphism in the Sri Lankan isolates. Lee *et al.* (1999) characterized a large number of *X. oryzae* pv. *oryzae* isolates by pathogenicity tests and genotypic analysis. The *X. oryzae* pv. *oryzae* isolates virulent to plant containing *Xa21* (bacterial blight resistance gene**),** were distributed over a wide region and have been recovered at a high frequency in Korea since 1994, even though the *Xa21* gene has not been used in commercial rice in Korea. These findings pose questions concerning the origin and increase of isolates virulent to *Xa21*. The bands of total protein pattern of isolates were similar to the bands of the reference isolate of *X. oryzae* pv. *oryzae* CFBP 2532. Modern taxonomic techniques have clearly confirmed that the two pathovars, *X. campestris* pv. *oryzae* and *X. campestris* pv. *oryzicola* are quite distinct from each other, from *X. campestris* pathovars, and from the rice "brown blotch" pathogen (numerical analysis of phenotypic features and protein gel electrophoregrams (Vera Cruz *et al.* 1984). Swings *et al.* (1990) have reclassified them as *X. oryzae* and provide up-to-date details of their distinctive characters. PCR techniques with the use of XOF and XOR primers can be applied to detect and identify both pathogen and pathovars of *X. oryzae*. A PCR technique was successfully used to detect of *X. oryzae* pv. *oryzae*. PCR is considered as the less time consuming, cost effective, and rapid method for the detection and identification of pathogenic bacteria, although many improved methods (biochemical test, serological assays, fatty acids, and methabolic profiling) have been developed so far. So, the PCR assay using a primer set (XOF and XOR) designed from the sequence of *hpa*A gene will be a useful tool for the detection and identification of *X. oryzae* pv. *oryzae* (Lee *et al.* 2004). To our knowledge, the occurrence and incidence of this disease on rice in different geographic regions of Iran have not been studied. This is the first report of bacterial blight of rice in the north region of Iran. Study on the biological control of bacterial blight on rice by antagonistic isolates in different parts of Iran and use of resistant cultivars could be a case study for future research.

ACKNOWLEDGMENTS

We are grateful to The Rice Center of Excellence in Iran for partial support of this project.

REFERENCES

Adhikari T.B., Mew T.W. 1994. Progress of bacterial blight on rice cultivars carrying different *Xa* genes for resistance in the field. Plant Dis. 78: 73–77

Adhikari T.B., Vera Cruz C.M., Zhang Q., Nelson R.J., Skinner D.Z., Mew T.W., Leach J. E. 1995. Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. Appl. Environ. Microbiol. 61: 966–971.

Akhtar M.A, Zakeri M., Abassi F.M. 2003. Inoculum build up of bacterial blight of rice in rice-wheat cropping area of Punjab in relation to zero tillage? Asian J. Plant Sci. 2: 548–550.

Anuratha C.S., Gnanamanickman S.S. 1987. *Pseudomonas fluorescens* suppresses development of bacterial blight symptoms. International Rice Research Newsletter 12: 1, 17.

Ardales E.Y., Leung H., Vera Cruz C.M., Mew T.W., Leach J.E., Nelson R.I. 1996. Hierarchical analysis of spatial variation of the rice bacterial blight pathogen across diverse agroecosystems in the Philippines. Phytopathology 86: 241–252.

Backer D. 2002. Method for Inoculating Rice with *Xanthomonas*. Plant Pathology Laboratory, Iowa State University Dr. Adam Bogdanove.

Dath A.P., Devadath S. 1983. Role of inoculum in irrigation water and soil in the incidence of bacterial blight of rice. Indian Phytopathol. 36: 142–144.

Hildebrand D.C. 1988. Pectate and pectin gel for differentiation of *Pseudomonas* sp. and other bacterial plant pathogens. Phytopathology 61: 1430–1439.

Hsieh S.P.Y., Buddenhagen I.W., Kauffman H.E. 1974. An improved method for detecting the presence of *Xanthomonas oryzae* in rice seed. Phytopathology 64: 273–274.

Hugh R., Leifson E. 1953. The taxonomic significance of fermentative versus oxidative methabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. 66: 24–26.

Kauffman H.E., Reddy A.P.K., Heisk S.P.V., Maraca S.D. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis. Rep. 57: 537–541.

Laemmli V.K. 1970. Cleavage of Structural Proteins During Assembly of the Head of Bacteriophage T4. Nature 227: 680–685.

Leach J.E., Rhoads M.I., Vera Cruz C.M., White F.F., Mew T.W. 1992. Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* with a repetitive DNA element. Appl. Environ. Microbiol. 58: 2188–2195.

Lee S.W., Choi S.H., Han S.S., Lee D.G., Lee B.Y. 1999. Distribution of *Xanthomonas oryzae* pv. *oryzae* strains virulent to *Xa21* in Korea. Phytopathology 89: 928–933.

Lee B.M., Young J.P., Dong S.P., Jeong G.K., Hee W.K., Tae H.N., Gilb L., Joung K.A. 2004. PCR-Base sensitive detection and identification of *Xanthomonas oryzae* pv. *oryzae*. Korean J. Microbiology 32: 256–264.

Lelliot R.A., Stead D.E. 1987. Methods for the diagnosis of bacterial disease of plant. Blackwell Scientific Pub. London.

Manceau C., Horvais A. 1997. Assessment of genetic diversity among strains of *Pseudomonas syringae* by PCR restricion fragment length polymorphism analaysis of rRNA operon with special emphasis on *P.syringae* pv. *tomato*. App. Environ. Microbiol. 63**:** 498–505.

Martins M.L., Araújo E.F., Mantovani H.C., Moraes C.A., Vanetti M.C.D. 2005. Detection of the apr gene in proteolytic psychrotrophic bacteria isolated from refrigerated raw milk. International J. Food Microbiol. 102: 203–211.

Mew T.W. 1987. Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol. 25: 359–382.

Mew T.W., Alvarez A.M., Leach J.E., Swing J. 1993. Focus on bacterial blight of rice. Plant Dis. 77: 5–12.

Mew T.W., Vera Cruz C.M., Medalla E.S. 1992. Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in the Philippines. Plant Dis. 76: 1029–1032.

Murty V.S.T., Devadth S. 1984. Role of seed in survival and transmission of *Xanthomonas campestris* pv. *oryzae* causing bacterial blight of rice. Phytopathologische Zeitschrift. 110: 15–19.

Nelson R.J., Baraoidan M.R., Vera Ceuz C.M., Yap I.V., Leach J.E., Mew T.W., Leung H. 1994. Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. Appl. Environ. Microbiol. 60: 3275–3283.

Ochiai H., Horino O., Miyajima H., Kaku H. 2000. Genetic Diversity of *Xanthomonas oryzae* pv. *oryzae* Strains from Sri Lanka. J. Bacteriology 90: 415–421.

Ogawa T. 1993. Methods and strategy for monitoring race distribution and identification of resistance genes to bacterial leaf blight (*Xanthomonas campestris* pv. *oryzae*) in rice. Jpn. Agric. Res. Q. 27: 71–80.

Ogawa T., Yamamoto T., Khush G.S., Mew T.W. 1991. Breeding of near-isogenic lines of rice with single genes for resistance to bacterial blight pathogen (*Xanthomonas campestris* pv. *oryzae*). Jpn. J. Breed. 41: 523–529.

Rahimian H. 1995. The occurrence of bacterial red streak of sugarcane caused by *Pseudomonas syringae* pv. *Syringae.* Iran. J. Phytopathol. 143: 321–324.

Sambrook J., Russel D.W. 2001. Molecular Cloning: a laboratory manual. Vol.2. Third Edition. NY, USA: Cold Spring Harbor Laboratory Press. p. 1659.

Schaad N.W., Jones J.B., Chun W. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. Thrid ed. APS. St. Paul. Minnesota, USA, 373 pp.

Sulsow T.V., Schorth M.N., Saka M. 1982. Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathol. 72: 917–918.

Swings J., Van Den Moore M., Vauterin L., Hoste B., Gillis M., Mew T.W., Kerster 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 pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. rev. Inst. J. Syst. Bacteriol. 40: 309–311.

Toenniessen G.H. 1991. Potentially useful genes for rice genetic engineering. p. 253–280. In: "Rice Biotechnology" (G.S. Khush , G.H. Toenniessen, eds.). CAB International, Wallingford, UK.

Vera Cruz C.M.V., Gossele F., Kersters K., Segers P., Van den Mooter M., Swings J., Ley J. D. 1984. Differentiation between *Xanthomonas campestris* pv. *oryzae*, *Xanthomonas campestris* pv. *oryzicola* and the bacterial "brown blotch" pathogen on rice by numerical analysis of phenotypic features and protein gel electrophoregrams. J. General Microbiol. 130: 2983–2999.

Yashitola J., Krishnaveni D., Reddy A.P.K., Sonti R.V. 1997. Genetic diversity within the population of *Xanthomonas oryzae* pv. *oryzae* in India. Phytopathology 87: 760–765.

POLISH SUMMARY

IZOLACJA I IDENTYFIKACJA *XANTHOMONAS ORYZAE* PV. *ORYZAE*, SPRAWCY BAKTERYJNEJ ZARAZY RYŻU W IRANIE

Bakteryjna zaraza wywoływana przez *Xanthomonas oryzae* pv. *Oryzae* jest jedną z ważnych chorób ryżu. Na porażonych roślinach ryżu powstają typowe objawy, takie jak zgorzel liści, która pojawia się na młodych roślinach po ich wysadzeniu. Są to jasno-zielone do szaro-brązowych wodniste smugi tworzące się w pobliżu wierzchołka oraz brzegów liści. W toku badań prowadzonych od 2004 do 2005 roku zbierano próby porażonych roślin ryżu z różnych rejonów w prowincji Guilan (Rasht, Lahijan, Foman, Anzaly, Talesh, Roudsarm Roudbar i Astara) w celu określenia sprawcy choroby. Aby izolować bakterie, porażoną tkankę lisci, łodyg i korzeni miażdżono w roztworze wodnym peptonu, nastepnie 100 µl homogenatu inkubowano na agarze odżywczym i pożywce węglanowo-dekstrozowej z wyciągiem drożdżowym oraz antybiotykiem (cyklohexamid, 50 $\mu g/ml^{-1}$). Izolowano pałeczkowate, gram-ujemne beztlenowe bakterie oraz bakterię tlenową. Pałeczki beztlenowe wytwarzały lewan na pożywce zawierającej sacharozę. Wszystkie te izobaty wywoływały reakcję nadwrażliwości (HR) na lisciach tytoniu i geranium, nie wytwarzały oksydazy, ureazy, nie rozkładały odczynnika Tweed 80 i indolu i nie powodowały zgnilizny na krojonych ziemniakach, wytwarzały H_2S i rosły w temperaturze 36°C. Ponadto wszystkie izolaty zużywały cytrynian, L-lizynę i cysteinę, wytwarzały kwas z arabinozy, galaktozy, myo-inozytolu, fruktozy, trehalozy, mannozy oraz hydrolizowały żelatynę. Na podstawie cech morfologicznych, fizjologicznych, biochemicznych oraz wyników reakcji PCR, w której użyto specyficzne startery, a także testów patogeniczności, sprawcę choroby określono jako *Xantomonas oryzae* pv. *oyzae*. Artykuł jest według autorów pierwszym doniesieniem o wystepowaniu w Iranie bakteryjnej zarazy ryżu.