Azmat S *et al.* (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 2, Article number 13547
DOI:10.15835/nbha52213547
Research Article

Morphogenetic characterization of *Stenotrophomonas maltophilia* infecting white stripe disease of rice (*Oryza sativa* L.)

Saira AZMAT[1a], Kashif RIAZ[2b], Ansa DILFEROZE[3], Akhtar HAMEED[4]*, Sajid FIAZ[5]*, Kotb A. ATTIA[6], Muhammad N. SUKHERA[3], Asmaa M. ABUSHADY[7,8], Muhammad W. ALAM[9], Subhan ALI[4]

[1]*Agriculture Extension and Adaptive Research, Agriculture Department, Government of the Punjab, Lahore 54000, Pakistan; sairaazmat786@gmail.com*
[2]*Director Smart Agriculture, Technologie Culture Intelligente Ferme D'Hiver Winter Farm, Brossard, Canada; kashifriaz@gmail.com*
[3]*University of Agriculture, Department of Plant Pathology, Faisalabad, 38000, Pakistan; Ansa.dilferoze@yahoo.com; nawazsukhera1@gmail.com*
[4]*Institute of Plant Protection, MNS University of Agriculture Multan 61000, Pakistan; akhtar.hameed@mnsuam.edu.pk (*corresponding author); subhanali@gmail.com*
[5]*University of Haripur, Department of Plant Breeding and Genetics, 22620 Haripur, Pakistan; sfiaz@uoh.edu.pk (*corresponding author)*
[6]*King Saud University, College of Science, Department of Biochemistry, P.O. Box 2455, Riyadh 11451, Saudi Arabia; kattia1.c@ksu.edu.sa*
[7]*Nile University, Biotechnology School, 26th July Corridor, Sheikh Zayed City, Giza, 12588, Egypt; aabushadyasmaa@agr.asu.edu.eg*
[8]*Ain Shams University, Agriculture College, Department of Genetics, Cairo, Egypt*
[9]*University of Okara, Department of Plant Pathology, 56300, Pakistan; waqar.alam@outlook.com*
[a,b]*These authors contributed equally to the work*

Abstract

Rice is a major cereal crop which ensure food security to more than half of the global population. Several biotic factors impact rice grain quality and its final production. White stripe disease, caused by pathogen *Stenotrophomonas maltophilia* is considered among the major limiting factor for reducing rice yields and quality. Present study was performed to understand the white stripe disease, which has been frequently misdiagnosed as bacterial leaf blight (BLB) due to similar symptoms. A survey was carried out based on accessibility and farmer participation to monitor incidence and sample collection. The survey was conducted in districts Faisalabad, Gujranwala, Sialkot, Sheikhupura, and Hafizabad, these districts were selected for their importance for rice cultivation in Pakistan. The total sample size was around 500 leaves distributed evenly throughout each study area. The results of study indicated presence of new pathogen of rice. These isolates were biochemically identified and confirmed by gram staining negative, 3% KOH positive, 5% salt tolerance positive, oxidase test negative, catalase test positive, starch hydrolysis test negative, nitrate reductase test positive, indole test negative, lactose test positive, maltose test positive, methyl red test negative, Voges-prokauer test negative and urea hydrolysis test negative. The pathogenicity test was confirmed that pathogen

Received: 05 Dec 2023. Received in revised form: 11 Mar 2024. Accepted: 03 Jun 2024. Published online: 28 Jun 2024.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

and the Sialkot isolate were the most aggressive isolate among the five isolates collected form the studied areas. The molecular characterization was accomplished by PCR and sequencing. The results of the phylogenetic study indicate that this pathogen belongs to a distinct group, as it is distantly related to *Xanthomonas oryzae* pv. *oryzae* (Xoo). This study provides important findings into a novel clade of pathogen causing white stripe disease in rice.

Keywords: bacterial leaf blight; disease resistant; epidemiology; pathogen; plant health; phylogenetic

Introduction

Rice is a major staple food that feeds over half of the world's population (Mohidem *et al*., 2022). It is an important crop around the world, accounting for more than 21% of human caloric requirements and up to 76% of Southeast Asian calorific intake (Zhao *et al*., 2020). China leads the rice production with 148.3 metric ton (Mt), followed by India 120 Mt, Bangladesh 35.3 Mt, Indonesia 34.9 Mt, and Vietnam 27.1 Mt (USDA, 2022). However, Pakistan's production is much lower and accounted about 7.60 Mt. Asia accounts for 90.6% of worldwide rice output, making it the world's leading producer (Bandumula, 2018).

Rice production faces several challenges including climate change, soil degradation, new emerging pathogens and poor nutrient management (Jamal *et al*., 2023), causing substantial decline in grain yield and quality (Ashfaq *et al*., 2022). Pakistan has reported numerous biotic (bacterial leaf blight, white stripe, rice stripe virus, leaf spot and rice blast) resistant rice varieties, (Brooke, 2012; Ashfaq *et al*., 2022). However, White stripe of rice caused by *Stenotrophomonas maltophilia* is an emerging global pathogen that cause serious infections in humans as well i.e., immunocompromised individuals (Brooke, 2012). It also causes significant damage to rice crops, leading to reduction in quality and yield which ultimately develop negative impact on the economy of the country (Hu *et al*., 2021). This disease exhibited symptoms that closely resemble with the bacterial leaf blight (BLB) of rice. The disease appears as white stripes on the leaves of the rice (Hu *et al*., 2021) and under severe cases it may cause approximately 20 percent of yield reduction. However, the percentage of yield losses may depend on number of variables i.e., crop stage, plant health, severity of infection and the environmental conditions (Riaz *et al*., 2016).

It has been reported from China that *Stenotrophomonas maltophilia* also causes Sanqi root soft rot disease in rice (Zheng *et al*., 2022). Sanqi root soft rot is characterized by straight rods of 0.5 by 1.5 µm in size and requires methionine for their growth. The bacterium is motile, reduces nitrate and catalase-positive (Brooke, 2012; Duan *et al*., 2020). The epidemiology of bacterium is complex and multidrug-resistant which makes it difficult to control (Hafiz *et al*., 2022). To manage this disease farmer utilized several methods, including cultural, biological, crop rotation and chemical control (Mojica *et al*., 2019). Moreover, proper land preparation and soil management are also helpful to prevent the spread of the disease (Cruz-Córdova *et al*., 2020). However, there is no effective control for this disease and researchers are working to develop disease-resistant rice varieties (Cruz-Córdova *et al*., 2020). The primary objective of this study was to explore and characterize a novel clade of pathogen causing white stripe disease in rice. The survey covered major rice growing regions i.e., Faisalabad, Gujranwala, Sialkot, Sheikhupura and Hafizabad of Punjab, Pakistan. The plants exhibiting symptoms such as water-soaked lesions, characterized by a greyish white coloration at the leaf margins were collected. Additionally, leaves showing BLB disease symptoms were also collected. The preceding data provides compelling evidence for the need of early diagnosis, enabling timely implementation of effective management strategies.

Materials and Methods

Survey and sample collection

A survey was carried out based on accessibility and farmer participation to monitor disease incidence and collection of white stripe disease samples from districts Faisalabad, Gujranwala, Sialkot, Sheikhupura and Hafizabad, Punjab Pakistan based on their importance for rice cultivation. The total sample size was around 500 leaves distributed evenly throughout each sampling area (Figure 1). The plants exhibiting symptoms such as water-soaked lesions, characterized by a greyish white coloration at the leaf margins were collected in brown paper bags. Additionally, leaves showing BLB disease symptoms i.e., water-soaked lesions, marginal bacterial ooze on infected leaves, were also be collected to observe the difference between both diseases. These samples were brought to the laboratory for isolation and stored in the refrigerator at 4 °C until further processing.

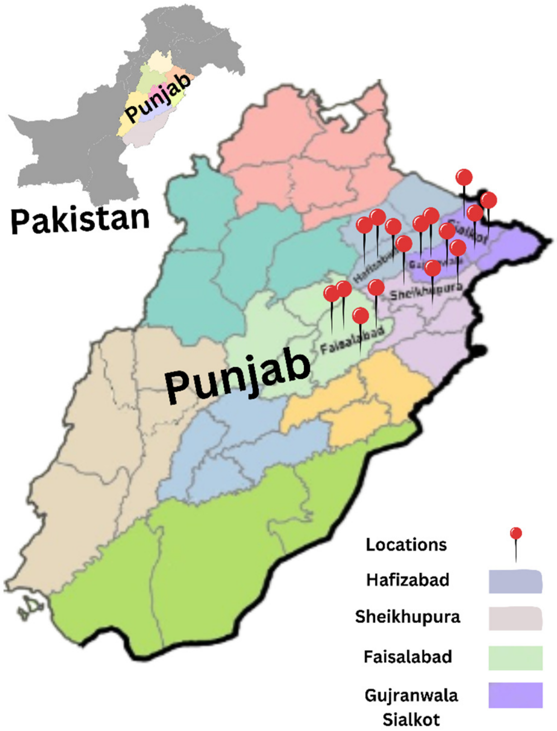

Figure 1. Location of survey areas for sampling of diseases infected leaves

Isolation and purification and multiplication of the pathogen

Nutrient agar (NA) media was prepared to isolate, multiply and purify the pathogen. A weighed amounts of water and nutrient agar were added in the conical flask and stirred to make homogenous mixture and covered with cotton plug, later autoclaved at 121 °C and 15 psi for 15 minutes along with petri plates. The disease samples were cut into 2-3 mm size with infected part and surface sterilized by dipping into 70% ethanol for 1 minute and dipped in distilled water for 2 minutes and placed for air dry in the laminar air flow. Samples were placed on already poured NA media plates, plates were wrapped and carefully labelled with date and sample number and placed into incubator at 28 °C for 24 hours. The observed bacterial colonies around the sample of rice were streaked on fresh NA media plate and again placed in the incubator at 28 °C. After re-streaking, the pure colonies of bacterium were observed on NA media plates and preserved in 50% glycerin solution at 4 °C for further evaluation.

Biochemical and physiological tests

All the isolates were identified morphologically by observing their colour, type of colony and performed biochemical tests viz., Gram staining, 3% KOH test, (Holt *et al*., 2000; Mubeen *et al*., 2015) 5% NaCl salt tolerance test, starch hydrolysis, oxidase test, nitrate reduction test, Indole test, Maltose test, Lactose test, Voges-Proskauer test, Methyl Red test and Urea hydrolysis test, respectively.

Pathogenicity test

The confirmation of the bacterial isolates' pathogenicity was undertaken by fulfilling Koch's postulates (Juhasz et al. 2013). To prepare the bacterial inoculum, the bacterial culture was grown at a 28 °C for 16 hours at 250 rpm. The obtained bacterial suspension was subjected to centrifugation and optimized to a final concentration of 10^8 cfu/ml with a spectrophotometer (Hoque and Mansfield, 2005). Rice seeds were sown in pots and inoculations were done by clip and dip method and pots were placed under greenhouse condition. After 15 days of inoculation data were recorded and re-isolation of the bacterium from disease showing leaves was performed and observed bacterial colonies to fulfil the Koch's postulate. The entire experiment was undertaken in greenhouse conditions.

Evaluation of most aggressive strain

Establishment of nursery

Nursery of a single rice variety, KSK-515 was brought from Rice Research Institute, Kala Shah Kaku, 31°44'26.36"N and 74°15'11.33"E Punjab, Pakistan.

Clip and dip inoculation

Each strain bacterial suspension was prepared in Nutrient broth (NB) media by shaking at 28 °C for 16 hours at 250 rpm.

Data recording

Data was recorded after every 6 days intervals of inoculation for three times (Table 1).

Table 1. Disease aggressive index scale by lesion length

Scale	Lesion length (cm)	Aggressivity Index
0	0	A-virulent
1	1-5	Least aggressive
2	6-10	Less aggressive
3	10.1-15	Moderately aggressive
4	15.1-20	Highly aggressive
5	20.1-25	Severely aggressive

Molecular characterization

DNA Extraction and PCR

The DNA of bacterial isolate was extracted by using modified CTAB method and DNA pellets were washed with ethanol and dried for PCR reaction (Iqbal *et al*., 2021). For amplification, specific primers were used. In PCR protocol denaturation (94 °C), annealing (50 °C) and extension (68 °C) was adjusted for 1 minute, 1 minute and 2 minutes for 34 cycles, respectively and a final auto-extension step of 72 °C for 5 minutes. After staining with ethidium bromide (100 µg/ml) was used, the amplified DNA was separated on a 1.0% (w/v) agarose gels (0.5× TBE buffer) and subsequently observed under ultraviolet trans-illuminator.

Statistical analysis

Completely Randomized Design (CRD) was used to measures the leaf area affected (LAA) percentage at that time in in-*vivo* conditions. The analysis of variance (ANOVA) on recorded data were performed at 5% significance level. Fisher's Least Significant Difference (LSD) test was used in context to ANOVA to compare the mean of all isolates formed lesion length (Steel *et al*., 1997).

Results

Survey and isolation

A sum of 150 isolates were isolated from collected samples for White Stripe and BLB. The relative occurrence of both diseases was determined by examining the isolated colonies. The twenty-seven isolates formed small, yellow, raised and circular colonies of *Stenotrophomonas maltophilia* (Figure 2), 99 isolates formed small, pale yellow and circular colonies of the *Xanthomonas oryzae* pv. *oryzae* (Xoo), 17 isolates formed small and irregular shape colonies of both the diseases and remaining 9 samples gave some unknown pathogen on NA media plates (Figure 3). *Stenotrophomonas maltophilia*, the causal agent of white stripe of rice were isolated from 18 percent of the total samples collected (Figure 4).

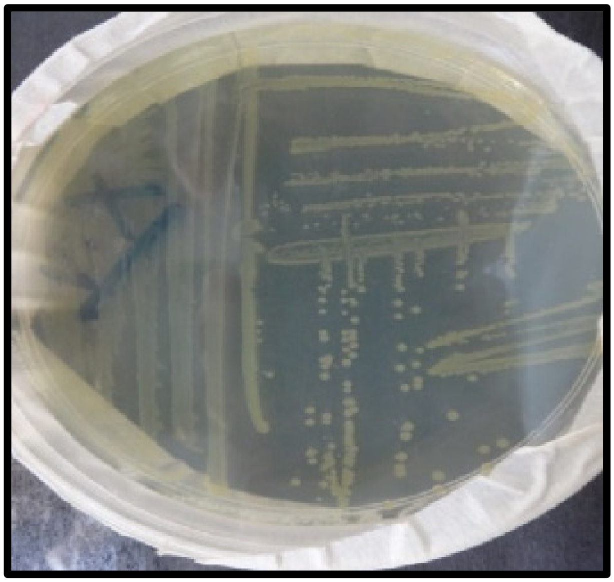

Figure 2. Sia-5 isolate colonies colour and shape

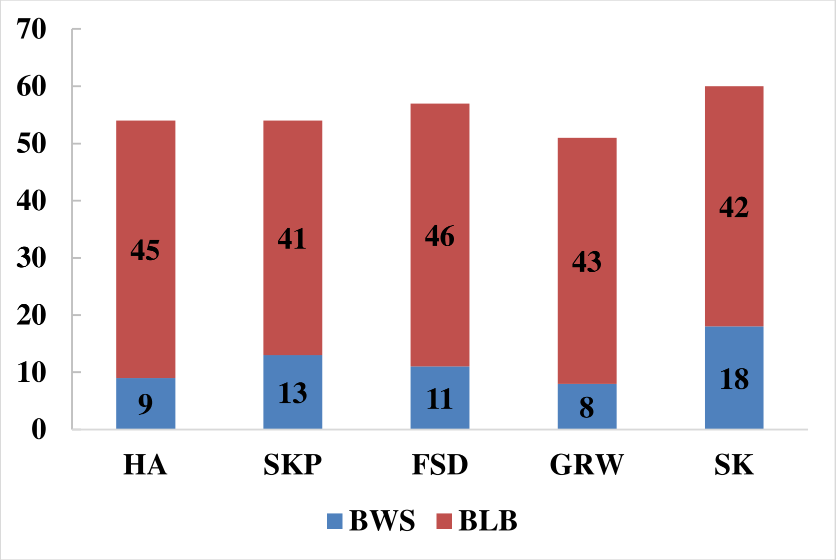

Figure 3. Relative percentage of BWS infections in Hafizabad (HA), Sheikhupura (SKP), Faisalabad (FSD), Gujranwala (GRW) and Sialkot (SK)

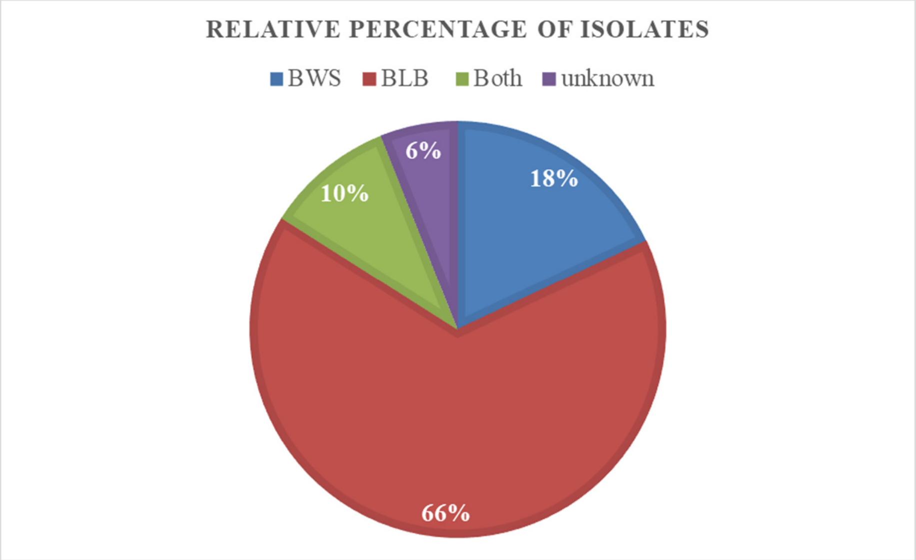

Figure 4. Relative percentage of isolates in all the five districts of Punjab under survey

Biochemical tests

For identification and biochemical characterization, the bacterial isolates were subjected to different biochemical tests. The isolates were determined to be gram negative by showing pinkish colour (Figure 5), 3% KOH positive by producing thread like string, 5% salt tolerance positive by showing salt sensitive, oxidase test negative by showing dark purple colour, catalase test positive by showing bubbles formation, starch hydrolysis test negative by not producing zone, nitrate reductase test positive by showing the red colour, indole test negative by showing the yellow colour of reagent layer, lactose test positive by shows a rise in hydrogen content, maltose test positive by showing colour change from red to yellow, methyl red test negative by showing yellow colour, Voges-prokauer test negative by showing yellow colour at the top of tube and urea hydrolysis test negative by showing yellowish colour.

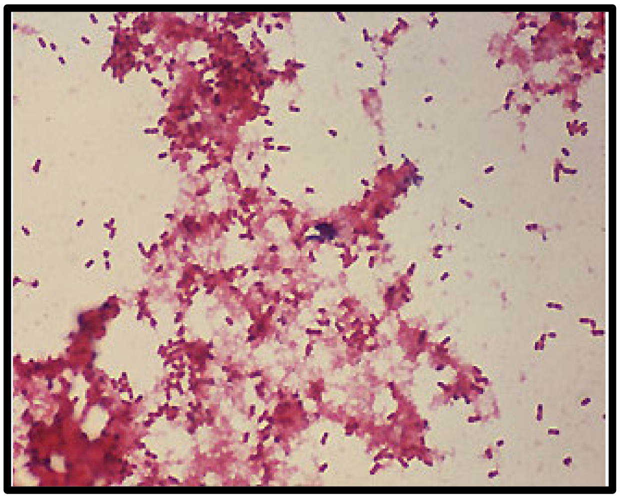

Figure 5. Sia-5 isolate result of Gram Staining under microscope

Pathogenicity test confirmation by fulfilment of Koch's postulates

After 15 days of inoculation, all the plants exhibited disease symptoms and re-isolation of the bacterium from disease symptoms were performed and observed bacterial colonies to fulfil the Koch's postulate.

Evaluation of most aggressive isolate of S. maltophilia through Clip and Dip inoculation method

After 6, 12 and 18 days of inoculation, all the plants exhibited disease symptoms and the data was noted by measuring the LAA percentage. The Sialkot isolate exhibited the highest level of aggressiveness compared to the other four isolates (Figure 6 and Table 2).

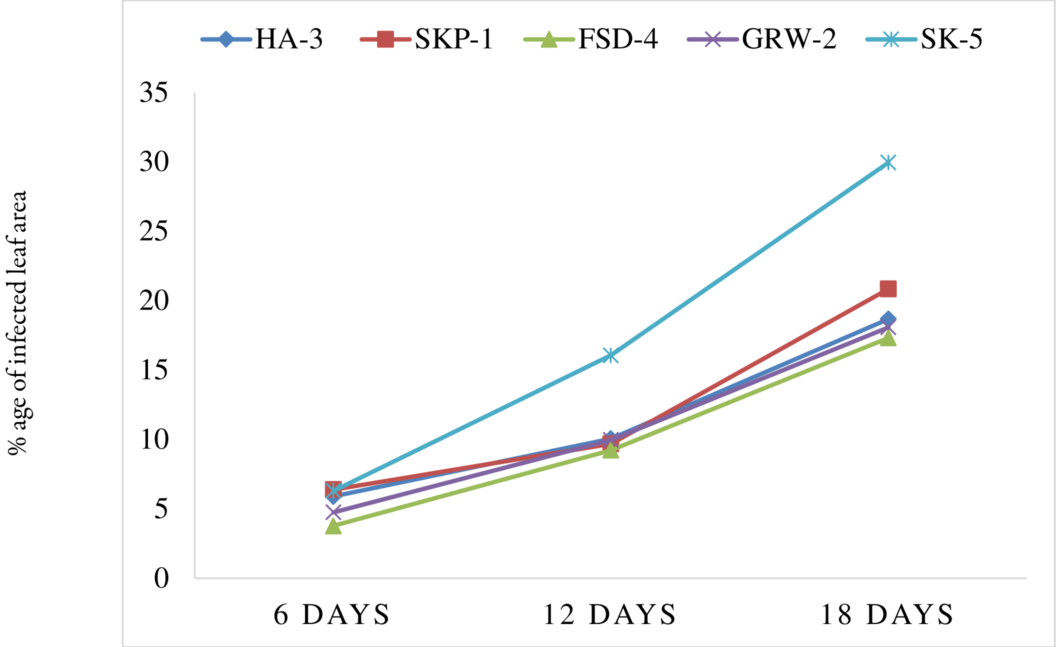

Figure 6. Aggressiveness of Sia-5 isolates of surveyed samples collected from different Hafiz Abad, Sheikhupura, Faisalabad, Gujranwala and Sialkot etc at three different intervals

Table 2. Lesion Length formed by isolates in 6, 12 and 18 days

Isolate	Lesion length (cm)			Average
	6 days	12 days	18 days	
HF-3	5.9	10.05	18.69	11.54
Shk-1	6.4	9.7	20.85	12.31
Fsd-4	3.78	9.23	17.32	10.11
Gjr-2	4.76	9.98	18.08	10.94
Sia-5	6.3	16.06	29.96	17.44

Molecular characterization

DNA was extracted through the process of lysing the bacterial pellets and universal primers were utilized for DNA amplification using PCR. This PCR product was then subjected to gel electrophoresis under fluorescent light. The identified DNA was commercially sequenced (Figure 7).

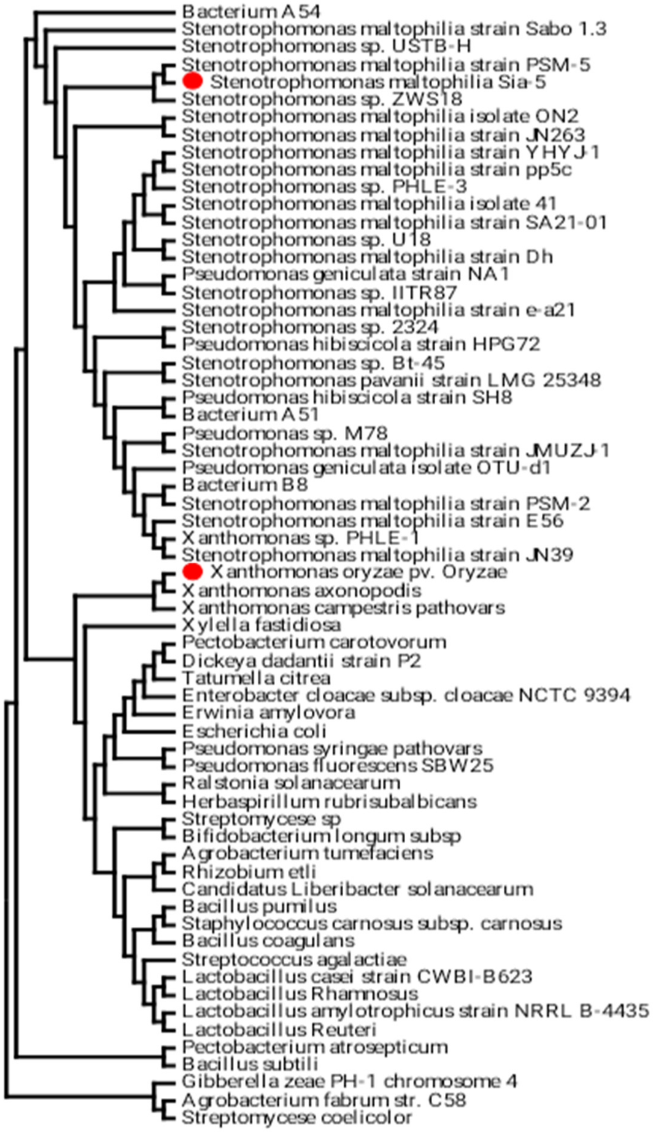

Figure 7. Dendrogram representing genetic relationships between isolates

Discussion

Rice production faces several challenges that can decrease its quality and production. These challenges are diverse in nature and can be caused by various factors. Pakistan has reported several diseases affecting rice crops, including bacterial leaf blight, white stripe, rice stripe virus disease, and leaf spot. White stripe of rice caused by *Stenotrophomonas maltophilia* is an emerging global opportunistic pathogen causing serious infection in humans (Brooke, 2012). The disease-causing white stripes to appear on the leaves of the rice plants, leads toward reduction in both yield and quality, which may have a negative impact net return (Hu *et al.*, 2021).

The primary objective of this study was to explore and characterize newly emerging disease in major rice growing regions of Punjab i.e., Faisalabad, Gujranwala, Sialkot, Sheikhupura and Hafizabad, respectively. Thorough examination of the plants during surveys it was observed the existence of additional symptoms along with BLB. However, these symptoms exhibit a significant similarity to the symptoms of BLB disease. Out of 150 isolates from collected samples, 37 developed small, yellow, elevated, and circular colonies, 99 formed light yellow and circular colonies, and 16 formed small and irregular colonies on NA medium plates. The isolates were determined to be gram negative by showing pinkish colour, 3% KOH positive by producing thread like string, 5% salt tolerance positive by showing salt sensitive, oxidase test negative by showing dark purple colour, catalase test positive by showing bubbles formation, starch hydrolysis test negative by not producing zone, nitrate reductase test positive by showing the red colour, indole test negative by showing the yellow colour of reagent layer, lactose test positive by shows a rise in hydrogen content, maltose test positive by showing colour change from red to yellow, methyl red test negative by showing yellow colour, Voges-prokauer test negative by showing yellow colour at the top of tube and Urea hydrolysis test negative by showing yellowish colour of the medium. Similar results were observed during the KOH and gram staining test performed by the Jonit *et al.* (2016) that confirmed the *Xoo* bacterium were gram-negative exhibiting pinkish colour under the microscope, respectively. Tests of both *Xoo* and *Stenotrophomonas maltophilia* were revealed that both pathogens exhibit similar behaviour under identical conditions.

To fulfill Koch's postulates all isolates were evaluated for a pathogenicity test. It has been shown that the plants exhibited identical symptoms to those observed in samples collected from the field. Following the pathogenicity test, Sialkot strain exhibited the highest level of aggression compared to the other four isolates. The complete identification was achieved through PCR. The PCR product was viewed under fluorescent light following gel electrophoresis and pure product was commercially sequenced. This sequence was evaluated using NCBI BLAST procedure and it was found the sequence was distinct from the rice BLB pathogen *Xoo*. The BLAST result showed similarity with *Stenotrophomonas* spp. and confirmed that the disease was caused by the *Stenotrophomonas maltophilia.* The highly similar sequences were downloaded and phylogenetic tree analysis were undertaken and it was observed this novel pathogen is not among the rice pathogens and found different from the *Xoo*. It exists between *Stenotrophomonas* spp, hence this pathogen was a new addition tonovel clade of rice pathogens. The present study was in line with research done in by Singh *et al.* (2014) who studied at the manner and confirmed that *Stenotrophomonas maltophilia* is the causal organism of white stripe disease of rice. It was also confirmed that the *S. maltophilia* is the casual organism of white stripe disease of rice in India and postharvest fruit rot of Lanzhou lily (Hu *et al.*, 2021).

Conclusions

This study helped in identification of a previously unnoticed disease known as White Stripe, which has been frequently misdiagnosed as BLB due to similar symptoms. White Stripe was found to be present in approximately 18% of rice-growing areas surveyed in Punjab, including districts like Hafizabad, Sheikhupura, Sialkot, Gujranwala, and Faisalabad of Pakistan. The isolated pathogen causing White Stripe was identified as

Stenotrophomonas maltophilia, and it helped to distinguished it from common rice pathogen *Xoo*. The phylogenetic analysis indicated that this pathogen represents a novel clade among plant pathogenic bacteria affecting rice. Based on these results, it has been recommended that further research and monitoring efforts should be dedicated to study White Stripe disease's epidemiology and implementation of disease management strategies, which may differ from those used for BLB, to minimize its impact on rice yields.

Authors' Contributions

AH: designed the study; MNS, KR helped SA to perform the experiments and analysis; AD, AH, SF, KAA, AMA, MWA, FA and SA helped in data analysis, software standardization and writing of original draft; AD, AH, SF, KAA and AMA provided technical expertise to improve the article and helped in funding acquisition. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors extend their appreciation to the Researchers Supporting Project number (RSP-2024R369), King Saud University, Riyadh, Saudi Arabia.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Ashfaq M, Rasheed A, Ali M, Sajjad M, Rasool B, Shaheen S, … Mubashar U (20220. Identifying and characterizing the main causal pathogen responsible for rice grain discoloration in Pakistan. Pakistan Journal of Agricultural Sciences 59(2).

Bandumula N (2018). Rice production in Asia: Key to global food security. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 88:1323-1328. *http://dx.doi.org/10.1007/s40011-017-0867-7*

Brooke JS (2012). *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. Clinical Microbiology Reviews 25(1):2-41. *https://doi.org/10.1128/cmr.00019-11*

Cruz-Córdova A, Mancilla-Rojano J, Luna-Pineda VM, Escalona-Venegas G, Cázares-Domínguez V, Ormsby C, Xicohtencatl-Cortes J (2020). Molecular epidemiology, antibiotic resistance, and virulence traits of *Stenotrophomonas maltophilia* strains associated with an outbreak in a Mexican tertiary care hospital. Frontiers in Cellular and Infection Microbiology 10:500783. *https://doi.org/10.3389/fcimb.2020.00050*

Duan Z, Qin J, Liu Y, Li C, Ying C (2020). Molecular epidemiology and risk factors of *Stenotrophomonas maltophilia* infections in a Chinese teaching hospital. BMC Microbiology 20:1-9. *https://doi.org/10.1186/s12866-020-01985-3*

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (2000). Bergey's Manual of Determinative Bacteriology (IX ed). Lippincott Williams and Wilkins, Philadelphia, U.S.A.

Hoque ME, Mansfield JW (2005). A simple and reliable method for pathogenicity tests of bacterial blight disease of rice. Bangladesh Journal of Botany 34:11-16.

Hu M, Li C, Xue Y, Hu A, Chen S, Chen Y, Zhou J (2021). Isolation, characterization, and genomic investigation of a phytopathogenic strain of *Stenotrophomonas maltophilia*. Phytopathology 111(11):2088-2099. *https://doi.org/10.1094/phyto-11-20-0501-r*

Iqbal M, ul Haq ME, Kamran M, Idrees M, Nazir S, Ullah I, Naz S, Ali S, Iqbal MZ (2021). Morpho-molecular characterization of *Xanthomonas axonopodis* Pv. *Citri* associated with kinnow (Mandarin) and its management. Pakistan Journal of Agricultural Research 34(1):8.

Jadhav RR, More AS, Apet KT, Dandnaik BP (2018). Cultural and bio-chemical characterization of *Xanthomonas axonopodi* spv. *citri*: causing citrus canker. International Journal of Current Microbiology and Applied Sciences 6:1682-1687.

Jamal MR, Kristiansen P, Kabir MJ, Lobry de Bruyn L (2023). Challenges and adaptations for resilient rice production under changing environments in Bangladesh. Land 12(6):1217. *https://doi.org/10.3390/land12061217*

Jonit NQ, Low YK, Tan GH (2016). *Xanthomonas oryzae* pv. *oryzae*, biochemical tests, rice (*Oryza sativa*), bacterial leaf blight (BLB) disease, Sekinchan. Journal of Applied & Environmental Microbiology 4:63-69. *https://doi.org/10.12691/jaem-4-3-3*

Juhasz CC, Leduc A, Boyer C, Guérin F, Vernière C, Pruvost O, Wonni I, Ouédraogo L (2013). First report of *Xanthomonas citri* pv. *citri* causing Asiatic citrus canker in Burkina Faso. Plant Disease 97(12):1653-1653. *https://doi.org/10.1094/pdis-06-13-0600-pdn*

Mohidem NA, Hashim N, Shamsudin R, Che Man H (2022). Rice for food security: Revisiting its production, diversity, rice milling process and nutrient content. Agriculture 12(6):741. *https://doi.org/10.3390/agriculture12060741*

Mojica MF, Rutter JD, Taracila M, Abriata LA, Fouts DE, Papp-Wallace KM, Bonomo RA (2019). Population structure, molecular epidemiology, and β-lactamase diversity among *Stenotrophomonas maltophilia* isolates in the United States. MBio 10(4):10-1128. *https://doi.org/10.1128/mbio.00405-19*

Mubeen M, Arshad HMI, Iftikhar M, Bilqees I, Arooj S, Saeed HMA (2015). *In-vitro* efficacy of antibiotics against *Xanthomonas axonopodis* pv. *citri* through inhabitation zone techniques. International Journal of Agriculture and Applied Sciences 7:69-71.

Riaz K, Riaz K, Dilferoze A, Alam M, Riaz A, Jameel S, ... Hannan A (2016). Emergence of a novel white stripe disease pathogen *Stenotrophomonas maltophilia* strain Sia5 in rice felds of Punjab under the climate change scenario. Phytopathology 106:120-120.

Singh NI, Swings IJ, Devi RT, Imotomba RK (2001). White stripe, a new disease of rice caused by *Stenotrophomonas maltophilia* in India. Indian Phytopathology 54(2).

Steel RG, Torrie JH, Deekey DA (1997). Principles and Procedures of Statistics. A biometrical approach 3rd edition. S.T. McGraw Hill book Co. Inc. New York, U.S.A.

USDA. World Rice Production 2021/2022. 2022. Retrieved 2021 September 1st from: *http://www.worldagriculturalproduction.com/crops/rice.aspx/*

Zhao M, Lin Y, Chen H (2020). Improving nutritional quality of rice for human health. Theoretical and Applied Genetics 133:1397-1413. *https://doi.org/10.1007/s00122-019-03530-x*

Zheng K, Su X, Xue Z, Zhang L, Chen Y, Wu k, Zhao Z (2022). First report of *Stenotrophomonas maltophilia* causing root soft rot of Sanqi (*Panax notoginseng*) in China. Plant Disease 106(2):755. *https://doi.org/10.1094/pdis-07-21-1353-pdn*

The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.

License - Articles published in ***Notulae Botanicae Horti Agrobotanici Cluj-Napoca*** are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.
© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

Notes:

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.