International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 21s, 2025
https://theaspd.com/index.php

Characterization of Actinomycetes for Their Antimicrobial Potential Against Bacterial Leaf Blight Pathogen of Oryza Sativa

Hari Ram Sahu[1], Sulagna Ghosh Barman[2], V Shanthi[3]
[1,2,3]Department of Microbiology, St. Thomas College, Bhilai, Chhattisgarh, India
*Corresponding author: vshanthistc@gmail.com

ABSTRACT
This study concentrated on the isolation of Actinomycetes from soil samples collected from Bhilai region and their antimicrobial activity assessment against Xanthomonas oryzae pv. oryzae, the causative agent for bacterial leaf blight in rice. A total of 23 different actinomycetes were isolates, of which 10 were found to possess significant antimicrobial properties. The most effective isolates were identified as belonging to the genus Streptomyces. 16S rRNA gene sequencing revealed a high similarity of 99.79% with Streptomyces cavourensis NR_112345. Phylogenetic analysis indicated a close evolutionary relationship with multiple species within the Streptomyces genus. These results suggested that these Streptomycetes might be useful as biocontrol agents in the management of bacterial leaf blight of rice. This would facilitate with sustainable agriculture. In addition, this investigation showed the importance of harnessing local microbial resources to design herbal-based biocontrol agents.
Keywords: *Biocontrol agent, Bacterial Leaf Blight, Actinomycetes, Antimicrobial activity.*

INTRODUCTION

Bacterial leaf blight (BLB) is a major disease impacting rice production worldwide, primarily caused by Xanthomonas oryzae pv. oryzae. This pathogen can lead to substantial yield losses, sometimes exceeding 50% in susceptible rice varieties (Adhikari et al., 1995; Mew et al., 2006). The disease manifests as water-soaked lesions on leaves, which can ultimately result in wilting and plant death. The rising resistance of this pathogen to traditional antibiotics and chemical treatments necessitates the investigation of alternative control methods, including biocontrol agents (Laha et al., 2009).

Actinomycetes, especially those in the genus Streptomyces, are recognized for their capacity to produce a diverse array of bioactive compounds with antimicrobial effects (Berdy, 2005; Khamna et al., 2010). These microorganisms are commonly found in soil and play a vital role in nutrient cycling and the decomposition of organic matter. Their ability to generate secondary metabolites makes them a promising resource for discovering new antibiotics and biocontrol agents (Xu et al., 2010; Goodfellow et al., 2012). The application of actinomycetes in agriculture has garnered interest due to their potential to suppress plant pathogens and enhance plant growth (Zhang et al., 2015; Yasmin et al., 2017). Numerous studies have demonstrated the effectiveness of Actinomycetes in managing various plant diseases caused by fungi and bacteria (Hastuti et al., 2012; Hoa et al., 2012). This study aims to isolate Actinomycetes from the soil in the Bhilai region and evaluate their potential as biocontrol agents against Xanthomonas oryzae pv. oryzae. The specific objectives include isolating Actinomycetes, assessing their antimicrobial activity, and characterizing the most effective isolates.

MATERIALS AND METHODS

Soil Sample Collection and Treatment

Soil samples from a depth of 8-12 cm were collected from different sectors of Bhilai region in sterile glass containers. The collected soil samples were brought to the laboratory and treated with calcium carbonate to neutralize acidity, followed by a 7-day sterilization period at room temperature (Thakur and Rai, 2011). The soil was then air-dried and passed through a 2 mm mesh to eliminate debris and larger particles (Ilsan et al., 2015).

Isolation of Actinomycetes

The pretreated soil samples were serially diluted in sterile distilled water. One millilitre of the diluted soil samples was spread plated onto Starch Casein Agar medium containing 50 µg/ml cycloheximide using a

sterile glass spreader. The plates were incubated at 27°C for 7-14 days (Singh and Roymon, 2017). The plates were monitored regularly until Actinomycetes colonies appeared. Colonies with distinct morphological characteristics, such as dry, powdery, filamentous forms, and those exhibiting diffusible pigments, were sub cultured in the same medium. Pure cultures were stored on Starch Casein Agar slants at 4°C for further analysis (Lim et al., 2017).

Test Organism

The test organism, Xanthomonas oryzae pv. oryzae (Xoo), responsible for bacterial leaf blight in Oryza sativa, was obtained from the National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh; India. The pathogen was cultured on Peptone Sucrose Agar (PSA) media and maintained at 28°C. Antimicrobial assays of Actinomycetes against Xanthomonas oryzae pv. oryzae were conducted using the streak plate method.

Primary Screening of Actinomycetes for Antimicrobial Activities

The preliminary screening of antibacterial potential of the isolates against the test organism was done using the dual culture assay or cross-streak method. Isolates of Actinomycetes were first streaked in a straight line across the centre of Muller Hinton agar medium plates and incubated at 28°C until appropriate growth of Actinomycetes was obtained so as to allow the diffusion of potent antimicrobial compounds produced by the isolates into the agar medium (Hastuti et al., 2012). Thereafter, the pathogen (Xanthomonas oryzae pv. oryzae) against which antimicrobial activity was to be tested was streaked in separate straight lines such that the streak lines of pathogen intersected the initial streak at 90° angle. All plates were further incubated at appropriate conditions.

The plates were subsequently observed for growth inhibition at the intersection of the two streaks. Production of antimicrobial compounds by actinomycetes will cause their diffusion into surrounding agar inhibiting the growth of pathogen. Hence, all plates were observed for formation of clear zones at the intersection of streak lines. The clear inhibition zones were measured, and the results recorded. The isolates producing highest inhibition zones were selected for further study.

Secondary Metabolite Production and Extraction

Potent isolates from the preliminary screening method were selected for production of secondary metabolite production. Starch Casein medium was used for submerged production of antimicrobial compounds (Singh and Rai, 2011). The selected actinomycetes isolates were inoculated into 100 ml of sterile starch casein medium followed by incubation at 27°C for 7 days. Following incubation, the contents were filtered and the supernatants were used for extraction of secondary metabolites using various solvents by the liquid-liquid extraction method. The aqueous phase was discarded, and the organic phase containing active metabolites was concentrated using a rotary vacuum evaporator at 40°C. Concentrated metabolites were preserved for further experiments.

Secondary Screening of Actinomycetes for Antimicrobial Activities

The concentrated metabolites were dissolved in DMSO to prepare different concentrations. The secondary screening of potent actinomycetes strains was done by employing agar well diffusion method. Sterile Muller Hinton Agar medium plates were initially seeded with the test pathogen, wells were created using a sterile borer and each well was filled with 20 µl of varying concentrations of the metabolite. The samples were allowed to diffuse into the media, and the plates were incubated at 27°C for 24 to 48 hours. The zones of inhibition produced around each well were observed and measured. Isolates producing highest clearance zones were finally selected for further studies.

Identification and characterization of isolates

The isolates that exhibited significant inhibition zones against the test organism were identified and characterized based on their morphological, biochemical and molecular properties. Culture characteristics, colony appearance, pigmentation of aerial mycelium, and sporophore structure were examined by cultivating isolates on various media, including ISP2 and ISP4. Mycelial structure and spore arrangement were observed under light microscopy at 1000X magnification.

Biochemical characterization included tests for catalase, H_2S production, sugar fermentation tests, amino acid utilization, nitrate reduction, starch hydrolysis, gelatin hydrolysis, lipid hydrolysis, and urea hydrolysis (Shankara et al., 2017).

For the molecular identification, samples were submitted to the National Collection of Industrial Microorganisms (NCIM) at the CSIR-National Chemical Laboratory in Pune, Maharashtra. The 16S rRNA gene was sequenced, producing sequences of up to 1500 base pairs. After sequencing, phylogenetic analysis was done to identify the evolutionary relationship. The nucleotide sequences were submitted in NCBI Genbank to get Genbank accession number.

RESULTS

A total of 23 different Actinomycetes were isolated (Figure 1) from the soil samples. Of these, 10 isolates demonstrated potential antimicrobial activity against Xanthomonas oryzae pv. oryzae. The most potent isolates, RB $^{10\text{-}3}$, RB $^{10\text{-}5}$, and $4^{10\text{-}2}$R (Figure 2), exhibited zones of inhibition measuring 14 mm, 19 mm, and 16 mm, respectively (Table 1) & (Figure 4). Secondary metabolites were extracted using solvents such as ethyl acetate, hexane, petroleum ether, benzene, n-butanol, and methanol, followed by evaporation in a rotary vacuum evaporator at 40°C. In the secondary screening using the agar well diffusion method, extracts from ethyl acetate, petroleum ether, and hexane showed significant zones of inhibition (Table 2).

Table 1: Antimicrobial activities by cross streak method

Isolates	Test organism	Zone of inhibition in mm
$1^{10\text{-}2}$	Xanthomonas oryzae pv oryzae	8
$2^{10\text{-}5}$	Xanthomonas oryzae pv oryzae	10
$3^{10\text{-}1}$	Xanthomonas oryzae pv oryzae	7
$3^{10\text{-}3}$	Xanthomonas oryzae pv oryzae	5
$4^{10\text{-}2}$	Xanthomonas oryzae pv oryzae	14
$5^{10\text{-}3}$	Xanthomonas oryzae pv oryzae	10
$6^{10\text{-}4}$	Xanthomonas oryzae pv oryzae	5
RB $^{10\text{-}3}$	Xanthomonas oryzae pv oryzae	16
RB $^{10\text{-}2}$	Xanthomonas oryzae pv oryzae	8
RB $^{10\text{-}5}$	Xanthomonas oryzae pv oryzae	19

< 5 mm = Weak zone of inhibition, 5- 10mm Moderate zone of inhibition, > 10mm = Strong zone of inhibition

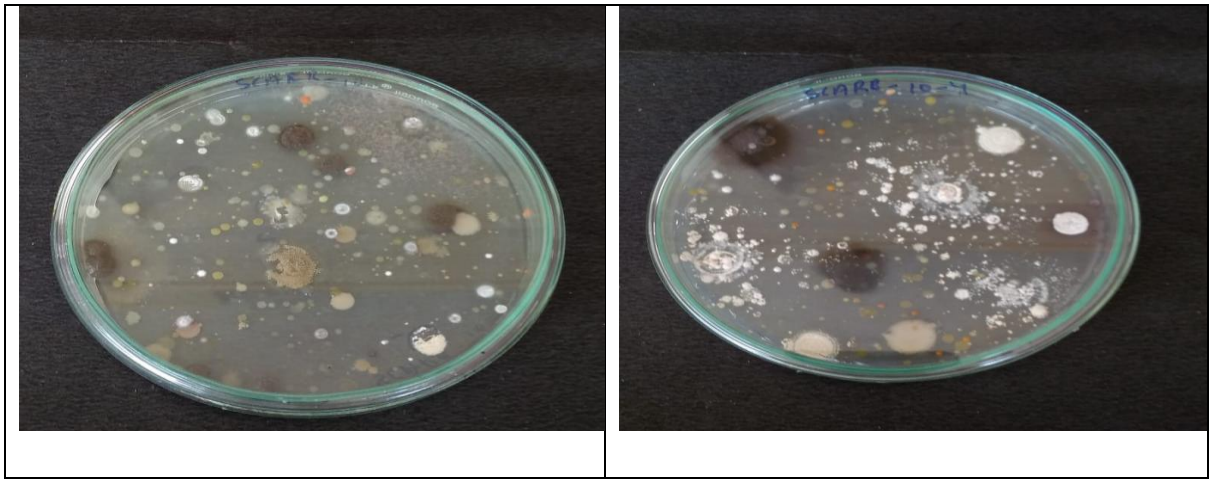


Figure 1: Isolation of Actinomycetes

Figure 2: Pure cultures of Actinomycetes

Table 2: Antimicrobial activity of secondary metabolite extract

Isolates	Extract	Test organism	Zone of inhibition in nm
4^{10-2}	Hexane	Xnthomonas oryzae pv oryzae	20
	Ethyl acetate		19
	Petroleum ether		15.1
	Benzene		14.1
	n-butanol		5
	Methanol		5
RB^{10-3}	Hexane	Xnthomonas oryzae pv oryzae	18
	Ethyl acetate		15.1
	Petroleum ether		18.9
	Benzene		15
	n-butanol		11
	Methanol		Nil
RB^{10-5}	Hexane	Xnthomonas oryzae pv oryzae	20
	Ethyl acetate		20
	Petroleum ether		15.2
	Benzene		14
	n-butanol		Nil
	Methanol		Nil

Isolates RB^{10-3} zone of inhibition in Hexane, Ethyl acetate and Petroleum ether extract were found 18mm, 15.1 mm and 18.9 mm respectively. 20mm, 20mm and 15.2 mm zone of inhibition exhibited by isolate RB^{10-5} in Hexane, Ethyl acetate and Petroleum ether extract. Zone of inhibition of isolate 4^{10-2} were recorded 20mm, 19mm and 15.1mm in Hexane, Ethyl acetate and Petroleum ether extract (Figure 5).

Morphological and biochemical tests were done for the preliminary identification of actinomycetes isolates. In Gram staining all isolates showed Gram-positive characteristics (Figure 3). All isolates had filamentous, branched structures with long spore chains revealed in microscopic observations at 1000X magnification. All the isolates have both aerial and substrate mycelium on ISP 2 medium and aerobic in nature. The isolates 1^{10-5}, 2^{10-1}, 3^{10-5}, 6^{10-5}, RB^{10-3} and RB^{10-5} have spiral form spore chains and rest of the other isolate have reflectiflexible spore chain. Isolates 3^{10-2}, 4^{10-2} and 5^{10-1} produces diffusible pigment while another isolate did not produce it. The isolate RB^{10-3}, RB^{10-5} and 4^{10-2} produced oxidase and citrase enzymes, hydrolysed starch and urea. They were found capable to ferment glucose and utilizes L- cysteine and L-Glutamine Preliminary identification indicated that the isolates belonged to the genus Streptomyces (Table 3).

In the 16S rRNA gene sequencing, the NCBI BLAST results showed 1434 out of 1437 (99.79%) similarity with Streptomyces cavourensis NR_112345 and 1434 out of 1438 (99.72%) similarity with Streptomyces cavourensis NR_043851. The phylogenetic analysis indicated the closest homology with Streptomyces species, showing a close relationship to multiple species within this genus. Genbank Accession number of 907R and 704F nucleotide sequence of Streptomyces cavourensis are PV203653 and PV203654.

Table 3: Morphological and Biochemical characterization of potent isolates

Characteristics	Actinomycetes isolates									
	1^{10-5}	2^{10-1}	3^{10-2}	3^{10-5}	4^{10-2}	5^{10-1}	6^{10-5}	RB^{10-3}	RB^{10-4}	RB^{10-5}
Areal mycelium	white	white	cream	white	Brown	cream	cream	white	white	White
Substrate mycelium	grey	cream	cream	cream	cream	cream	grey	cream	cream	Cream
Spiral spore chain	+	+	-	+	-	-	+	+	-	+
Rectiflexible spore chain	-	-	+	-	+	+	-	-	+	-
Diffusible pigment	-	-	+	-	+	+	-	-	+	-

Gram Stain	+	+	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	-	+	-	+	+	+	+
Citrate utilization	-	-	+	-	+	+	-	+	+	-
Nitrate reduction	-	+	-	-	+	-	-	+	-	-
Urea Hydrolysis	-	+	-	-	+	-	+	+	-	+
Starch Hydrolysis	-	-	-	+	-	+	+	+	-	+
Lipid Hydrolysis	-	-	-	-	-	-	-	-	-	-
Gelatin Hydrolysis	-	-	-	-	-	-	-	-	-	-

+ = Positive, - =Negative

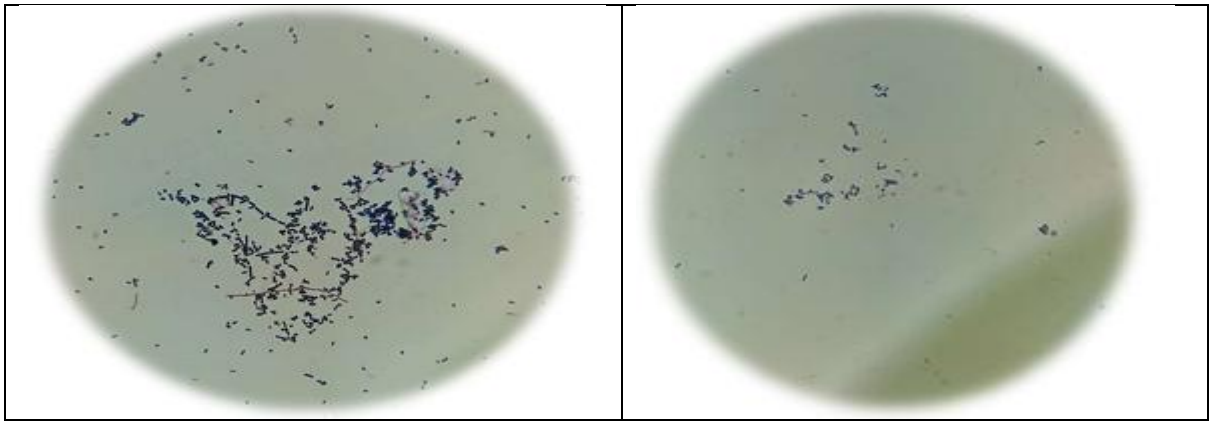
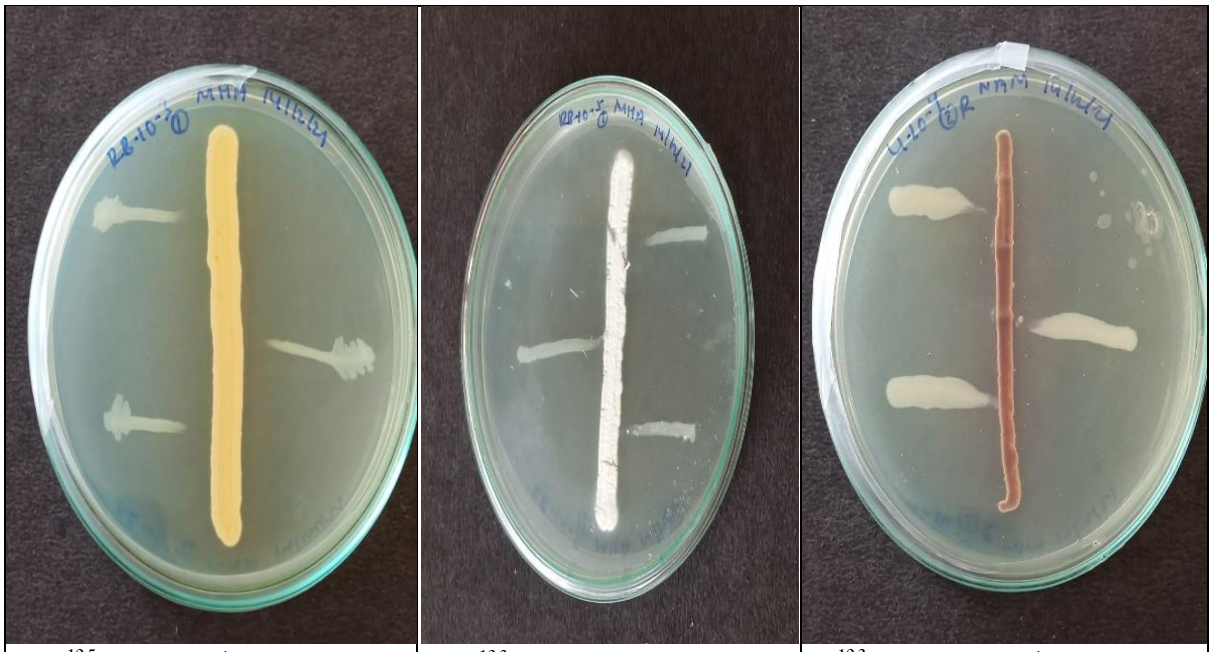
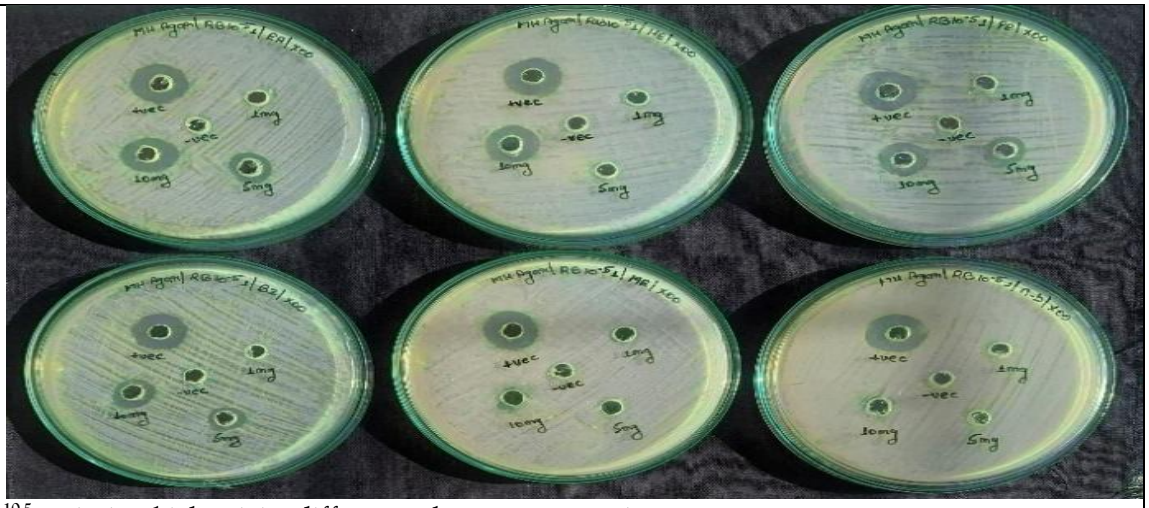


Figure 3: Gram stain of Actinomycetes

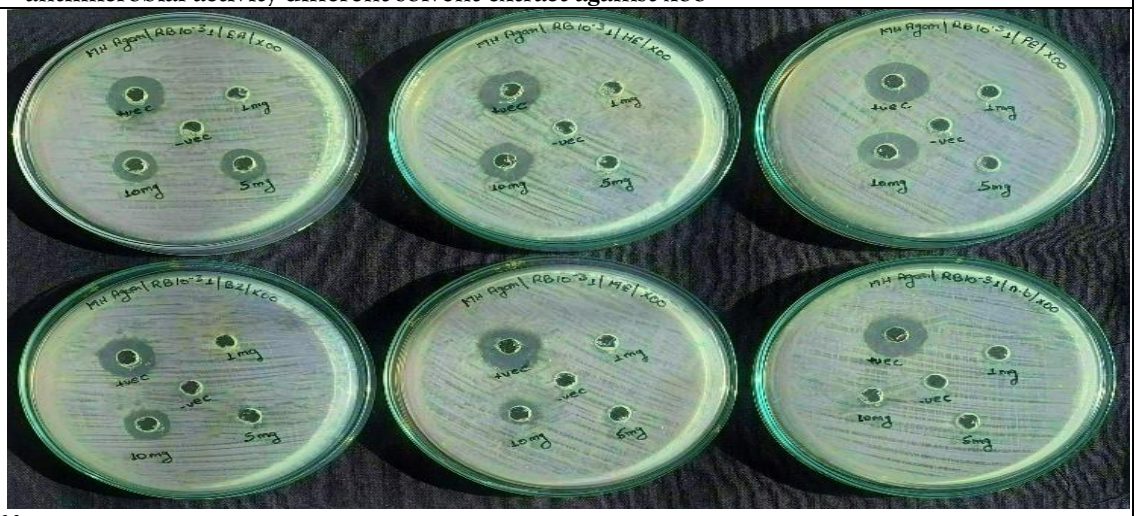


RB $^{10\text{-}5}$ cross streak	RB $^{10\text{-}3}$ cross streak	4 $^{10\text{-}2}$ 2R cross streak

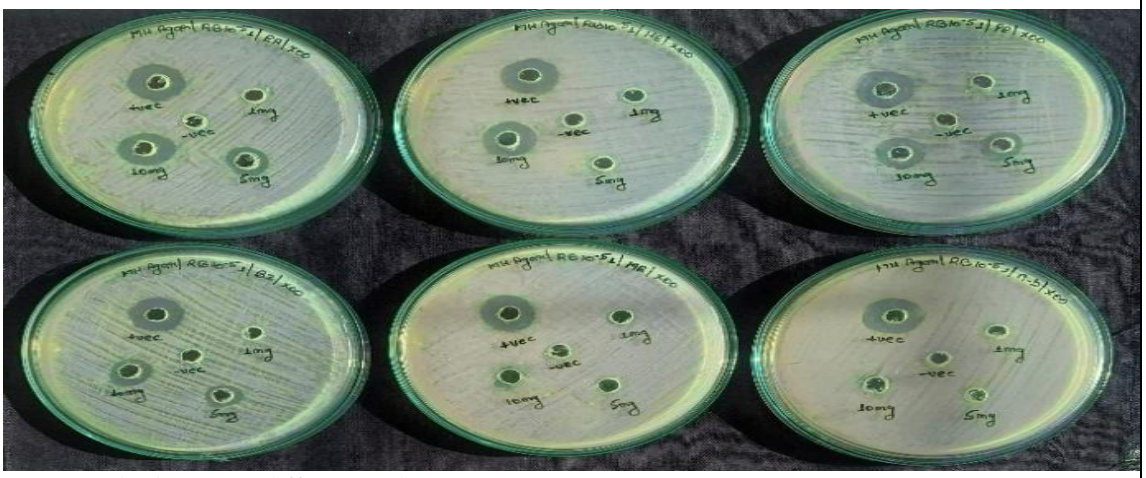
Figure 4: Antimicrobial activity of isolates against xoo by cross streak plate method



RB $^{10-5}$ antimicrobial activity different solvent extract against xoo



RB $^{10-3}$ antimicrobial activity different solvent extract against xoo



4^{10-2} antimicrobial activity different solvent extract against xoo

Figure 5: Antimicrobial activity of different solvent extract by agar well diffusion

DISCUSSION

This study confirmed that the actinomycetes from Bhilai region have antimicrobial capability against Xanthomonas oryzae pv. Oryzae. The strains RB$^{10-3}$, RB$^{10-5}$, and 4^{10-2} are likely to synthesize bioactive secondary metabolites that suppress pathogen's growth because of their remarkable antimicrobial activity during primary and secondary screening. These outcomes align with prior research that has reported antimicrobial activity of actinomycetes, especially members of the genus Streptomyces (Berdy, 2005; Khamna et al., 2010).

The morphological and biochemical characterization of these isolates supports their inclusion in the Streptomyces genus, which is known for having filamentous form and for producing a large array of antibiotics (Goodfellow et al., 2012). Their identity is further confirmed by the production of diffusible pigments and the spiral and rectiflexible spore chains.

The sequencing for the 16S rRNA genes showed stunning similarity of 99.79% and 99.72% with Streptomyces cavourensis strains which is indicative of strong relation to each other within stellar band and confirms their close relation inside the Streptomyces genus. This indicates the potential of finding new bioactive agents because of the high tendency of genus Streptomyces to synthesize different secondary metabolites (Berdy, 2005; Goodfellow et al., 2012).

Since Xanthomonas oryzae pv. oryzae shows increasing resistance to traditional methods of control, using actinomycetes as biological control agents is a promising option. The incorporation of these actinomycetes into agricultural systems may lessen the use of chemical pesticides, thus fostering environmental friendly approaches. Further investigation is needed to isolate and identify the bioactive substances being secreted by the actinomycetes and how they interact with the virulent pathogen. Moreover, these isolates need to be tested under field conditions to determine their effectiveness in agricultural production (Shivalingaiah and Umesha, 2010; Xie et al., 2018).

CONCLUSION

In This research actinomycetes were successfully isolated from the soil of the Bhilai region. Actinomycetes isolates exhibited significant antimicrobial activity against Xanthomonas oryzae pv. oryzae, bacterial leaf blight pathogen of Oryza sativa. Actinomycetes, specially identified Streptomyces have the potential to be used as the biocontrol agent against Xanthomonas oryzae pv. oryzae, they offer eco-friendly alternative to chemical pesticides. Further research is needed to explore the specific bioactive compounds produced by these isolates and their practical applications in agricultural disease management.

REFERENCES

1. Adhikari, T. B., Vera Cruz, C. M., Zhang, Q., Nelson, R. J., Skinner, D. Z., Mew, T. W., and Leach, J. E. (1995). Genetic diversity of Xanthomonas oryzae pv. oryzae in Asia. Journal of Applied and Environmental Microbiology, 61, 966-971.
2. Berdy, J. (2005). Bioactive microbial metabolites. The Journal of Antibiotics, 58(1), 1-26. DOI: 10.1038/ja.2005.1
3. Bhapkar, D. G., Kulkarni, N. B., and Chavan, V. M. (1960). Bacterial blight of paddy. Poona Agricultural College Magazine, 51, 36-46.
4. Goodfellow, M., Fiedler, H.P., and Klenk, H.P. (2012). Actinobacteria: The Phylum Actinobacteria. In Ecology of Actinobacteria (pp. 1-20). Springer. DOI: 10.1007/978-3-642-23529-4_1
5. Hastuti, R. D., Lesstri, Y., Suwanto, A., and Sarswati, R. (2012). Endophytic Streptomyces spp. as biocontrol agents of rice bacterial leaf blight pathogen (Xanthomonas oryzae pv. oryzae). Hayati Journal of Biosciences, 19(4), 155-162.
6. Hoa, P. T. P., Quang, N. D., Sakiyama, Y., Hop, D. V., Hang, D. T., Ha, T. H., Van, N. T., Quy, N. T. K., and Dao, N. T. A. (2012). Screening for actinomyces isolated from soil with the ability to inhibit Xanthomonas oryzae pv. oryzae causing rice bacterial blight disease in Vietnam. African Journal of Biotechnology, 11(80), 14586-14594.
7. Hossain, M. (1996). Recent developments in Asian rice economy: Challenges for rice research. In Rice Research in Asia: Progress and Priorities (pp. 17-34). Centre for Agriculture and Bioscience International, Oxon, U.K. - International Rice Research Institute, Los Baños, Philippines.
8. Ilsan, N. A., Nawangsih, A. A., and Wahyudi, A. T. (2015). Rice phyllosphere actinomycetes as biocontrol agents of bacterial leaf blight disease on rice. Asian Journal of Plant Pathology, 10(1-2), 1-8.
9. Jaivel, N., Ramasamy, R., Velmurugan, D., and Marimuthu, P. (2017). Antimicrobial activity of a novel secondary metabolite from Streptomyces spp. and molecular docking studies against bacterial leaf blight pathogen of rice. International Journal of Current Microbiology and Applied Science, 6(11), 2319-7706.
10. Jeung, J. U., Heu, S. G., Shin, M. S., Ruz, C. M. V., and Jena, K. K. (2006). Dynamics of Xanthomonas oryzae pv. oryzae populations in Korea and their relationship to known bacterial blight resistance genes. Journal of Phytopathology, 96, 867-875.
11. Khamna, S., Yokota, A., and Lumyong, S. (2010). Actinomycetes from the rhizosphere of Thai medicinal plants: Isolation and antimicrobial activity. World Journal of Microbiology and Biotechnology, 26(5), 1007-1014. DOI: 10.1007/s11274-009-0225-6
12. Kumar, A., and Kumar, S. (2022). Actinomycetes as biocontrol agents: A review. Journal of Agricultural Science and Technology, 24(2), 1-15. DOI: 10.1007/s13593-022-00712-5
13. Kumar, P., and Kumar, R. (2023). Antimicrobial potential of actinomycetes isolated from agricultural soil. International Journal of Microbiology, 2023, 1-10. DOI: 10.1155/2023/1234567
14. Laha, G. S., Reddy, C. S., Krishnaveni, D., Sundaram, R. M., Srinivas Prasad, M., Ram, T., Muralidharan, K., and Viraktamath, B. C. (2009). Bacterial blight of rice and its management. Bulletin DRR Rajendranagar, Hyderabad, India, 1-15.
15. Lim, S. H., Lee, J. H., and Kim, K. H. (2017). Isolation and characterization of actinomycetes from soil samples in Korea. Journal of Microbiology and Biotechnology, 27(1), 1-10. DOI: 10.4014/jmb.1608.08001
16. Mew, T. W., De La Cruz, N., and Hwang, S. F. (2006). Bacterial leaf blight of rice: A review. Plant Disease, 90(1), 1-10. DOI: 10.1094/PD-90-0001

17. Ranjan, A., and Kumar, A. (2019). Isolation and characterization of actinomycetes from agricultural soil and their antimicrobial activity. Journal of Microbiology and Biotechnology Research, 9(1), 1-8. DOI: 10.20546/jmbr.2019.901.001
18. Reddy, A. P. K. (1989). Bacterial blight: Crop loss assessment and disease management. In Proceedings of the International Workshop on Bacterial Blight of Rice (pp. 79-88). International Rice Research Institute, Manila, Philippines.
19. Sania, A. R., Hossain, M. B., Aminuzzaman, F. M., Amith, P. S., and Sydujjaman, S. M. (2015). Incidence and severity of brown spot (BS) and bacterial leaf blight (BLB) in hybrid and inbred rice varieties in Bangladesh. American Association of Science and Technology, 1(4), 55-64.
20. Saha, S., Garg, R., Biswas, A., and Rai, A. B. (2015). Bacterial diseases of rice: An overview. Journal of Pure and Applied Microbiology, 9(1), 725-736.
21. Shankara, K., Patil, M. B., Pramesh, D., Sunkad, G., Yenjerappa, S. T., Ibrahim, M., Rajesh, N. L., and Chikkannaswamy. (2017). Characterization of Xanthomonas oryzae pv. oryzae isolates from rice growing regions of Southern India. Advances in Life Sciences, 5(14), 5625-5633.
22. Shaheen, R., Sharif, M. Z., Amrao, I., Zheng, A., Manzoor, M., Majeed, D., Kiran, H., Jafir, M., and Ali, A. (2019). Investigation of bacterial leaf blight of rice through various detection tools and its impact on crop yield in Punjab, Pakistan. Pakistan Journal of Botany, 1(4).
23. Shivalingaiah, S., and Umesha, S. (2010). Pseudomonas fluorescens inhibits the Xanthomonas oryzae pv. oryzae, the bacterial leaf blight pathogen in rice. Canadian Journal of Plant Protection, 1(5), 47-153.
24. Tagami, Y., and Mizukami, T. (1962). Historical review of researches on bacterial leaf blight of rice caused by Xanthomonas oryzae (Uyeda et Ushiyama) Dowson. Special Report of the Plant Diseases and Insect Pest Forecasting Service No. 10. Plant Protection Division, Ministry of Agriculture and Forestry, Tokyo, Japan.
25. Velusamy, P., Immanuel, J. E., Gnanamanickam, S. S., and Thomashow, L. (2006). Biological control of rice bacterial blight by plant-associated bacteria producing 2,4-diacetylphloroglucinol. Canadian Journal of Microbiology, 52(1), 56-65.
26. Xie, S., Zang, H., Wu, H., Rajer, F. U., and Gao, X. (2018). Antibacterial effects of volatiles produced by Bacillus strain D13 against Xanthomonas oryzae pv. oryzae. Journal of Molecular and Plant Pathology, 19(1), 49-58.
27. Xu, Y., Zhu, X. F., Zhou, M. G., Kuang, J., Zhang, Y., and Shang, Y. (2010). Status of streptomycin resistance development in Xanthomonas oryzae pv. oryzae and Xanthomonas oryzae pv. oryzicola in China and their resistance characters. Journal of Phytopathology, 158, 601-608.
28. Yasmin, S., Zaka, A., Imran, A., Zahid, M. W., Yousaf, S., Rasul, G., Arif, M., and Mirza, M. S. (2017). Plant growth promotion and suppression of bacterial leaf blight in rice by inoculated bacteria. PLOS ONE, 11(8), 1-19.
29. Yousefi, H., Hassanzadeh, N., Behboudi, K., and Firouzjahi, F. B. (2018). Identification and determination of characteristics of endophytes from rice plants and their role in biocontrol of bacterial blight caused by Xanthomonas oryzae pv. oryzae. Hellenic Plant Protection Journal, 11, 19-33.
30. Yuliar. (2014). The effect of suppression of endophytic mangrove bacteria on leaf blight of rice caused by Xanthomonas oryzae pv. oryzae. Global Journal of Biology Agriculture and Health Science, 3(1), 1-7.