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Exploiting the Potential of Chitinolytic Bacteria Against Dry Root Rot Pathogen Infecting Black Gram

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ABSTRACT: Black gram (*Vigna mungo* L.) is a vital pulse crop extensively cultivated across India, valued for its high protein content and soil-enriching properties. However, its productivity is frequently hampered by dry root rot, a devastating disease caused by the soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid, particularly under drought and high-temperature conditions. To provide a sustainable and eco-friendly solution, this study explored the biocontrol potential of chitinolytic bacteria against *M. phaseolina*. A field survey was conducted in major black gram-producing regions of Tamil Nadu to assess disease incidence. The pathogen was isolated from infected plants and identified through morphological methods. Simultaneously, bacterial isolates were obtained from partially degraded cow horn samples and screened for chitinolytic activity using different culture media. Among the 27 isolates tested, three strains BCH2, BCH10, and BCH14 demonstrated significant chitinase activity. Of the media tested, M3 medium supported the highest enzyme production. Dual culture assays were conducted to evaluate antagonistic potential against *M. phaseolina*. All three isolates inhibited fungal growth, with BCH14 showing the most prominent suppression zone, indicating strong antagonistic ability. The results suggest that chitinolytic bacteria, particularly BCH14, possess promising biocontrol efficacy and could serve as a viable alternative to chemical fungicides. This study contributes to the development of eco-friendly, microbe-based strategies for effective management of charcoal rot in black gram, supporting sustainable agriculture and reducing dependency on synthetic agrochemicals.

Keywords: Blackgram, Dry root rot, chitinolytic bacteria, biocontrol, sustainable agriculture.

INTRODUCTION

Blackgram (*Vigna mungo* L.), a member of the *Fabaceae* family, is one of the most important pulse crops and has been a staple in the human diet since ancient times. It is highly nutritious, containing 24% protein, 60% carbohydrates, 3.2% minerals, 0.9% fibre, and is a good source of iron (9.1 mg/100g), calcium (154 mg/100g), and phosphorus (385 mg/100g). India is both the largest producer and consumer of black gram, cultivating it on about 4.1 million hectares. The country produces between 2.2 and 2.8 million tonnes annually, with an average yield of 540 kg per hectare, contributing 13.05% total pulse production. Major producing states include Maharashtra, Uttar Pradesh, Tamil Nadu, Karnataka, Andhra Pradesh, and Rajasthan. Although blackgram is important for both nutrition and agriculture, its cultivation faces several challenges from biotic and abiotic stresses. These stresses significantly reduce both the yield and quality of the seeds. Black gram seeds are often infected by various seed-borne fungi, either on the surface, inside the seed, or both, leading to major losses in quantity and quality. Charcoal rot, caused by the fungus *Macrophomina phaseolina* (Tassi) Goid, is one of the most common and harmful diseases of black gram (Pandey *et al.*, 2020). This pathogen can survive in soil for many years and infects plants at any stage of growth (Choudhary *et al.*, 2022; Nisha *et al.*, 2025). The disease causes blackening and rotting of the roots, which eventually leads to wilting and death of the plant in severe cases (Khan and Javid 2023). During infection, *M. phaseolina* produces toxins, such as botryodiplodin and phaseolinone, which facilitate the

invasion of susceptible plants from soil reservoirs, particularly during the overwintering period (Abbas *et al*., 2019). Chitinases are a group of enzymes that catalyze the hydrolysis of chitin, a major structural component of fungal cell walls. Chitinolytic microorganisms are known to protect plants by breaking down the fungal cell wall, which weakens its structure, leads to cell death, and stops fungal growth (Inbar and Chet 1991). Among them, bacterial chitinases have shown strong antifungal activity and are considered promising agents for the biological control of plant-pathogenic fungi (Ordentlich *et al.,* 1988).

MATERIALS AND METHODS

A. Survey and Symptomatology of Pathogen

A field survey was carried out in the major blackgram (*Vigna mungo* L.) growing regions of Tamil Nadu, namely Chengalpattu, Perambalur, and Tiruchirapalli districts to assess the prevalence of charcoal rot. Disease incidence was calculated using the formula proposed by Wheeler (1969), and the observed symptoms were carefully documented for diagnostic and research purposes.

B. Isolation of the charcoal rot pathogen of blackgram

The pathogen *Macrophomina phaseolina,* responsible for charcoal rot, was isolated from black gram plants showing typical symptoms like dark brown lesions and bark shredding. Infected tissues, about 1 cm in size, were cut and surface sterilized with 70% ethanol for 30 seconds, then rinsed three times with sterile distilled water. The tissues were dried with sterile tissue paper and placed on Potato Dextrose Agar (PDA) medium supplemented with streptomycin (Choudhary *et al*., 2011). The plates were incubated at 28 ± 2°C for five days to promote fungal growth. Emerging fungal colonies were purified using the hyphal tip method (Dhingra and Sinclair 1978). Mature cultures were then observed for mycelial characteristics.

C. Isolation of chitinolytic bacteria from partially degraded cow horn

Soil sample from partially degraded cow horn was collected and processed using serial dilution and spread plate techniques. One millilitre of each dilution was plated in triplicate on minimal salt medium (MSM) containing colloidal chitin (10, 50, and 250 ppm) as the sole carbon source (Kuddus & Ahmad, 2013). The MSM was prepared by combining Na_2HPO_4, KH_2PO_4, NH_4Cl, NaCl, yeast extract, agar, and colloidal chitin in 1 litre of sterile water. The plates were kept at 28°C for 3 days, and later chitin-degrading bacteria were identified by the formation of clear zones around the colonies. The isolated colonies were subsequently transferred to nutrient agar plates and incubated at 28°C to promote further growth. Positive colonies were preserved as pure cultures and stored in glycerol stock for future use.

D. Qualitative assay for chitinolytic activity using NA minimal medium

To detect chitinolytic activity, bacterial isolates were grown on a minimal Nutrient Agar (NA) medium supplemented with bromocresol purple dye. The presence of chitinase activity was confirmed by the appearance of a clear halo zone around the bacterial colonies, indicating the breakdown of chitin.

E. Comparative evaluation of growth media for enhanced chitinase production by chitinolytic bacteria

To study chitinase production, selected chitinolytic bacteria were grown in four different nutrient media: LB Broth, Nutrient Broth, M2 (chitin 10g/l, peptone1g/l, $(NH_4)_2SO_4$ 2g/l), and M3 (chitin 10g/l, peptone1.8g/l, $(NH_4)_2SO_4$1.6g/l, KH_2PO_4 0.5g/l, K_2HPO_4 0.5g/l, $Mg(SO_4)\cdot 7H_2O$ 2g/l). Each medium contained 0.4% colloidal chitin and was sterilized before use. About 5 mL of each medium was inoculated with a bacterial isolate and incubated at 30°C for 3 days in a shaking incubator. After incubation, the cultures were spun at 8,000 rpm for 5 minutes to remove the cells. The clear liquid (supernatant) was then used to measure chitinase activity using a colour test method at 540 nm, as described by Ohtakara (1988).

F. Screening of chitinolytic bacterial isolates against the charcoal rot pathogen against M.phaseolina

Chitinolytic bacterial isolates were assessed for their antagonistic activity against *M. phaseolina* using the dual culture technique described by Dennis and Webster (1971). The antagonistic effect of the bacterial isolates was quantified as Percentage Inhibition (PI) over control, following the formula proposed by Vincent (1947).

$PI = [(C - T) / C] \times 100$

Where C - radial growth (in mm) of the pathogen (control),

T - radial growth observed in the presence of the antagonistic organism.

RESULTS AND DISCUSSION

A. Survey and Symptomatology of Pathogen

A field survey carried out in the major blackgram-growing districts of Tamil Nadu, Chengalpattu, Perambalur, and Tiruchirapalli, confirmed a widespread incidence of charcoal rot in the commonly cultivated variety, VBN 11. In Trichy district, Solanganallur village recorded the highest percentage of disease incidence (PDI) at 52%, while Kuruvampatti village in the same district showed the lowest incidence at 31% (Table 1).

The manifestation of symptoms like bark shredding and root rot reflects the aggressive nature of *M. phaseolina* (Noor, 2022) (Fig. 1). These findings underscore the importance of effective disease management strategies, particularly the use of biocontrol agents, to control charcoal rot and sustain blackgram cultivation (Gopalakrishnan *et al.*, 2011).

B. Isolation of the charcoal rot pathogen of blackgram

The pathogen was isolated and grown in PDA, and the pathogen developed whitish-grey to black aerial mycelial growth, ranging from sparse to dense, on Potato Dextrose Agar (PDA) medium (Fig. 2). Various morphological and cultural variations were observed in *M. phaseolina* (Table 2). Kaur *et al.* (2013) revealed

that, *M. phaseolina* strains produced a wide range of mycelial characteristics, from sparse to dense growth and colours varying between whitish-grey and black, indicating considerable strain variability. Such morphological differences may influence the pathogen's level of aggressiveness, presenting difficulties in effective field management (Iqbal & Mukhtar 2014).

C. Isolation of chitinolytic bacteria from partially degraded cow horn

A total of 27 isolates having antagonistic activity were obtained from the dilutions 10^{-3} and 10^{-6} (Fig. 3). The isolates exhibited differences in their colony morphology, ranging from smooth to irregular shapes with wavy, lobate, or rough edges, as well as the presence of clear zones (Table 3). Among all the isolates screened in chitin media, three isolates exhibited extracellular enzyme production. Akindolire *et al.* (2025) examined the morphological characters of bacterial isolates capable of producing hydrolytic enzymes from psychrophilic anaerobic digestion system (PAD) revealed a wide range of colony traits, including differences in shape, elevation, margin, and pigmentation. This variety in morphology indicates a diverse bacterial population with the potential to produce various bioactive compounds, highlighting cow horn as a valuable resource for discovering prospective biocontrol agents (Jayachandran *et al.*, 2016).

D. Qualitative assay for chitinolytic activity using NA minimal medium

Clear zones were observed around the three bacterial colonies grown on Nutrient Agar (NA) medium supplemented with bromocresol purple dye, indicating chitinase activity. These chitinolytic bacteria were able to degrade the colloidal chitin present in the medium, resulting in the formation of halos and a colour change around the colonies. This visual change, caused by a shift in pH due to chitin degradation, confirmed the production of chitinase enzymes by the isolates. The results demonstrated that minimal NA medium with bromocresol purple is effective for detecting chitinolytic activity. Additionally, the bacteria also utilized other nutrients present in the medium, such as peptone and beef extract (Fig. 4). The incorporation of bromocresol purple, a pH-sensitive dye, allowed for easy visual identification of enzymatic activity. This assay effectively validated the chitinase-producing potential of the isolates and proved to be a reliable screening method for identifying chitinolytic bacteria (Agrawal & Kotasthane 2012; Kuddus & Ahmad 2013).

E. Comparative evaluation of growth media for enhanced chitinase production by chitinolytic bacteria

The chitinase activity of the selected isolates varied clearly across the four different media tested. Among them, the M3 medium showed the highest enzyme production, indicating it had the best nutrient combination to boost chitinase activity (Fig. 5). M2 medium also showed good results, but slightly lower than M3. Luria-Bertani (LB) broth supported a moderate level of enzyme production, while Nutrient Broth showed the lowest chitinase activity. These results suggest that the type of culture medium greatly affects enzyme production, with M3 being the most effective for increasing chitinase activity in the tested bacterial isolates.

While M2 medium also facilitated considerable chitinase activity, it was slightly less effective than M3. In comparison, LB broth showed moderate enzyme production, and Nutrient Broth resulted in the lowest activity (Kuddus & Ahmad 2013).

F. Screening of chitinolytic bacterial isolates against the charcoal rot pathogen against M.phaseolina

A total of 27 chitinolytic bacterial isolates were tested against *M. phaseolina*, and three of them showed strong ability to reduce the fungal growth in the lab. Among these, *Alcaligenes faecalis* showed the highest inhibition zone (11 mm), followed by the *uncultured bacterium* (9.5 mm), and *Sphingobacterium thalpophilum* (7 mm), compared to the control (Fig. 6). In all three cases, the fungal growth appeared greyish white, and the development of pycnidia and microsclerotia was delayed (Table 4). These three effective isolates were chosen for further study.

In addition, a dual culture test using *Trichoderma longibrachiatum* against *S. rolfsii* and *M. phaseolina* showed that the fungus was directly suppressed, likely through antibiotic-like action (Sridharan *et al*., 2020). The delay in fungal structure development suggests that these bacterial isolates could be promising biocontrol agents (Gopalakrishnan *et al.*, 2011).

Table 1: Survey and Symptomology.

Sr. No.	District	Village	GPS coordinates	Isolate	Variety	PDI%
1.	Chengalpattu	Baburayanpettai	Lat 12.363625 Long 79.86259	MP1	VBN 11	48%
2.	Trichy	Solanganallur	Lat 10.945206 Long 78.608619	MP2	VBN 11	52%
3.	Trichy	Kuruvampatti	Lat 10.942608 Long 78.60139	MP3	VBN 11	31%
4.	Perambalur	Chathiramanai	Lat 11.171164 Long 78.78529	MP4	VBN 11	45%

(Survey carried out in black gram (*Vigna mungo* L.) cultivating areas of Tamil Nadu to evaluate the incidence of charcoal rot.)

Table 2: Morphological characterization of *M. phaseolina.*

Sr. No.	Isolation	Radial mycelial growth (in mm)	Mycelial character	Sporulation (Days)
1.	MP1	90	Greyish black mycelium, raised margin	5
2.	MP2	88	Greyish black mycelium with raised margin	4
3.	MP3	87	Grey to white with fluffy mycelium, raised margin	7
4.	MP4	90	Greyish black mycelium with raised margin	5

(Variations observed in different isolates of *M. phaseolina* collected from different zones of Tamil Nadu.)

Table 3: Morphological character of chitinolytic bacteria.

Sr. No.	Bacteria isolates	Color	Growth	Characters
1.	BCH 1	Yellow	Slimy Colony	Smooth, Round Shape
2.	BCH 2	Dirty White	Rough	Irregular, Undulate, Swarming
3.	BCH 3	Whitish Yellow	Slimy	Smooth, Round
4.	BCH 4	Light Brown	Slimy	Smooth, Umbonate
5.	BCH 5	Yellowish Brown	Slimy	Smooth, Round
6.	BCH 6	Brown	Slightly Slimy	Slight Smooth, Irregular Undulate
7.	BCH 7	Whitish Brown	Slimy	Slight Smooth, Raised
8.	BCH 8	Dark Brown	Slightly Slimy	Irregular, Lobate
9.	BCH 9	Yellowish Brown	Slightly Slimy	Smooth, Irregular, Filamentous
10.	BCH 10	Light Yellow	Slightly Slimy	Filamentous, Irregular, Undulate
11.	BCH 11	Brownish	Slimy	Filamentous, Irregular, Lobate
12.	BCH 12	Whitish Brown	Slightly Slimy	Irregular, Undulate, Translucent, Rugose
13.	BCH 13	Dirty White	Slimy	Irregular, Lobate
14.	BCH 14	White	Slimy	Irregular, Umbonate
15.	BCH 15	Pure White	Slimy	Undulate, Wavy, Irregular
16.	BCH 16	Dirty White	Slimy	Irregular, Undulate, Wavy
17.	BCH 17	Yellowish White	Slimy	Serrate, Scalloped Margin
18.	BCH 18	Dirty White	Smooth	Irregular, Serrate
19.	BCH 19	Yellowish Brown	Slimy	Irregular, Lobate, Swarming
20.	BCH 20	Light Brown	Slightly Slimy	Round, Umbonate, Serrate Margin
21.	BCH 21	Dirty White	Rough	Irregular Raised Colony Elevation
22.	BCH 22	White	Slimy	Round, Convex
23.	BCH 23	Yellowish Brown	Slightly Slimy	Irregular Raised Colony
24.	BCH 24	Brown	Slimy	Round, Entire Margin, Convex
25.	BCH 25	Dirty Brown	Slightly Slimy	Irregular, Undulate
26.	BCH 26	Dirty Brown	Slimy	Irregular, Lobate
27.	BCH 27	Light Brown	Slimy	Irregular Convex Colony, Undulate

(The bacterial isolates were morphologically characterized based on sliminess, colour and colony characters)

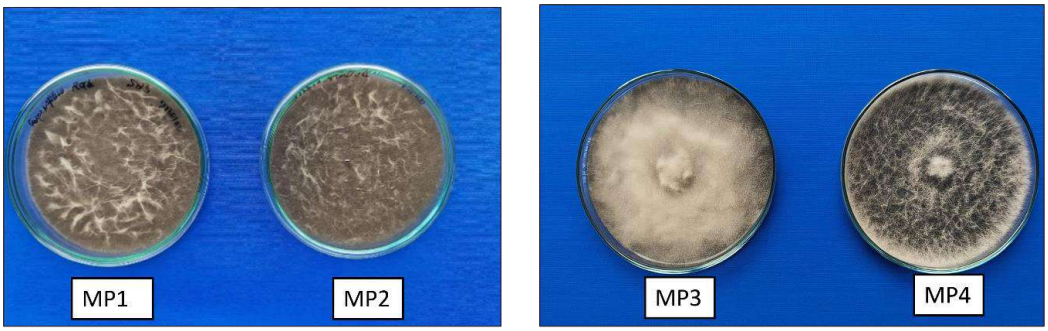
Table 4: Screening of chitinolytic bacterial isolates against the *M. phaseolina* infection of blackgram – Dual Culture Technique (Dennis and Webster 1971).

Sr. No.	Name	Inhibition zone (mm)	Mycelial growth (mm)	Per cent inhibition over control (%)
1.	*S. thalpophilum*	7^{b} (15.16)	33^{b} (33.22)	63.33
2.	Uncultured bacterium	9.5^{ab} (17.67)	28.5^{bc} (30.80)	68.33
3.	*A. faecalis*	**11^{a} (19.02)**	27.5^{c} (30.24)	69.44
4.	Control	0^{c}	90^{a} (60.62)	0
SE(d)		1.061	1.650	
CD		2.945	4.604	

*Values are the mean of two replications

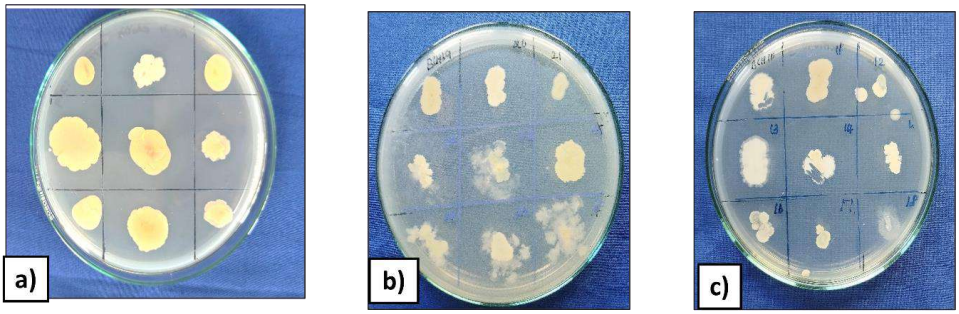
Yellowing of leaves, wilting of plants, bark shredding, dark brown lesions on the stem at ground level, and affected plants can be easily pulled out with rotten roots.

Fig. 1. Survey and Symptomology.



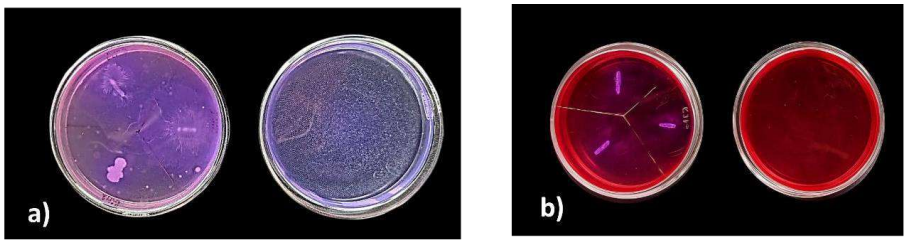
MP1-Baburayanpettai, MP2-Solanganallur, MP3-Kuruvampatti, MP4-Chathiramanai

Fig. 2. Isolation of *Macrophomina phaseolina* infecting blackgram.



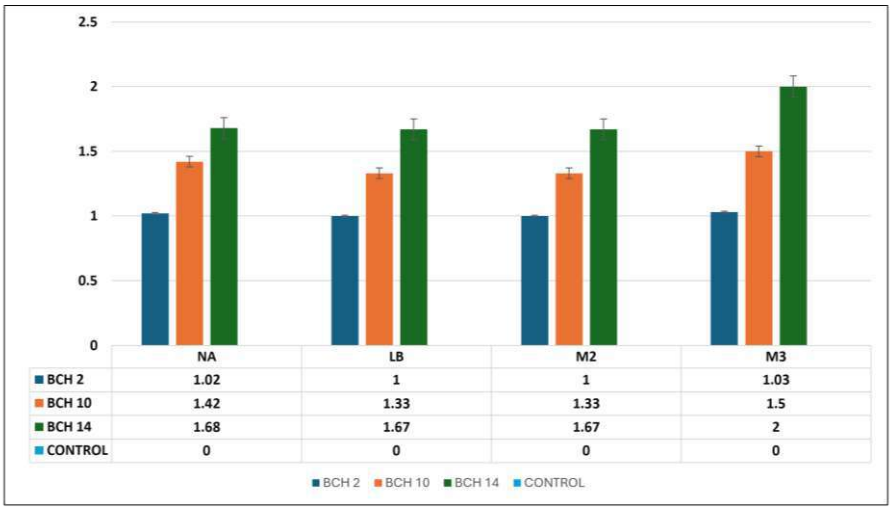
27 isolates having antagonistic activity were obtained from the dilutions 10^3 and 10^6

Fig. 3. Different isolates of chitinolytic bacteria isolated from cow horn samples.



The selected bacteria utilized and degraded the peptone and beef extract present in the medium. Formation of clear zones around bacteria.

Fig. 4. Minimal media of NA for selected chitinolytic bacteria.



The chitinase activity of the isolates varied across the four media. M3 medium showed the highest enzyme production, indicating it best supported chitinase induction. M2 also showed good activity but less than M3. LB broth supported moderate enzyme levels, while Nutrient Broth had the lowest.

Fig. 5. Comparative analysis of growth media for enhanced chitinase production in chitinolytic bacteria.

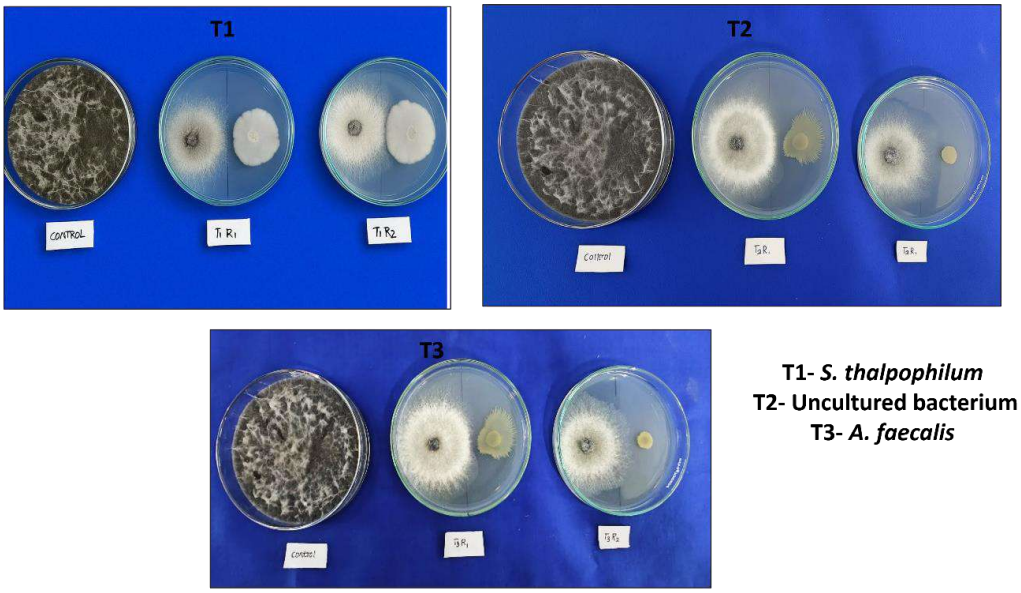


Fig. 6. Screening of chitinolytic bacterial isolates against the charcoal rot pathogen of blackgram.

CONCLUSIONS

This study emphasises the potential of chitinolytic bacteria as an environmentally friendly approach for managing charcoal rot disease in black gram, caused by *Macrophomina phaseolina*. A field survey conducted across major black gram-growing regions in Tamil Nadu confirmed the presence of the disease. The pathogen was isolated and identified through morphological observations and molecular techniques. Out of 27 bacterial isolates obtained from a cow horn sample, three strains, BCH 2, BCH 10, and BCH14, exhibited strong chitinase activity, which helps break down fungal cell walls. These isolates formed clear zones on chitin-containing media and showed good enzyme production, especially in M3 medium. BCH 14 performed the highest level of effectiveness. These bacteria also grew well in special media designed to detect chitinase activity. Overall, the findings suggested that chitinolytic bacteria, especially BCH 14, could serve as a safe and natural alternative to chemical fungicides. Applying these helpful microbes in agriculture can effectively manage plant diseases, lower the reliance on chemical treatments, and promote environmentally friendly farming. This approach provides a promising way to control charcoal rot in black gram while sustainably enhancing crop health and productivity.

FUTURE SCOPE

Chitinolytic bacteria offers great potential for controlling *Macrophomina phaseolina* because of its strong biocontrol abilities and eco-friendly traits. Recent improvements in formulation methods, like

biopesticide and fungicide using multiple strains together, should improve its effectiveness in the field. Additionally, molecular tools and omics approaches will help create genetically improved strains with better resistance to pests and environmental stress. Using Chitinolytic bacteria in sustainable plant protection strategies could significantly reduce the need for chemical fungicides and pesticide.

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Conflicts of Interest. None.

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