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Research Article

ISOLATION AND CHARACTERIZATION OF POTENTIAL BIOCONTROL RHIZOSPHERIC BACTERIA EFFECTIVE AGAINST WHITE ROT (*SCLEROTIUM CEPIVORUM* Berk) AFFECTING GARLIC (*ALLIUM SATIVUM* L.) UNDER LABORATORY CONDITIONS

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ABSTRACT. Garlic (*Allium sativum* L.) is the second most widely used Allium used as food, medicine, condiment and cash crop vegetable. Its productivity and yield is affected by both abiotic and biotic factors. White rot (*Sclerotium cepivorum*) is one of the biotic agents that aggressively attack garlic. The main aim of this study was to isolate and screen potential antagonists for controlling this pathogen. Consequently, 23 rhizospheric bacterial isolates were screened. The isolates' biocontrol potential against the pathogen was tested under laboratory conditions using microbiological procedures. Among these isolates, 11 (47.8%) of them inhibited the radial growth of the pathogen with growth inhibition zone ranging 60 - 88%. The isolates also showed wide morphological and cultural diversity. Of the isolates, 9 (82.8%) solubilized phosphate at solubilization indices of 2.3–3.72 cm. Among the isolates 6(54. 6%), 10(90.9%), 5 (45.5%), 100% and 5 (45.5%) were chitinase, cellulase, protease, ammonia and cyanide producers, respectively. Of the biocontrol traits tested, 6 (54.55%) of them were endowed with all the expected biocontrolling characteristics. Most of the isolates showed high resistance to extreme environmental stresses including pH, temperature and salt concentrations. Moreover, the isolates also showed high tolerance to the tested antibiotics and heavy metals. In the all the tested parameters conducted under laboratory conditions, isolates WUGR-8, WUGR-14 and AAUGPR- 92 showed the top performance. Consequently, these isolates can be recommended as candidate microbial inoculants for greenhouse applications.

Keywords: *Biocontrol, Garlic, phenotypic characterization, Rhizobacteria, White rot*.

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the family Alliaceae and is the second most widely used Allium after onion [1]. It is a significant bulb vegetable crop used as a condiment of foods, adds a taste to foods, and makes food more palatable and digestible [2]. It has been recognized for its medicinal value in the control and treatment of hypertension, worms, germs, bacterial and fungal diseases, diabetes, cancer, ulcer, and rheumatism [3]. Garlic grows best at higher elevations ranging from 1800–2800 meters above sea level where cool weather conditions prevail.

Garlic contains 30–35 percent dry matter, 6–7% protein, 0.2 percent total lipid, 23–28 percent carbohydrate, 0.7–0.9 percent fiber, 1.1–1.4 percent ash matter, and vitamins B1, B2, B6, and C, as well as antibiotics garlicin and allistatin, enzymes, amino acids, universal substances, and trace elements [4]. Garlic contains antihypertensive, anticancer, anticoagulant & fibrinolytic, anthelmintic, wound healing potential, antiatherosclerosis &hypolipidemia, antifungal, antibacterial, anti-diabetic, anti-inflammatory, antioxidant, and hepatoprotective properties, in addition to its nutritional benefits [5]. It's mostly used as a condiment and a source of revenue for some small-scale farmers [6, 7]. It's also used as a spice and flavoring in cuisine, besides as a medicinal plant [8].

Garlic output in Ethiopia varies for a variety of reasons. In 2006/07, the total cultivated production area was 9,266 hectares, with an anticipated annual production of 683,000 quintals [9]. According to the same author, total production increased from 79,421 to 222,548 tonnes of bulbs from 6,042 hectares of land in 2001/02 to 21,258 ha of land in 2012/13. However, from 13.20 and 10.47 t ha, respectively [7, 10], its productivity has fallen. Inappropriate agronomic practices, limited improved variety, and a lack of disease control and insect pest management practices were among the production hurdles that contributed to Ethiopian garlic's low yield [11].

White rot, the destructive disease that causes large losses in garlic is one of the 66 diseases that damage the Allium crop globally [12]. White rot is a dangerous fungal disease that significantly reduces the potential yields of onion and garlic crops in Ethiopia. Once established in the area, the disease generates large numbers of poppy seed-sized sclerotia that can live in the soil for many years, rendering the field useless for garlic for up to 40 years in Ethiopia [13]. Chemical fungicides have been used to control this disease, but they have health and environmental risks. As a result, this control strategy is costly, pollutes the environment, and causes harm to non-target organisms. Biocontrolling agents, on the other hand, are less expensive, environmentally friendly, effective, and non-toxic to beneficial organisms. In the research area, no relevant disease control techniques against these bacterial and fungal phytopathogens of garlic are currently being pursued. The exorbitant costs of pesticides besides their unfriendly environmental impact necessitate the use of biocontrol technologies as preferable alternatives. *Pseudomonas* and *Bacillus* spp. have been widely used as biocontrol techniques in low-input agriculture, according to innovations in sustainable agriculture [14]. Furthermore, *Trichoderma* is used as a fungal microbial inoculant to suppress the effect of the garlic pathogen [15].

Antifungal bacteria use antibiotics, siderophores, lytic enzymes, (fungal cell wall disintegrating) hydrogen cyanide (HCN), competition for nutrition and parasitism, and the emission of powerful antifungal volatile organic chemicals to limit or control the pathogen's effects [16]. *Pseudomonas* is a rhizospheric bacterium that produces a wide range of metabolites (antibiotics, siderophores, volatiles, and growth-promoting compounds), competes aggressively with other microorganisms, and responds to environmental challenges [17]. *Bacillus* species having a unique ability to grow quickly while also being resistant to harsh environmental conditions create volatile chemicals with a wide spectrum of biocontrol potential [18].

This study was aimed at isolating and characterizing rhizobacteria collected from the rhizosphere of the healthy standing plant of garlic and evaluating their biocontrol potential against white rot (*Sclerotium cepivorum*) attacking garlic under laboratory conditions.

MATERIALS AND METHODS

Sample collection

Soil samples were taken from Ferede kebele, Woreilu District, South Wollo Zone in Amhara National Regional State, from healthy standing garlic plants growing among white rot (*Sclerotium cepivorum*) infected garlic plants. Moreover, parts of infected garlic plants were collected. They were then placed in sterilized plastic polythene bags and transported to the Wollo University Biotechnology Laboratory, where they were maintained in a 4°C refrigerator until the pathogen White rot was isolated [19]. Furthermore, strains that were previously identified [20] and kept at Wollo University's Biotechnology Laboratory refrigerator were used as a sample source and were replenished by regular culturing.

Isolation and identification of the Sclerotium cepivorum

The disease-infected parts of garlic including the bulb, stem, and leaf were cut into small pieces and surface sterilized with 0.5% sodium hypochlorite solution for two minutes and washed three times with sterilized distilled water [21]. They were dried between two layers of sterilized filter papers to remove the excess water and placed on sterilized potato dextrose agar (PDA) media containing (dextrose 20 g/l, potato extract 4.0 g/l, Agar 15 g/l). The plates were incubated at 28±2 °C for 5-7 days and grown fungal cultures were purified by using hyphal tip isolation techniques (Brown, 1924). The fungal isolates were identified according to the guideline [22]. For further investigation, the pure cultures were stored on PDA slants at 4 °C.

Isolation, purification and designation of antagonistic rhizobacteria

As the source of bacterial antagonists, the rhizosphere soil attached to the roots of healthy garlic plants growing among white-rot-infested ones was used. Approximately 1 g of rhizosphere soil was homogenized with 9 mL sterilized distilled water and shaken for 30 min., and suspensions were serially diluted until 10^{-6} with sterile water (Han et al., 2015). An aliquot (0.1 ml) of dilution was plated on a nutrient agar medium (NA) supplemented with 100 $\mu g ml^{-1}$ of cycloheximide to suppress fungal growth [19] and incubated at 28±2°C for 3- 5 days. Single and seemingly different colonies were selected and purified through repeated re-streaking and incubation until pure isolates were obtained.

Dual culture inhibition test

The antagonistic activity of the rhizobacteria against the white-rot was tested using the dual culture technique as described by [23]. A loop full of the bacterial isolates was equidistantly spot inoculated on the margins of potato dextrose agar (PDA) plates amended with sucrose (0.5%) and incubated at 28 ± 2 °C for 48 h. A 4 mm diameter of the fungal pathogens from PDA grown culture was placed at the center of each bacterial isolate grown plate and incubated at 28±2°C for 5 days. Plates containing fungal discs without rhizobacteria were included as controls. Fungal growth inhibition by the bacterial isolates was assessed by the presence of the inhibition zone on the dual culture, and the fungi inhibited by bacterial isolates were estimated by measuring the percent radial growth inhibition zone (PIRG) by using the equation described by [23]:

$$PIRG = C\text{-}T/C*100$$

Where PIRG= ercentage inhibition of radial growth, C=radial growth measurement of the pathogen in control, T=radial growth of pathogen in the presence of antagonistic bacteria.

The isolates that showed better inhibition were selected for further plant growth promotion and biocontrol tests.

Designation of the isolates

The purified isolates that displayed better inhibition test during dual culture test were designated as WUGRB (Wollo University Garlic Rhizobacteria) represented by different numbers (Table 1).

Morphological and Biochemical characterization of the isolates

Colony morphology

The bacterial isolates were determined by growing them on nutrient agar and incubating at 28±2 ℃ for 2-3 days as described by [19]. Then they were selected based on their shape, elevation, surface, color, and pigmentation features.

Gram reaction

The Gram reaction type of the rhizobacterial isolates was determined using the KOH technique [24]. A drop of 3% KOH was poured on a clean microscope slide, and a loop of rhizobacterial colony was adequately mixed with it for l minute. The mixture was raised about 1 cm from the slide using an inoculating loop, and the presence and lack of visible stringiness (viscosity) were recorded as Gram-negative and Gram-positive bacteria, respectively.

Production of hydrolytic enzymes

Chitinase production

Using the method outlined by [25], the ability of the test isolates to generate chitinase was tested by growing them on colloidal chitin agar (CCA) media. The CCA contained 0.4% colloidal chitin, 007% K_2HPO_4, 003% KH_2PO4, 003% NaCl, 0001% $FeSO_4.7H_2$, 005% $MgSO_47H_2O$, 00001% $MnCl_2.4H_2O$, 002% yeast extract, and 2% agar. Bacterial cultures of 48 hr. old were injected on CCA media and incubated for 3 days at 28±2 °C.

The presence of a clear zone around the indicated chitinase production.

Cellulase and Protease production

According to [26], the isolates' cellulase production potential was determined on a carboxy-methylcellulose agar medium with yeast extract plates containing (g l^{-1}) $NaNO_3$ (2), K_2HPO_4 (1), $MgSO_4$ (05), KCl (05), CMC sodium salt (2), peptone (02), and agar (17), and incubated for 3–5 days at 28±2 °C. Cellulase production was suggested by the formation of distinct halos around bacterial colonies. According to [27], the isolates' protease activity was evaluated on skim milk agar (skim milk powder 10 g l^{-1}, agar 15 g l^{-1}). Protease activity was demonstrated by the formation of clear zones around the colonies.

Hydrogen cyanide and Ammonia production

According to [28], all isolates were evaluated for HCN production by streaking them on a slant NA media with filter paper strips bathed in picric acid and 2% sodium carbonate. After sealing the test tubes with parafilm, they were incubated at 28±2°C for 3–5 days. The color change of the yellow filter paper strips to brown or red was used to determine HCN production. Each rhizobacterial isolate was cultured in peptone broth (10 mL) and incubated at 28±2 °C for 48 to 72 hours to determine ammonia production. After incubation, the bacterial suspension was given 0.5 mL of Nessler's reagent. Ammonia production was recognized by the change in color from yellow to brown [29].

Plant growth promotion properties of the isolates

Phosphate Solubilizing Test

By inoculating each isolate on pikovaskaya agar medium containing (g/l): Glucose (10), tricalcium phosphate (5), ammonium sulphate (0.5), yeast extract (0.5), magnesium sulphate (0.1), sodium chloride (0.2), manganese sulphate (0.002), and agar (15), the isolates' ability to solubilize phosphate was determined. The medium's pH was adjusted to 7.0. The plates were incubated for 3–5 days at 28±2 °C. For phosphate solubilization, the growth and establishment of clear zones around the colonies were studied [19]. Solubilizers of tricalcium phosphate (TCP) were chosen from isolates that produced a distinct halo zone surrounding them, and their qualitative solubilizing indices (PSI) were computed according to [23]:

$$PSI = \frac{\text{Halo zone diameter (Z) + colony diameter (C)}}{\text{Colony diameter (C)}}$$

Nitrorgen fixation test using N-free media

Using nitrogen-free media, a qualitative assessment of nitrogen fixation was carried out [30]. The following composition (g/l) of nitrogen-free medium is used: The following ingredients were added: ager (15), $CaCO_3$ (1), K_2HPO_4 (1), $MgSO_4.7H_2O$ (0.2), NaCl (0.2), $Fe_2SO_4.7H_2O$ (0.1), $Na_2MoO_4.2H_2O$ (5), 50ml glucose solution, and 1000ml distilled water. The bacterial isolates were subsequently cultured for 5 days at 28±2 °C, with the production of a pellicle at the subsurface level deemed a positive test for N-fixation.

Indole acetic acid (IAA) production

Indole-3-acetic acid production (IAA) by the bacterial isolates was evaluated using the method described by [31]. Bacterial cultures were grown in LB (Luria-Bertani) medium composition supplemented with 1-tryptophan (1.02 g/l) and incubated at 28±2 °C for 3–5 days. The cultures were then centrifuged at 7000 rpm for 3 min, and 1ml of the supernatant was added to 2ml of Salkowski reagent (60% of perchloric acid, 3 ml 0.5 M $FeCl_3$ solution). The development of a pink coloration indicated the production of IAA.

Physiological characterization

The growth of each isolate on NA medium adjusted at different pH (4.0, 4.5, 5.0, 9.0, 9.5, and 10) and 2, 3, 4, 5, 6, 7, 8, and 9% NaCl [32], besides incubation temperatures of

4, 10, 15, 35, 40, and 45 °C [33]. The plates were incubated at 28±2 °C for 3-5 days. Resistance was determined by measuring the growth of rhizobacterial isolates.

Resistance to heavy metal test

Resistance to heavy metal (HM) was determined by growing bacterial cultures on solid NA media containing filter-sterilized HM at concentrations (μgml^{-1}) of Hg (HgO) (50), (As_2O_3) (100), Ni ($NiCl_2$) (100), Pb ($Pb(CH_3COO)_2$) (100), Cd ($CdCl_2$) (100), and Cr ($CrCl_2$)(10) and monitoring colony growth after 3–5 days of incubation at 28±2°C [34]. After incubation, presence and absence of growth was recorded.

Antibiotic resistance test

Resistance to concentrations of antibiotics were determined by preparing the fresh solutions of filter sterilized (0.22 µm) antibiotics in NA medium containing one of the following filter sterilized antibiotics (μgml^{-1}): ampicillin (30), chloramphenicol (40), erythromycin (30), nalidixic acid (20), neomycin (20), streptomycin (10) and tetracycline (30), and novobiocin as described by [35] and incubated for 5 days at 28±2 °C.

Statistical analysis

The information gathered was subjected to an analysis of variance (ANOVA). Duncan Multiple Range Test (DMRT) was used to compare the treatments. The level of significance was determined using SPSS version 20 at $P<0.05$.

RESULTS AND DISCUSSION

Isolation of White rot

The incubation of the infected part of the garlic plant leaves resulted in the growth of White rot (*Sclerotium cepivorum*). The White rot mycelium grown in young PDA agar plates changed into black sclerotium which indicated the morphology of White rot.

Isolation of the antagonistic rhizobacteria

At the end of serial dilution and three days of incubation, five rhizobacterial isolates that showed variation in margin, shape, texture, elevation, and pigmentation were obtained. Moreover, among isolates stored at the stock culture of Wollo University Biotechnology Laboratory, six isolates showed different bacterial colony morphology.

Morphological characterization and Gram reaction of the isolates

The isolates showed wide variation in colony morphology and gram reaction (Table 1). Regarding the morphological properties of the isolates, 5 (45.5%) and 6 (55.5%) of the isolates were raised and flat in elevation, whereas 6 (55.5%) and 5 (45.5%) were circular and irregular in shape. All the bacterial isolates were opaque. Seven (63.6%) and 4 (36.4%) were rough and wrinkled in texture, respectively. Likewise, 8 (72.7%), 2 (18.2%), and 1(0.9%) were white, pink, and orange in pigmentation, in order. In their margin, the isolates were undulate (81.8%), entire (9.1%), and curled (9.1%). The Gram reaction test confirmed that 7 (63.6%) and 4 (36.4%) of the rhizobacterial isolates screened for characterization were Gram-positive and Gram-negative, respectively.

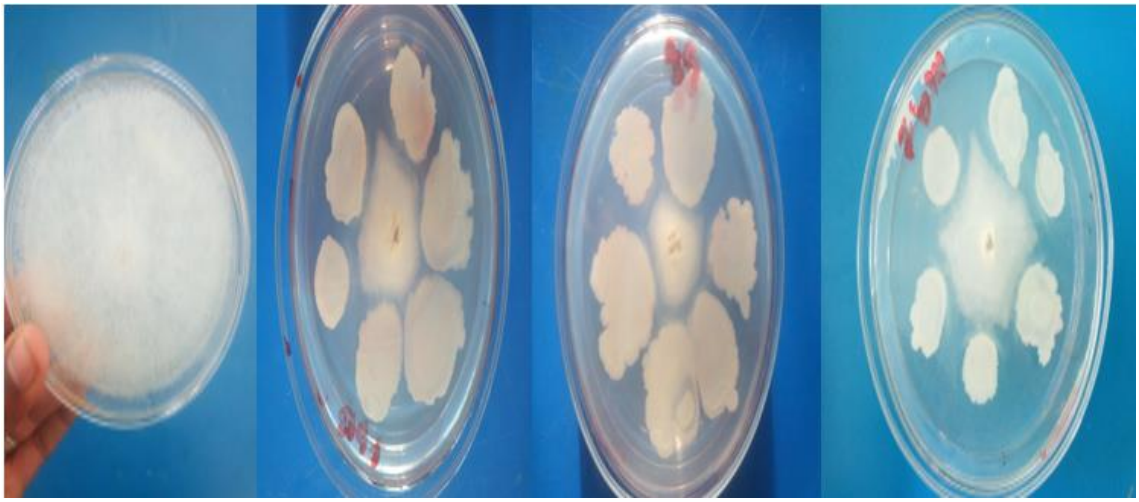
Table 1. *Morphological and cultural characterization of the isolates*

Isolates	Margin/ Border	Edge / Surface	Pigmentation	Elevation	Opacity	Form	Gm reaction
WUGRB-8	Undulate	Wrinkled	White	Flat	Opaque	Irregular	+
WUGRB-9	Undulate	Rough	White	Flat	Opaque	Circular	-
WUGRB-10A	Curried	Rough	White	Flat	Opaque	Circular	+
WUGRB-13	Undulate	Rough	White	Flat	Opaque	Irregular	-
WUGRB-14	Undulate	Wrinkled	White	Flat	Opaque	Irregular	-
AAUGPR-38	Undulate	Rough	White	Raised	Opaque	Irregular	-
AAUGPR-53	Undulate	Rough	Orange	Raised	Opaque	Circular	+
AAUGPR-90	Undulate	Wrinkled	Pink	Raised	Opaque	Circular	+
AAUGPR-91	Undulate	Wrinkled	White	Flat	Opaque	Irregular	+
AAUGPR-92	Undulate	Rough	Light pink	Raised	Opaque	Circular	+
AAUGPR-98	Entire	Rough	White	Raised	Opaque	Circular	+

Gm=Graham reaction

Dual culture inhibition

Among a total of 23 rhizobacterial isolates, 11 (47.8%) of them inhibited the growth of the fungal pathogen, white rot. The isolates showed a fungal radial growth inhibition ranging from 60-92.4% with the maximum (92.36±3.20) and minimum (60.0±0.00) growth inhibition recorded by isolates WUGRB -9 and WUGRB -53, respectively (Table 2). The radial growth of the fungal pathogen was highly restricted by the antagonistic rhizobacterial isolates as compared to the control treatment (Fig 1).



Control WUGR- 8 WUGR- 9 WUGR- 14

Fig. 1. *Selected representative isolates inhibiting white rot growth*

Table 2. *White rot growth inhibition by selected rhizobacterial isolates*

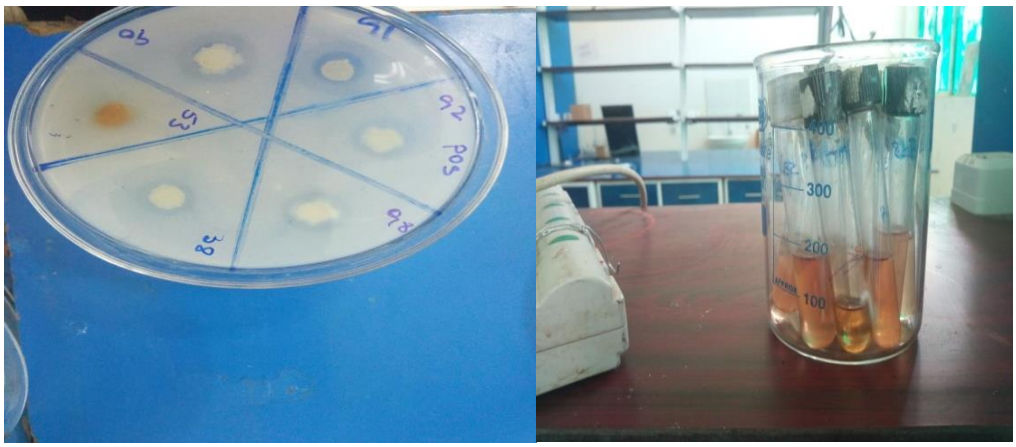
Isolate code	Source of the isolates	Strain	Average inhibition(cm)	% PIGR
CONTROL	-	-	-	0.00±0.00^{e}
WUGRB-8	Wereilu	UN	1.3	69.9±6.75^{cd}
WUGRB -9	Wereilu	UN	0.18	92.36±3.20^{a}
WUGRB -10	Wereilu	UN	0.7	84.4±0.00^{ab}
WUGRB -13	Wereilu	UN	0.9	79.93±3.86^{c}
WUGRB -14	Wereilu	UN	1.46	66.66±1.15
WUGRB -38	Stock culture	UN	1.5	66.0±0.0 0^{d}
WUGRB -53	Stock culture	UN	1.8	60.0±0.00^{d}
WUGRB -90	Stock culture	UN	1.5	66.00±0.00^{d}
WUGRB -91	Stock culture	UN	0.5	88.00±0.00^{b}
WUGRB -92	Stock culture	UN	1.06	76.23±2.54^{c}
WUGRB -98	Stock culture	UN	1.5	66.0±0.00^{d}

PIRG=percent of radial growth inhibition, UN=Unidentified

Testing the rhizobacterial isolates for plant growth promoting traits

Phosphate solubilization

The tested isolates showed variation in their phosphate solubilizing capacity (Table 3). Among the isolates, 9 (81.8%) of them solubilized phosphate. The isolates solubilized phosphate at PSI ranging 2.3-3.6 cm. The maximum solubilization potential (3.6 cm) was obtained from the isolates designated by WUGRB 91, 92, whereas the minimum solubilizing ability (2.3 cm) was shown by isolate WUGR-98. Among the isolates, 8 (72.7%) of them were indole acetic acid producers. In general, the isolates showed better phosphate solubilizing efficiency (Fig 2).



Phosphate solubilization Indole acetic acid production

Fig. 2*. Representative phosphate solubilizing and IAA producing isolates*

Table 3. *Plant growth promotion traits of selected isolates*

Isolate	Phosphate solubilisation			N- fixation	IAA production
	Colony diameter	Halo zone diameter(cm)	% PSI		
Control	-	-	0.00 ± 0.00^{d}	-	-
WUGRB-8	1.3	1.8	2.4 ± 0.2^{c}	+	+
WUGRB-9	-	-	-	+	+
WUGRB-10	-	-	-	-	-
WUGRB-13	0.5	0.9	2.8 ± 0.11^{b}	-	-
WUGRB-14	0.5	0.7	2.4 ± 0.20^{c}	+	+
WUGRB-38	0.8	1.25	2.6 ± 0.42^{b}	-	+
WUGRB-53	0.6	1.5	3.4 ± 0.94^{ab}	+	+
WUGRB-90	1.0	1.45	2.5 ± 0.11^{bc}	-	+
WUGRB-91	0.7	1.65	3.6 ± 1.0^{a}	+	+
WUGRB-92	0.8	2.05	3.6 ± 0.34^{a}	+	+
WUGRB-98	1.1	1.4	2.3 ± 0.10^{cd}	-	-

PSI= phosphate Solubilization Index, N-= Nitrogen fixation, IAA= Indole acetic acid

Nitrogen fixation and indole acetic production

The ability of the isolates to fix nitrogen and produce indole acetic acid was presented (Table 3). Of the isolates, 6 (54.6%) fixed nitrogen and 8 (72.7%) showed a positive result for indole acetic acid production. Isolates WUGRB 8, 14, 53, 91, and 92 were positive for all plant growth-promoting traits (Fig. 2).

Production of hydrolytic enzymes and bioactive compounds

The potential of the isolates to produce hydrolytic enzymes such as chitinase, protease and cellulase, and ammonia and hydrogen cyanide was presented (Table 4).

Table 4. *Evaluating the isolates for having other biocontrol traits*

Isolates	Biocontrolling traits				
	Chitinase	Protease	Cellulase	HCN	NH_3
WUGRB- 8	-	+	+	-	+
WUGRB- 9	-	+	+	-	+
WUGRB- 10	+	+	-	-	+
WUGRB- 13	-	+	-	-	+
WUGR- 14	+	+	+	+	+
WUGRB- 38	-	+	+	+	+
WUGRB- 53	+	+	+	+	+
WUGRB- 90	+	+	+	+	+
WUGRB- 91	+	+	+	+	+
WUGRB- 92	+	+	+	+	+
WUGRB- 98	+	+	+	+	+
%	63.6	100	81.8	72.7	100

Note: NH_3 = Ammonia, HCN=Hydrogen cyanide

All the isolates produced ammonia, whereas 8 (72.7%) of them produced hydrogen cyanide. Regarding the isolate's ability to produce hydrolytic enzymes, 100%, 81.8%, and

63.6% of the isolates produced protease, cellulase, and chitinase, in order. The minimum biocontrol property is shown by the isolate WUGRB 13, whereas isolates WUGRB 14, 53, 90, 91, 92, and 98 produced all the tested hydrolytic enzymes and bioactive compounds.

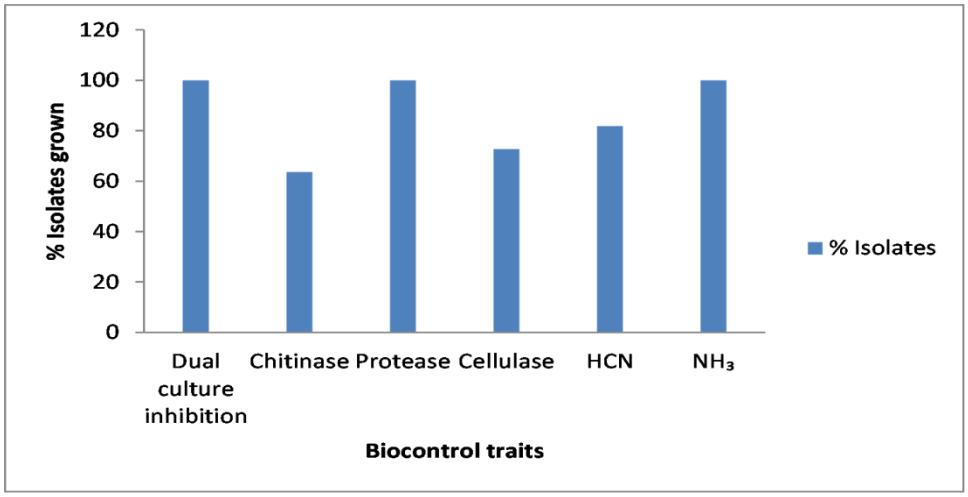


Fig. 3. *The potential of the isolates to show biocontrol traits*

Most of the isolates that inhibited the radial growth of the fungus showed better performance in their biocontrolling properties by producing protease, ammonia and hydrogen cyanide (Fig 4).

Hydrogen cyanide production | Ammonia production | Protease production

Fig. 4. *Biocontrol activities of some isolates*

Physiological Tolerance of the Isolates

The isolate's potential to intrinsically resist extreme pH, temperature and salinity was determined (Fig. 2-4). Among the isolates, 7 (63.6%) of them grew at pH 4 and 5, whereas, 9 (81.8%) grew at pH 6. On the other hand, all (100%) of the isolates showed growth at pH 7- pH 9.5. Seven (72.7%) grew at pH 10. Isolates WUGRB 8, 14, 53, 91, and 92 showed growth at all the tested pH ranges (pH 4-10). Moreover, few isolates

(45.5%) grew at pH 11. The overall findings showed that an increment of pH resulted in the reduction of bacterial growth. Isolates WUGRB- 8, 91, and 92 grew at the tested pH ranges.

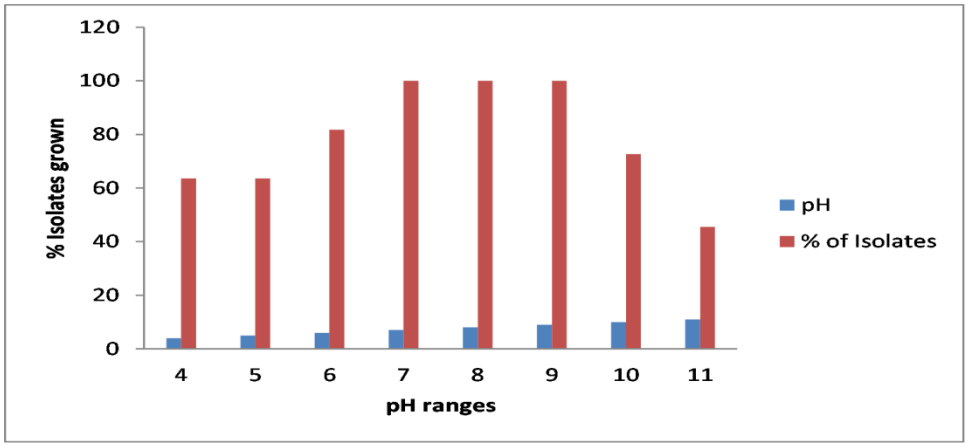


Fig 5. *pH tolerance of rhizobacteria*

Variation in bacterial growth to varying salt concentrations was observed (Fig. 6). Regarding the resistance of the isolates to different concentrations of salt, all the rhizobacterial isolates grew at a range of 1-6% salt concentrations. Nine (81.8%) isolates grew at 5-6% salt concentrations. On the other hand, 7 (63.6%), 5 (45.5%), and 3 (27.7%) of the isolates grew at salt concentrations of 7, 8, and 9%, respectively. Likewise, an increase in salinity showed a sharp decrease in the growth of the isolates. Isolates WUGRB- 8, 53, and 91 resisted all the salt concentrations.

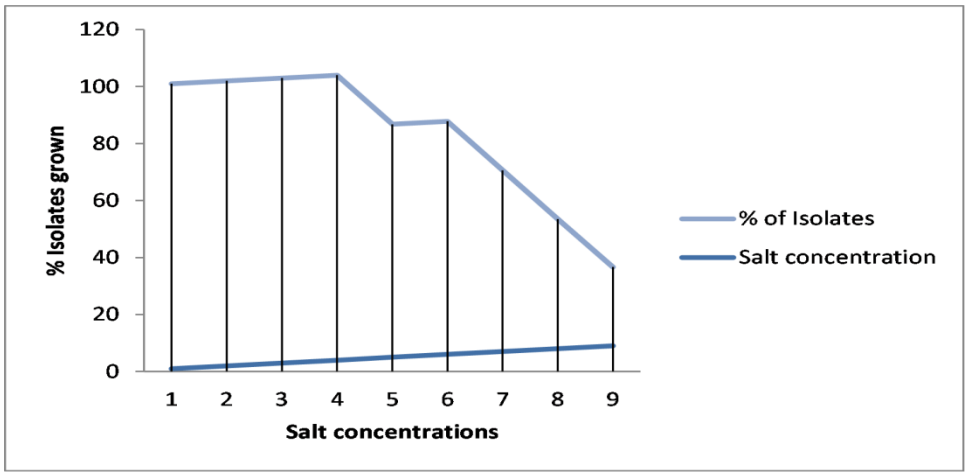


Fig. 6. *Salt resistance of the rhizobacterial isolates*

The isolates showed growth variation for different temperature ranges (Fig. 7). Only 5 (63. 6%) of the isolates grew at 4 ℃, whereas 8 (72.7%) showed growth at 10 ℃. Nine isolates (81.8%) showed growth at 15 ℃. All the isolates grew at temperature ranges of 20- 30 ℃. At temperatures of 40 ℃ and 50 ℃, 72.7 and 27.3% of the isolates showed

growth resistance. Similarly, isolates WUGRB-8, 9, and 92 grew at both low and high-temperature ranges.

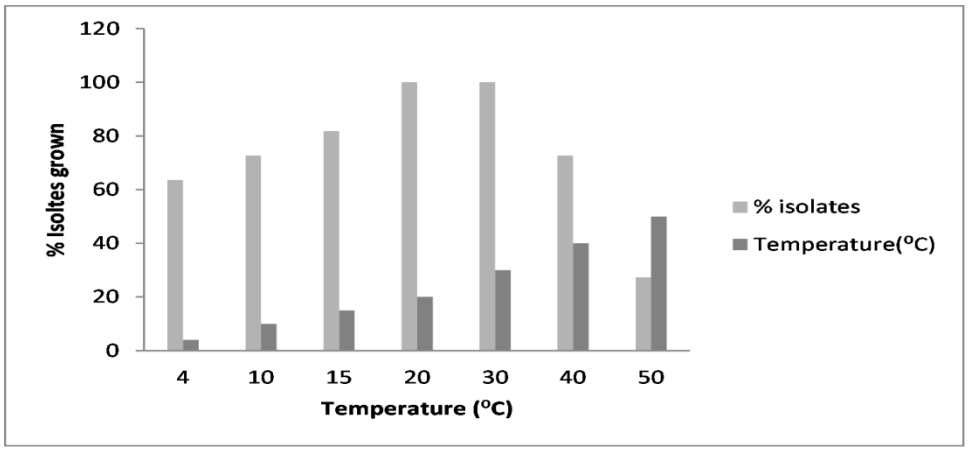


Fig 7. *Temperature tolerance of the rhizobacterial isolates*

Antibiotic and Heavy metal resistance

The isolates' resistance and sensitivity to different antibiotics were determined (Fig. 8). Out of the tested isolates, 9.1% of the isolates showed growth resistance to Neomycin (30 µg) and streptomycin (10 µg), whereas 18.2% and 36.4% grew in nutrient medium supplemented with 30 µg chloramphenicol and 30 µg nalidixic acid. Similarly, 45.5%, 54.5%, 72.7%, and 81.8% of the isolates showed resistance to ampicillin (25 µg), erythromycin (15 µg), tetracycline (30 µg), and novobiocin (5 µg). The isolates WUGRB- 14, 53, and 92 were resistant to 75% of the tested antibiotics.

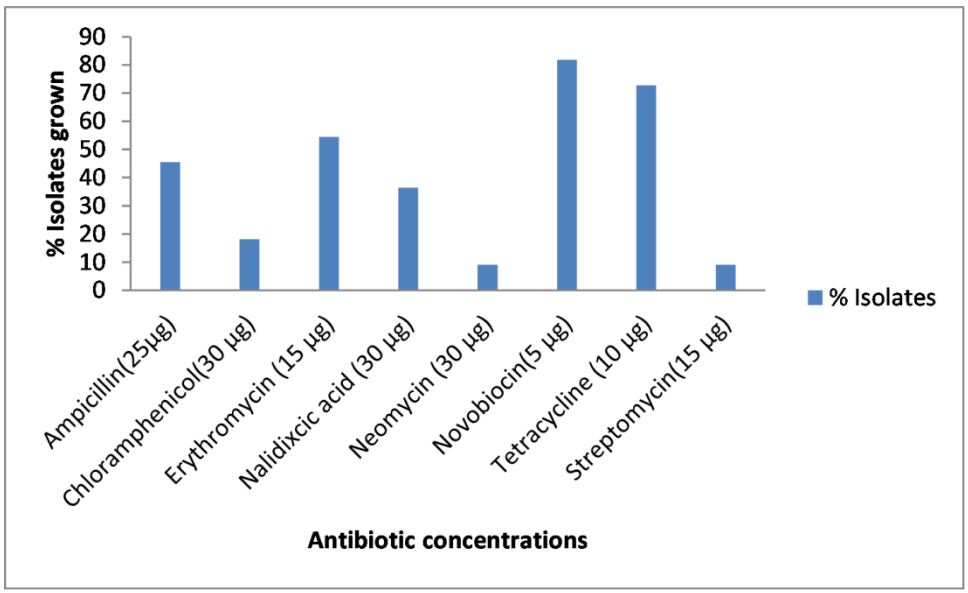


Fig. 8. *Antibiotic resistance and sensitivity test*

The isolates showed resistance and sensitivity variation to different heavy metal concentrations (Fig. 9). All isolates grew at 100 µg/ml of As_2O_3. Among the isolates, 36.4% and 45.5% of the isolates were sensitive to $CrCl_2$ and HgO, respectively.

Moreover, 63.4%, 72.7%, and 90.9% of the isolates showed growth resistance in $CdCl_2$, $Pb(CH_3COO)_2$, and $NiCl_2$, in order. Among the isolates, isolates WUGRB-14, 53, and 92 showed resistance to all the tested heavy metals.

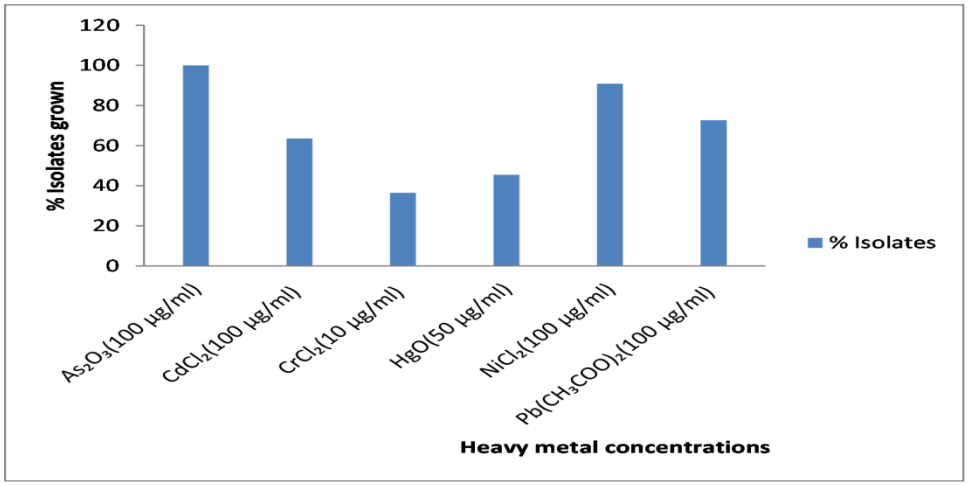


Fig. 9. *Tolerance and Sensitivity of the isolates to heavy metals*

This study which aimed at screening garlic rhizospheric bacteria is vital in selecting potential rhizobacteria endowed with plant growth and biocontrol traits. Consequently, 15 rhizospheric bacterial antagonists having an inhibiting effect against the phytopathogens, *Sclerotium cepivorum* Berk were isolated of which 11 (47.8%) of the isolates inhibited the radial growth of the pathogen which was similarly reported in [36]. Regarding fungal pathogen growth inhibition, 60- 92% of the isolates showed antifungal property by inhibiting the radial growth of the fungal pathogen, *Sclerotium cepivorum.* Similarly, [37] reported that *Bacillus* isolates inhibited the radial growth of *Sclerotium cepivorum* by 76.8-82.7% under dual culture conditions.

Concerning the hydrolytic properties of the isolates, 6 (54.6%), 10 (90.9%), and 5 (45.5%) of them were positive for Chitinase, cellulase, and protease production in the same order. Similarly, 92 % of protease producing rhizobacteria [20] and 60 and 50% of chitinase and cellulase producing rhizobacteria, respectively [38] were reported indicating the effectiveness of the study isolates. The presence of such bacterial isolates is a highly important strategy to inhibit fungal spore germination and lysis of hyphal tips [39] and to resist environmental, mechanical, and chemical stresses [40].

Among 11 isolates tested for production of bioactive compounds, 11 (100%) and 5 (45.5%) were found ammonia and hydrogen cyanide producers. Previous report [40] described that all rhizobacteria isolated from chickpea and green gram, produced ammonia which coincided with the findings of this study. Similarly, 50% of Chick pea rhizobacteria produced ammonia implying that the study isolates had biocontrol properties which is important in inhibiting the pathogenic fungal growth through affecting their respiratory system [41], inhibiting the electron transport system and hence disruption of energy supply to the cells [42].

The isolates solubilized phosphate at PSI ranging 2.3-3.6 cm. [43, 44] similarly reported that lentil and chick pea rhizosphere rhizobacteria produced solubilisation indices of 1.34–2.25 cm and 1.44–3.06 cm, respectively which is less than the findings of

this study indicating that the study isolates are promising isolates. Among the isolates, 72.7% of them showed a positive result for indole acetic acid production. Similarly, [44] also found rhizobacterial isolates that produced indole acetic acid. Regarding the nitrogen fixation under in vitro conditions, [20] reported Grass pea rhizobial isolates that fixed nitrogen which is nearly close to the findings of this study.

The isolates of this study showed growth at both lower pH (4) and higher pH (11). Similarly, [45] reported isolates that grew at nearly same pH ranges 10-12 pH. The investigation of this isolates can be used as microbial candidates for application at both alkaline and acidic conditions. All the isolates grew at salt concentrations of 1-9% with high growth at lower concentrations and low growth at higher concentrations. Other findings [46] reported similar findings that are in line with the current finding. Regarding temperature tolerance of the isolates, the isolates of this study showed growth resistance at all tested temperature ranges (4- 50 ℃) which is similarly reported by [46, 47].

The isolates showed resistance and sensitivity to the tested antibiotics with a better resistance to stronger antibiotics such as tetracycline. Such same variation in resistance and sensitivity to antibiotics were reported by [47]. Some isolates showed resistance to the highly toxic metals such as chromium and mercury. Likewise, [20] reported nearly similar variation in heavy resistance and tolerance of the isolates.

CONCLUSION

Garlic is an all-purpose bulb vegetable crop. It is used as food, medicine, and as an economically important vegetable. However, its productivity and yield are declining due to a number of biotic and abiotic factors. Among the biotic factors affecting garlic is white rot. The application of chemical pesticides to control white rot is a common strategy. Nevertheless, applying chemical pesticides has harmful effects on humans, animals, and the environment and is economically unaffordable for farmers. The application of biopesticides is a better alternative to chemical pesticides. Consequently, rhizobacteria were isolated, tested, and screened for having biocontrol potential to inhibit and control the growth of white rot.

In the current study, the isolates inhibited the growth of white rot under a dual culture test. Moreover, these rhizobacterial isolates were endowed with the potential to produce cell wall-degrading lytic enzymes and antifungal bioactive metabolites. Most of the isolates also showed better plant growth and stress tolerance properties that can enhance their use as biocontrolling agents under stressful soil conditions. Among the isolates, isolate WUGRB-91 was positive for all lytic enzymes and bioactive metabolite production. Moreover, it showed better white rot growth inhibition next to Isolate WUGRB-9. Thus, isolate WUGRB-91 can be recommended as a good candidate microbial biocontrol agent under greenhouse and field conditions.

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