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Isolation and Characterization of Rhizobacteria with Biocontrol activities against Faba bean (*Vicia faba* L.) Chocolate spot disease causing *Botrytis fabae*

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ABSTRACT

This study has aimed at isolating and characterizing rhizobacteria having biocontrol traits against chocolate spot (*Botrytis fabae* L.) that attacks faba bean. Accordingly, a total of 52 rhizobacterial isolates were recovered from rhizosphere of faba bean and screened for biocontrol activities against the test pathogen using dual culture method. Of these, only 50% of them showed inhibition against the pathogen. All the 26 isolates with antagonistic activities against *Botrytis fabae* were characterized using standard microbiological methods. The isolates inhibited the radial growth of the pathogen at an inhibition percentage ranging to 52.5-85.8%. Among these isolates, 17(65.38%) of them were Gram negative. Regarding the isolates' plant growth promoting properties, 26(100%), 23(88.5) and 21(80.8%) were positive for nitrogen fixation, phosphate solubilisation and indole acetic acid production, respectively. The minimum and maximum phosphate solubilisation indexes of the isolates were 2.3 and 4.1 cm, respectively. The hydrolytic enzyme production test showed that 25(96.2%), 15(57.69%) and 14(53.84%) of isolates produced protease, chitinase and cellulase, respectively. Moreover, all (100%) and 6(23.07%) of the rhizobacterial isolates produced ammonia and hydrogen cyanide, respectively. Several isolates showed better growth at lower and higher temperature ranges and salt concentrations. Similarly, the majority of these isolates showed better resistance to the tested antibiotics and heavy metals with a decreased growth response to tetracycline and mercury. Elite isolates (WUFBI 6A and WUFBI 9D) which showed the highest performance in all the tests can be recommended as inoculants for application under greenhouse conditions and field test.

Keywords: Biocontrol, Chocolate spot, Faba bean, Inhibition, Rhizobacteria.

INTRODUCTION

Faba bean (*Vicia faba* L.) is the fourth most important food legumes next to pea, chickpea and lentil in the world (Torres et al., 2006). Faba bean is highly nutritious crop with high protein content (up to 35% in dry seeds), and is a good source of many nutrients, such as K, Ca, Mg, Fe, and Zn (Lizarazo et al., 2015). In addition, its seeds contain several other bioactive compounds including polyphenols (Turco et al., 2016), carotenoids (Neme et al., 2015), and 51-68% of carbohydrates (Landry et al., 2016). Besides food and feed, it is an excellent nitrogen fixer due to the crops ability to form endosymbiotic association with root nodulating bacteria (rhizobia) group called *Rhizobium leguminosarum* bv. Vicia (Sahile et al., 2008).

The production of Faba bean in Ethiopia was reported as 10,067,518.28 quintals (Central Statistical Agency, 2020) indicating insignificant production rate. However, the total population of Ethiopia is projected from 86 million to over 115 million implying the unmatched Faba bean production and population growth. The production of Faba bean needs to be boosted so as to satisfy the food sustainability of the growing populations of the country. There are abiotic and biotic factors affecting faba bean production. Abiotic stresses like heat, drought, frost, and salinity induce yield losses. Drought can cause a decrease in output up to 79 percent during the reproductive period of the faba bean (Ammar et al., 2014). The biotic factors, on the other hand, included the fungal diseases ascochyta blight (caused by *Ascochyta fabae*), chocolate spot (caused by *Botrytis fabae*), rust (caused by *Uromyces viciae*-fabae and *Sclerotinia sclerotiorum* (Lib.), root rot (caused by Pythium spp.), stemphylium blight (caused by *Stemphylium botryosum* Wallr.), the parasitic plant broomrape (*Orobanche crenata* Forsk.), virus and aphid species are the most important biotic stresses worldwide that decrease Faba bean yield (O'Sullivan & Angra, 2016).

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Checholate spot is the major fungal pathogen that contributes to Faba bean production decline. Different management options have been developed to reduce the yield losses of Faba bean caused by chocolate spots worldwide of which the application of chemical fungicides, procymidone and mancozeb reduced Checolate spot severity (Sahile et al., 2008). However, their application brought the development of resistance in *Botrytis cinerea* and in *Botrytis fabae* against fungicides has been reported (Sahile et al., 2008). Nonetheless, biological control is the key strategy to inhibit the fungal pathogen which is harmful to Faba bean, especially rhizobacteria are the most important biocontrol agent for the suppression of fungal disease of Faba bean. Plant growth-promoting rhizobacteria have been shown to colonize the rhizosphere quickly, reduce soil-borne diseases at the root surface, and are advantageous to the plant by increasing growth (Moeinzadeh et al., 2010).

Plant growth promoting rhizobacteria (PGPR) colonize plant roots and enhance plant growth by a wide variety of mechanisms (Ashrafuzzaman et al., 2009). The main mechanisms by which plant growth promoting rhizobacteria directly contribute to plant growth include phytohormone production, such as auxins, cytokinins and gibberellins, enhancing plant nutrition by solubilization of minerals such as phosphorus and iron, production of siderophores and enzymes, and lowering ethylene levels (Bhattacharyya & Jha 2012). The indirect benefits of PGPR include bacterial antagonism against phytopathogenic fungi via the production of lytic (fungal cell wall degrading) enzymes such as chitinase, cellulase, glucanase and protease, antibiotic production (Whipps, 2001), hydrogen cyanide (HCN) production (Voisard et al., 1989), competition for nutrients, niche exclusion, induced systemic resistance, and antifungal metabolite production (Lugtenberg & Kamilova, 2009). There is a growing interest in using bacterial biocontrol agents to manage soil-borne diseases, partially in reaction to public concerns about the harmful effects of synthetic fungicides and partly due to the lack of effective control for soil-borne pathogens (Schmiedeknecht et al., 2001). Chemical fungicides are not always efficient in controlling phytopathogens (Huang et al., 2012). They are not only pollutants to the environment but also have negative health consequences. Biological control of soil borne illnesses and plant growth enhancement through the introduction of certain microbes to seed or planting materials have been investigated in this direction in recent years (Swelim et al., 2003).

The application of rhizobacteria is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. Promoting agricultural efficiency through increasing plant growth by applying eco-friendly alternatives is essential and timely for sustainable agricultural production. Thus, the application of potentially selected microbial inoculants is one of the preferred alternatives. Limited research was conducted on Faba bean by previous researcher (Keneni et. al., 2010) that involved isolation and characterization of acid tolerant Faba bean rhizobia. However, the isolation and characterization of Faba bean rhizobacteria and their plant growth and biocontrol effect on Checolate spot is not yet conducted in the study area. Thus, the main aim of this study was to isolate rhizobacteria from Faba bean rhizosphere soil and characterize them for screening potential isolates that inhibit the growth of the fungal pathogen, *Botrytis fabae* causing checolate spot on Faba bean under laboratory conditions.

MATERIALS AND METHODS

Description of the Study Area:

The sampling sites of this study were Kutaber and Dessie Zuria which are two districts of South Wollo, Ethiopia selected based on their high Faba bean production potential and prevalence of Faba bean spot disease history and no inoculation of its rhizobacteria bacteria. Rhizosphere soil samples were collected from from 52 sampling sites from October to December, 2020 during their flowering stage and brought to Biotechnology laboratory at Wollo University and kept at 4 ^{0}C refrigerator for further work

Collection, isolation and purification of Pathogenic and biocontrol Agents:

Faba bean leaves infected with Chocolate Spot were collected using microbiological procedures. After a thorough rinse with sterilized distilled water, the infected plant part was sterilized by dipping it in 1 % sodium hypochlorite (NaOCl) for 1-2 minutes. The contaminated plant component was surface-sterilized before being placed on PDA media and cultured at 28±2 ^{0}C for 5- 7 days (Somasegaran & Hoben, 1994). Based on their morphology and identification key guidelines, the pathogen was recognized as *Botrytis fabae* after culturing (Barnett & Hunter, 1987).

Rhizosphere soil samples were collected from Faba bean growing fields of some district areas of South Wollo during 2020. Serial dilution and spread plating methods were used for the isolation of rhizobacteria. Ten grams of rhizosphere soil was taken in 250 ml Erlenmeyer flask containing 90 ml sterile distilled water and mixed by shaking for 30 min (Vincent, 1970). Ten folds of serial dilutions were conducted up to 10^{-1} to $10^{-6}$of which 10^{-3} up to 10^{-6} dilutions was taken for streaking on nutrient agar. Aliquots of 0.1 ml were spread on plates

containing nutrient agar media and incubated at 28± 2^0C for 2- 3 days. After 3 days, the bacterial colonies were selected based on morphological features.

Well-separated colony was selected and re-cultured on nutrient agar repeatedly for obtaining pure culture. The purified colonies were stored at 4°C for immediate work and at -20°C for further work. All isolates were designated as WUFPGPR (Wollo University Faba Bean Plant Growth Promoting Rhizobacteria).

Morphological Characterization of the Antagonists:

The colony morphology and the gram reaction of the isolates were determined using standard protocol. The morphology of the bacterial isolates was determined by growing them on nutrient agar and incubating at 28± 2 ^{0}C for 2-3 days as described by Somasegaren and Hoben (1994), whereas the gram reaction of the rhizobacterial isolates was determined using the KOH technique (Buck 1982). 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 lifted up about 1 cm from the slide using an inoculating loop, and the presence and absence of visible stringiness (viscosity) were recorded as Gram-negative and Gram-positive bacteria, respectively.

Dual Culture Antifungal Inhibition Test:

All rhizobacteria isolates were assessed for potential antagonistic activity against *Botrytis fabae* on PDA media amended with sucrose (0.5 %) using the dual culture technique (Rangeshwaran & Prasad, 2000). A loop full of rhizobial isolates were spot inoculated near the edge of the plates and incubated at 28± 2 ^{0}C for 48 hrs. After two days, a 4 mm fungal disc was cut and placed at the center of the plates. The plates inoculated with phytopathogen but not bacteria were included as a control. The plates were incubated at 28± 2 ^{0}C for 5-7 days. To calculate the percent radial growth inhibition (PIRG), the following equation (Riungu et al., 2008) was used.

PIRG = ((C-T)/C)*100,

Where C is radial growth of fungus in control plates (mm) and T is radial growth of fungus on the plate inoculated with antagonist (mm).

Hydrolytic enzyme production:

Hydrolytic enzyme production potential of the isolates was determined according to standard protocols. The ability of the isolates to produce chitinase was determined as described by Nisa et al. (2010) using the composition media: 0.4% pure chitin, 0.07% K_2HPO_4, 0.03% KH_2PO_4, 0.03% NaCl, 0.001% $FeSO_4.7H_2O$, 0.05% $MgSO_4.7H_2O$, 0.0001% $MnCl_2.4H_2O$, 0.02% yeast extract, and 2% agar and incubated 28± 2 ^{0}C for 2- 3 days. The presence of clear zone around the bacterial colonies indicated the production of chitinase enzyme. Cellulase production potential of the isolates was determined by growing them on carboxy-methylcellulose agar medium containing (g/l): 2g of $NaNO_3$, 1g of K_2HPO_4, 0.5g of $MgSO_4$, 0.5g of KCl, 2g of CMC sodium salt, 0.2g of peptone and 15g agar. The plates were incubated 28± 2 ^{0}C for 2- 3 days and the formation of a halo zone around the colony indicated cellulase activity according to Samanta et al. (1989). Protease production activity of isolates was also assessed on skimmed milk agar (skim milk powder 10 g/l, agar 15 g/l) (Smibert & Krieg, 1994). The plates were incubated at 28± 2 ^{0}C for 2- 3 days and clear zones formed around the colonies indicated the production of protease enzyme by the bacterial isolates.

Ammonia and cyanide production:

The bacterial isolates were evaluated for the production of ammonia by using peptone water. Freshly prepared cultures were inoculated into each tube with 10 mL peptone water and incubated at 28± 2 ^{0}C for 2- 3 days. After incubation, each tube received 0.5 ml of Nessler's reagent, and the color change from blue to pale yellow signified a positive ammonia production test (Cappuccino & Sherman, 1992). According to Lorck (1948), all isolates were evaluated for HCN production by streaking them on a slant NA media inserted with filter paper strips dipped in picric acid and 2% sodium carbonate. After being sealed with parafilm, the test tubes were incubated 28± 2 ^{0}C for 2- 3 days in which a color change of the yellow filter paper strips to brown indicated hydrogen cyanide production.

Phosphate Solubilization Test:

Phosphate solubilization test was done on Pikovskaya's (PVK) agar medium, containing the following (g/L): 0.5g of $(NH_4)_2SO_4$, 0.1g of $MgSO_4 \cdot 7H_2O$, 0.02g of NaCl, 0.02g of KCl, 0.003g of $FeSO_4 \cdot 7H_2O$, 0.003g of $MnSO_4 \cdot H_2O$, 5g of Ca $(PO_4)_2$, 10g of glucose, 0.5g of yeast extract, 15g of agar and 1000 L of distilled sterilized water and incubated at 28±2 ^{0}C for 5 days. After incubation, the phosphate solubilisation index (PSI) of the isolates was calculated by (Nautiyal, 1999):

PSI = (HZD+CD)/CD,

Where PSI is the Phosphate Solubilizing Index, HZD is Halo Zone Diameter and CD is Colony Diameter.

Indole Acetic Acid (IAA) test:

The isolates' potential to produce indole-3-acetic acid was evaluated using the method described by Loper and Schroth (1986). Bacterial cultures were grown in nutrient broth supplemented with 1-tryptophan (1.02g/l) and incubated at 28±2^{0}C for 3 days. The cultures were then centrifuged at 5000 rpm for 5 min and 1ml of the supernatant was added to 2ml of Salkowski reagent (60% of perchloric acid, 3ml 0.5 M $FeCl_3$ solution). The development of a pink coloration indicated the production of IAA.

Nitrogen Fixation test using N-free Media:

The nitrogen fixation was estimated qualitatively using nitrogen-free medium based on the following composition: 15g agar, 1g $CaCO_3$, 1g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.2g NaCl, 0.1g $Fe_2SO_4.7H_2O$, 5g $Na_2MoO_4.2H_2O$, and 50ml distilled water adjusted at a final pH of 7. The emergence of a pellicle at the subsurface level after 5 days of incubation at 28 ±2 ^{0}C was considered a positive test for N fixing (Rodrigues et al., 2016).

Resistance to Heavy Metal and antibiotic test:

According to Mohamed et al. (2012), resistance to heavy metal (HM) was determined by growing cultures on solid nutrient agar media containing filter-sterilized HM at concentrations (μgml^{-1}) of Hg (HgO) (50), As (As_2O_3) (100), Ni ($NiCl_2$) (100), Pb ($Pb(CH_3COO)_2$) (100), $Cdcl_2$(50) and $Crcl_2$(10) and monitoring colony growth at 28± 2^{0}C for 3-5 days. As described by Amarger et al. (1997), resistance to low concentrations of antibiotics was determined by preparing fresh solutions of filter-sterilized (0.22mm) antibiotics in nutrient agar medium containing one of the following filter-sterilized antibiotics (μg ml^{-1}): ampicillin (30), chloramphenicol (40), erythromycin (30), nalidixic acid (20), neomycin (20), streptomycin (10) and tetracycline (30).

Physiological Tolerance Test of Antagonists:

The ability of rhizobacterial isolates to grow at different concentration of salt was tested by the method as described by Suresh et al. (2014). It was done by streaking the rhizobial isolates on nutrient agar medium containing 1, 2, 3, 4, 5, 6, 7, 8, and 9% of NaCl. Isolates were tested for their temperature tolerance ability by streaking the isolates on a nutrient agar plate adjusted at different temperature levels (4, 10, 15, 40, and 50°C). All the plates were kept at 28±2^{0}C for 3-5 days.

Statistical Analysis:

All experiments were set in triplicate and the data is average of three. Phosphate solubilizing and dual culture inhibition data were analysed by One way ANOVA and the treatment means were compared following Duncan's test by using SPSS version 20 and computed at $p<0.05$ significant value.

RESULTS

Isolation, purification and identification of the pathogen

After collection of the samples from the research site, isolation and purification of the rhizobacterial isolates were done. Among a total of 52(100%) isolates of Faba bean rhizobacteria collected from Kutaber and Tita districts of South Wollo Zone, 26 (50%) of them showed inhibition against the fungal pathogen, *Botrytis fabae* causing chocolate spot (Table 1). During isolation and identification of the pathogen, the *Botrytis fabae* was identified as fungal pathogen causing Chocolate Spot of Faba bean based on color and morphology as observed on PDA media. Moreover, the identification of the pathogen was proved true by using the identification keys.

Cultural characterization of the rhizobacterial isolates

The variation of the rhizobacterial isolates in various morphological parameters was presented (Table 2). The colony morphological properties of the isolates showed, 5 (19.2%) and 21 (71.8%) of the isolates were irregular and round in shape, whereas 8(30.8%) and 18(68.2%) were flat and entire, respectively. All the rhizobacterial isolates were entire in margin. Of the isolates, 34.6%, 26.9%, 30.8% and 0.07% were white, yellow, creamy and orange in their order of pigmentation. As the Gram reaction test showed, only 11 (42.3%) of the isolates were Gram positive.

Physiological characterization of the isolates

The potential of the bacterial isolates for temperature tolerance was presented (Fig. 1). All the isolates grew between the temperature ranges 25- 35 °C. Eight (30.8 %) of the isolates grew at 5 °C. Whereas, 12(46.2%),18(69.2%) and 21 (80.8%) of the isolates grew at temperature of 10, 15 and 20°C. Twenty (76.9%) and 15 (56.7%) and of isolates grew at 40- 50 °C. Of the tested rhizobacterial isolates all grew at salt concentrations of 1-5%. Likewise, 21 (80.76%), 19(73.07%), 18(69.23%) and 16(61.53%) showed growth at salt concentrations of 6, 7, 8 and 9% respectively (Fig. 2). In both temperature and salt concentration tests, the growth of the isolates showed declining trend at extreme temperature ranges and salinity.

The antibiotic and heavy metal resistance of the isolates were tested. The ability of the isolates to resist different heavy metals was presented (Fig.3). Among the tested isolates, 22(84.61%), 13(50%),

Table 1: The growth response of the isolates to some plant growth and biocontrol traits

Isolates	Isolation site	Ammonia production	Hydrogen cyanide	PSI (cm)	Nitrogen fixation	PIGR (%)
WUFBI 8C	Kutaber	+	_	3.4 ± 0.4^{abc}	+	67.5 ± 2.5^{def}
WUFBI 17	Kutaber	+	_	2.3 ± 0.2^{c}	+	54.1 ± 3.8^{gh}
WUFBI 8E	Kutaber	+	_	2.9 ± 0.7^{abcd}	+	76.6 ± 1.4^{bc}
WUFBI 5C	Kutaber	+	_	2.9 ± 0.6^{abcd}	+	68.3 ± 6.2^{cdef}
WUFBI 6C	Kutaber	+	_	2.8 ± 0.3^{bcd}	+	57.5 ± 2.5^{gh}
WUFBI 6B	Kutaber	+	_	2.5 ± 0.5^{bcd}	+	52.5 ± 2.5^{h}
WUFBI 9D	Kutaber	+	+	2.7 ± 0.4^{bcd}	+	70.8 ± 3.8^{bcde}
WUFBI 6G	Kutaber	+	+	-	+	70.8 ± 5.2^{bcde}
WUFBI 5B	Kutaber	+	_	3.4 ± 0.3^{abc}	+	75.0 ± 5.0^{bcde}
WUFBI 15B	Kutaber	+	_	3.2 ± 0.6^{abc}	+	75.0 ± 5.0^{bcde}
WUFBI 7J	Kutaber	+	+	3.1 ± 0.8^{abcd}	+	75.8 ± 1.4^{bcd}
WUFBI 8D	Tita	+	_	2.8 ± 0.6^{bcds}	+	$70.8\pm3.8b^{cde}$
WUFBI 7M	Tita	+	_	-	+	$75.0\pm2.5b^{cde}$
WUFBI 6K	Tita	+	_	3.8 ± 0.8^{ab}	+	77.5 ± 2.5^{b}
WUFBI 6D	Tita	+	_	2.9 ± 0.9^{abcd}	+	$76.6\pm1.4b^{c}$
WUFBI 4D	Tita	+	_	2.8 ± 0.6^{bcd}	+	67.5 ± 5.0^{def}
WUFBI 4B	Tita	+	_	3.0 ± 0.9^{abcd}	+	66.6 ± 3.8^{ef}
WUFBI 6A	Tita	+	_	2.0 ± 0.2^{d}	+	$67.5\pm6.6d^{ef}$
WUFBI 4C	Tita	+	_	3.1 ± 1.3^{abcd}	+	74.1 ± 3.8^{bcde}
WUFBI 10A	Tita	+	+	-	+	60.8 ± 9.5^{fg}
WUFBI 9B	Tita	+	_	2.9 ± 0.3^{abcd}	+	$75.0\pm5.0b^{cde}$
WUFBI 1B	Tita	+	_	3.5 ± 0.2^{abc}	+	68.3 ± 6.2^{cdef}
WUFBI 7F	Tita	+	+	4.1 ± 0.5^{a}	+	69.1 ± 3.8^{bcde}
WUFBI 6I	Tita	+	_	2.9 ± 0.4^{abcd}	+	77.5 ± 2.5^{b}
WUFBI 16B	Tita	+	+	3.5 ± 0.8^{abc}	+	85.8 ± 1.4^{a}
WUFBI 4E	Tita	+	_	3.5 ± 0.8^{abc}	+	85.8 ± 2.8^{a}
Total		100%		3.0±0.7	100%	70.8±8.8

WUFBI = Wollo University Faba bean Bacterial Isolates, PSI = Phosphate Solubilizing Index, PIGR = Percent Inhibition Growth Radial, Data represented by different letters in superscript are significantly different

Table 2: The morphological characterization of selected rhizobacterial isolates

Isolates	Isolation site	Shape	Color	Elevation	Margin	Gram reaction
WUFBI 8C	Kutaber	Round	White	Elevated	Entire	-
WUFBI 17	Kutaber	Round	Creamy	Elevated	Entire	-
WUFBI 8E	Kutaber	Round	Creamy	Elevated	Entire	+
WUFBI 5C	Kutaber	Round	Yellow	Elevated	Entire	+
WUFBI 6C	Kutaber	Round	Yellow	Elevated	Entire	-
WUFBI 6B	Kutaber	Round	White	Elevated	Entire	-
WUFBI 9D	Kutaber	Round	White	Flat	Entire	+
WUFBI 6G	Kutaber	Round	Yellow	Flat	Entire	-
WUFBI 10A	Kutaber	Irregular	yellow	Elevated	Entire	-
WUFBI 5B	Kutaber	Round	Creamy	Elevated	Entire	-
WUFBI15B	Kutaber	Round	Creamy	Elevated	Entire	+
WUFBI 7J	Tita	Round	Orange	Flat	Entire	+
WUFBI 8D	Tita	Round	Orange	Flat	Entire	+
WUFBI 7M	Tita	Irregular	Yellow	Flat	Entire	-
WUFBI 6K	Tita	Round	yellow	Flat	Entire	-
WUFBI 6D	Tita	Irregular	yellow	elevated	Entire	-
WUFBI 4D	Tita	Irregular	Creamy	Elevated	Entire	+
WUFBI 4B	Tita	Round	Creamy	Elevated	Entire	+
WUFBI4C	Tita	Round	Creamy	Elevated	Entire	-
WUFBI 9B	Tita	Round	Creamy	Elevated	Entire	-
WUFBI 1B	Tita	Round	White	Elevated	Entire	-
WUFBI 16B	Tita	Round	White	Elevated	Entire	+
WUFBI 6I	Tita	Irregular	White	Elevated	Entire	+
WUFBI 4E	Tita	Round	White	Elevated	Entire	-
WUFBI 6A	Tita	Round	White	Flat	Entire	+
WUFBI 7F	Tita	Round	White	Flat	Entire	-

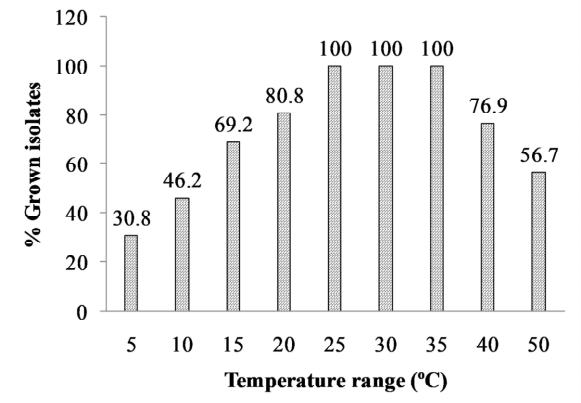

Fig. 1: Temperature tolerance of isolates

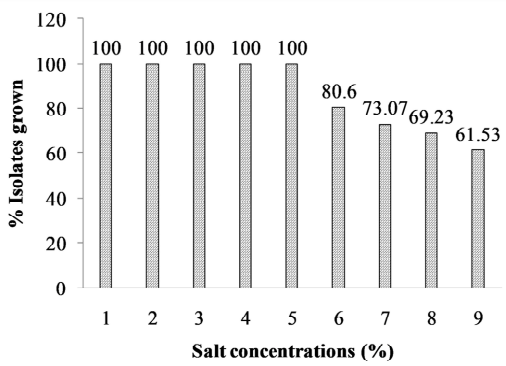

Fig. 2: Salt tolerances of isolates

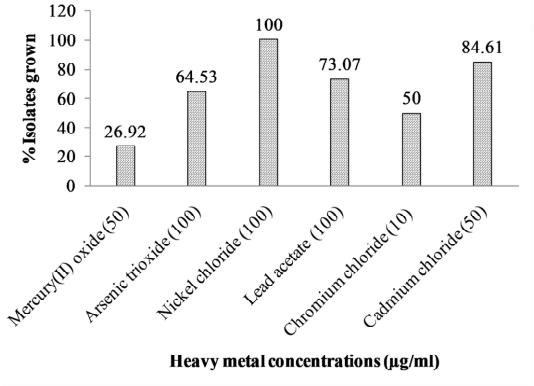

Fig. 3: Heavy metal resistance of isolates

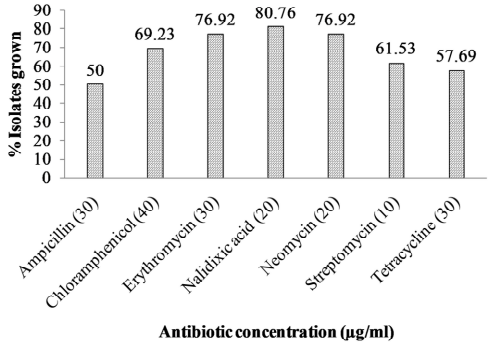

Fig. 4: Antibiotic resistance of

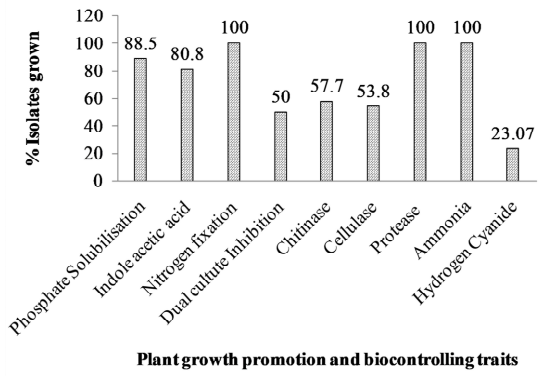

Fig. 5: Tests for plant growth and biocontrol traits

16(64.53%), 19(73.07%), 26(100%) and 22(84.61%), 13(50%), 16(64.53%), 19(73.07%), 26(100%) and 7(26.92%) resisted Cadmium (Cd), mercury (Hg), arsenic (As), lead (Pb), nickel (Ni) and chromium (Cr), respectively. The rest isolates were sensitive to the tested heavy metals. All the isolates showed variations in the six tested antibiotics (Fig. 4). Of the isolates, 21(80.76%), 20(76.92%), 1(69.23%), 20(76.92%), 13(50%), 15(57.69%) and 16(61.53%) showed resistance to nalidixic acid, novobiocin, chloramphenicol, erythromycin ampicillin, tetracycline and streptomycin, respectively. Whereas, the remaining isolates were sensitive to the mentioned antibiotics.

The capacity of the isolates to exhibit plant growth promoting traits such as phosphate solubilisation, indole acetic acid production and nitrogen fixation was determined (Table 2). The phosphate solubilisation ability of the isolates was tested. Among the tested isolates, 23(88.5%) solubilized phosphate (Fig. 5), from 2.0 – 4.1 PSI ranges (Table 2). The minimum (2.0 cm) and maximum (4.1cm) solubilisation was recorded by isolates WUFBI6A and WUFBI 7F, respectively. The isolates' potential to produce IAA was also determined (Table 2). Among the isolates, 21(80.8 %) of them were positive for the production of IAA and the rest were not capable. In addition, the nitrogen fixation test was tested for the selected isolates. All isolates, 26(100%) were positive for nitrogen fixation which was confirmed by formation of conspicuous pellicles that were an indication of better growth and biological nitrogen fixation efficiency.

Among 52(100%) isolates, only 26(50%) of them inhibited the radial mycelial growth of the pathogen, *Botrytis fabae* and has been selected for further characterization. The fungal radial growth inhibition percentage ranged from 52.5% - 85.8%. Of these isolates, 12(46.2%) of them showed better

pathogen inhibition potential above 75%. All isolates tested for their ability to produce different hydrolytic enzymes were presented (Fig. 5). All the 26(100%) isolates were positive for protease, whereas 14(53.8%) and 15(57.7%) of the isolates were positive for cellulase and chitinase production, respectively. However, 8(30.8%) of isolates including WUFBI 17, WUFBI 9D, WUFBI 6G, WUFBI 7J, WUFBI 8D, WUFBI 6K, WUFBI 6A, and WUFBI 9B were positive for the tested hydrolytic enzymes. All the isolates were positive for ammonia and only six (23.07%) of the total showed cyanide production.

DISCUSSION

This study which aimed at screening potential rhizobacteria that can enhance plant growth and have biocontrol role against *Botrytis fabae* causing Checolate spot of Faba bean were conducted. Consequently, rhizosphere soil from the healthy standing Faba bean plant and infected plant were collected and isolated. Out of these isolates, 26 (50%) showed antagonistic inhibition properties against the pathogen, *Botrytis fabae* on dual culture test with an inhibition percentage ranging between 52.5%- 85.8%. Similarly, Tamiru and Muleta (2018) reported 0.0-70.5%, implying the better performance of the study isolates.

The isolates showed variation in their phosphate solubilisation potential in which they solubilized phosphate at 2.0 – 4.1cm PSI ranges with isolate WUFBI 7F displaying the highest PSI. Previous study by Tamiru and Muleta (2018) reported PSI of Faba bean and groundnut rhizobacteria at a range of 1.0 – 1.9 and 1.06 – 1.86, respectively indicating the better effectiveness of the study isolates. Some rhizobacterial isolates of this study produced indole acetic acid which is also reported by Adal et al. (2018) from Grass pea rhizobacterial isolates which is important in improving overall plant growth by developing development of roots that are useful for absorption of nutrients (Aeron et al., 2011). All the isolates of this study fixed nitrogen, however, Abu et al. (2019) reported only 10% of Faba bean rhizobacteria were capable of fixing nitrogen on N- free media indicating the better nitrogen fixing effectiveness of the study isolates that could be important to the biogeochemical cycling in the terrestrial ecosystem (Bhattacharyya & Jha, 2012).

Most of the isolates of the current study (57.69%) produced chitinase which is consistent with the findings of Adal et al. (2018) who reported 60% of chitinase producing rhizobacteria which is an important strategy to inhibit fungal spore germination, germ tube elongation and lysis of hyphal tips (Ordentlich et al., 1988). Similarly, all and 14 (53.8%) of the isolates produced protease and cellulase, respectively showing the better performance of the isolates of this study as compared to the findings of Kavitha et al. (2013) who reported 50% cellulase and protease producing rhizobacteria. All the isolates were positive for ammonia production, whereas only 6 (23.07%) of them showed cyanide production. Similarly, Prashar et al. (2013) reported that 98% and 94% of *Bacillus* isolates and *Pseudomonas* produced ammonia and cyanide, respectively. The production of ammonia and cyanide can assist plants in meeting their nitrogen requirements and reduce pathogen invasion (Mbai et al., 2013).

All the tested isolates showed varied colony morphological characteristics which were similarly reported by Wubie and Adal, (2021) indicating variation of rhizobacterial isolates in their morphological characteristics. The isolates showed tolerance to both lower temperature ranges (4-15^{0}C) and higher temperature (40- 50 ^{0}C) which nearly coincided with the findings of Tsegaye et al. (2019) who reported 20% and 40.7% rhizobacterial isolates that showed growth at 4 ^{0}C and 40 ^{0}C.The finding of this study is important to prevent the impact of abiotic stresses on plant growth by inhibiting protein synthesis and photosynthesis via inactivating enzymes (Zhu, 2001). Regarding salinity, isolates that grew at both lower and higher salt concentrations were recorded which was similarly reported by Shoukry et al. (2013) indicating that the isolates can be applied at soils of higher salinity since they are helpful for alleviating the harmful effects of osmotic stress. The study isolates showed resistance variation to different antibiotics at 57.7 – 80.7% of resistance which is a useful marker for their characterization (Zahran, 2012) and is important for identifying their competitiveness in soil (Brockwell et al., 1995). Some isolates of the current study resisted heavy metals known in their toxicity implying that isolates of this study can be applied at farming soils of industrial areas.

In conclusion, among the rhizobacteria characterized and screened for deserving biocotrol potential against *Botrytis fabae*, some rizobacterial isolates were obtained as having diverse bio control attributes. Thus, these rhizobacteial isolates are recommended to be applied under greenhouse conditions.

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COMPETING INTERESTS

The authors have declared that they have no competing interest.

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