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Biochemical Characterization of *Ralstonia solanacearum (L.)* isolates from Edible Ginger *(Zingiber officinale* Rosc.*).*

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Abstract

Bacterial wilt caused by Ralstonia solanacearum (L.) limits quality and yield of edible ginger (Zingiber officinale) production in Ethiopia. The present study was initiated to undertake biochemical characterization of some isolates of ginger bacterial wilt agent (Ralstonia solanacearum) in a greenhouse and laboratory experiments. Among 50 bacterial isolates isolated from ginger rhizome, only eight isolates were selected based on the morphological, physiological, cultural, biochemical and pathogenicity studies that were carried out in the laboratory. All the isolates were grown on TZC plates at 28 ±20C for 48hr the virulent (colonies with pink or light red color or characteristic red center and whitish margin) and avirulent (smaller, off-white and non-fluidal colonies) isolates of R. solanacearum were identified in TZC medium containing 0.005% TTC. All isolated 50 isolates from ginger rhizomes had relatively close colony appearance on TZC medium resembling to R. solanacearum. Eight isolates were showed typical symptoms of wilting, whereas the remaining 42 were nonpathogenic and could be rated as saprophytic although they had similar colony appearance with R. solanacearum. Based on the morphological studies it was found that all the isolates were gram-negative, motility, rod shaped, pink color, non-capsulated and non-spore forming. Cultural characteristics of isolates on Tetrazolium Chloride (TZC) Agar selective medium showed light to red color with the characteristic red center which resembles the pathogen Ralstonia solanascearum. Biochemical and physiological characteristic revealed that similarities among isolates and strains were grouped in biovar III and corresponds to race 4 of the pathogen. Hence, further study is necessary to check the ability of the antagonists to control the disease under field conditions.

Keywords: *Bacterial wilt, Bio-chemical characterization, Ginger, Identification, Ralstonia solanacearum*

Introduction

Ginger (*Zingiber officinale* Rosc.), belonging to the family Zingiberaceae, is an important commercial crop grown for its aromatic rhizomes which are used as a spice and a medicine (Sharma et al., 2010). Ginger rhizome is typically consumed as a fresh paste, dried powder, slices preserved in syrup, candy (crystallized ginger), as a beverage or as flavoring agent. It is cultivated in almost all the tropical and subtropical parts of India (Tarafdar and Saha, 2007). India is the largest producer of ginger in the world accounting for about one-third of the total world output followed by Thailand and Japan. It is an important crop that earns a sizeable amount of foreign exchange for the country (Tarafdar and Saha, 2007). Ginger is known to have been introduced to Ethiopia as early as in the 13th century (Jansen, 1981). It is cultivated in South, Southwestern and Northwestern parts of Ethiopia as cash crop, and is among the important spices used in every Ethiopian kitchen for the preparation of pepper powder, stew, bread, etc. It has also some use in traditional medicine for the treatment of flu and stomach ache (Jansen, 1981; Girma Hailemichael and Digafie Tilahun, 2004).

In Ethiopia, the bacterial disease has been reported on Potato, Tomato, Pepper, Enset, Banana and ornamentals but ginger bacterial wilt is not yet reported and new to Ethiopia. However, ginger is now well known in Ethiopia as fresh or dry rhizomes for sale on most markets. It is mainly grown in the south and Southwestern part of the country with high land coverage and farmers percentage in the cultivation. In different parts of the world, like most cultivated crops, ginger is affected by biotic and abiotic factors. In most ginger producing countries like India, ginger is affected by different pathogens. Bacterial wilt (*Ralstonia solanacearum* soft rot/rhizome rot, Leaf spot (*Phyllostica zingiberi* Nematodes were commonly known to cause disease on ginger crop. Ginger bacterial wilt disease caused by R. *solanacearum* is known as most limiting factor in Hawaii. Ethiopia, in the history of spice research achievements, no

disease prevalence's have been recorded on recently, in 2011/12 and 2012/13 cropping season regional plant health clinics have reported that ginger crop is being infected by unusual disease. Based on such regional reports, survey was conducted during the 2011/12, 2012/13 and 2013/ cropping seasons in the Southern Nations, Nationalities and People and Gambela Regional States to investigate the status and causal agent of the recently occurred ginger disease (Habetewold kifelew *et al*., 2015).

Bacterial wilt of ginger was found widespread in all area surveyed. Among the different places surveyed, wilt incidence were found ranged from 93.5% sheka zone to 10.7 Gamogofa zone in 2012 survey season. The first bacterial wilt syndrome were reported from Benchmaji zone Bebeka coffee estate farm, then after it progress to the neighboring zone Sheka within a short period of time and cause up to 67% yield loss. During 2012 the diseases were less prevalent around Wolayta zone even if the area have been known for producing ginger as a major crop. At the same year even if the diseases is less prevalent at Wolayta area which is different in geography and agro-ecology to that of Benchmaji and Sheka zones, we collect and check the seed rhizomes from wolayta and surrounding for latent infection, from the laboratory result the seed rhizomes were found 100% latently infected. In subsequent year 2014 survey result, SNNPRS (Dawro, Wolayta, Kenbata tenbaro, Hadiya, Gomogofa, Konta, Alaba, Sheka, and Bench maji) and Gambella region (Majang) zone ginger were found devastated by the bacterial wilt and cause yield loss up to 98%. (Habetewold kifelew *et al*., 2015). In many tropical and subtropical regions the pathogen has been widely distributed and associated with a wide range of hosts (Agrios, 2005).

Biochemical characterizations of the *Ralstonia solanacearum* are one of the most important areas of identification. *R. solanacearum* is a rod-shaped bacterium with an average size varying from 0.5 to 0.7 by 1.5 to 2.5 µm and it is considered as an organism strictly aerobic (Denny & ayward, 2001). The principal biochemical characteristics are catalase positive, xidase positive, and nitrate reduction. The pathogen does not hydrolyze starch and does not readily degrade gelatin. In broth culture, the organism is inhibited by concentrations of sodium chloride (NaCl) greater than 2%. Liquid and solid (agar) growth media are commonly used for culture of the bacterium. On solid agar medium, individual colonies are usually visible after 36 to 48 hours of growth at 28oC and Kelman's tetrazolium chloride (TZC) agar is regularly used for its isolation (Kelman 1954). After two days on TZC medium, virulent wild-type colonies are large, elevated, fluidal, and either entirely white or with a pale red center. For most strains, the optimal growth temperature is 28-32oC; however some strains that are pathogenic on potato have a lower optimal growth temperature of 27^{o}C.

Since the crop is economically and medicinally important, studying these pathogens and controlling them will increase the yield and quality of ginger product, there by supporting the supply and quenching the demand. To know the best ginger varieties that can be able to grow and give higher yields in different agro ecological areas of the country, a test of infection by these pathogens against every available cultivar at the study sites is also important. Knowing the pathogens at biochemical level and provides a new way to seek for effective prevention and treatment strategies. In addition, a laboratory study of inter- specific interaction of the pathogenic species with other soil microbes will provide an excellent clue for the development of a biological control system against the pathogens. In view of the above facts the present study was under taken with the objective to undertake characterization of biochemical and morphological strains of *R. solanacearum* (*Smith)* of *R. solanacearum* isolates collected from major ginger growing areas in south western part of Ethiopia.

Materials and methods

Description of the Study area

The sampling site were Durame and Hadaro Tunto in(Kembata Tenbaro) and Areka in (Welayita) Zones of south west of Ethiopia. Durame is a town in southeastern Ethiopia the administrative center of the Kembata Tembaro Zone in the Southern Nations, Nationalities and Peoples Region (SNNPR).The town has a latitude and longitude of 7°14′N and 37°53′E with an elevation of 2101 meters above sea level. Durame has an estimated total population of 12,849 of whom 6,461 are men and 6,388 are women (Teferra and Leikun., 1999; EARI, 2009; Fitsum Markos, 2013; CSA, 2007).

Sample Collections

Samples were collected from three ginger growing areas of the Kembat Tenbaro and Welayita zone of SNNP region (Durame and Hadaro Tunto in Kembata Tenbaro and Areka in woliata zone) South western parts of Ethiopia. Total 50 samples of infected ginger rhizomes and Rhizosphere soil were collected from study site (14 from Durame,17 from Hadaro,19 from Areka), and brought to the laboratory for the isolation of *R. solanacearum.* Diseased organs of the plants were taken from the rhizome of all the present cultivars in the study sites. The isolates were collected from the farmer's field of all the agro climatic regions in three different altitudinal ranges (high, medium and low). Roots with adhering soils of healthy ginger plants were collected sub-sampled and transferred into sterile plastic bags. The method that was used for the collection of ginger pathogens from the plant materials was based on the examination and sampling of observed diseased sample. Standard sampling methods was used in the process developed by Aneja (2005). Samples were collected in 8 to 12 km intervals from farmer's field samples were kept in clean plastic bags (envelopes) and were brought to the mycology laboratory and then were stored at 4°C for further study. The in vitro study was carried out in the Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University (AAU) where as the in vivo study was conducted in the Green house College of Natural and Computational Sciences, at AAU.

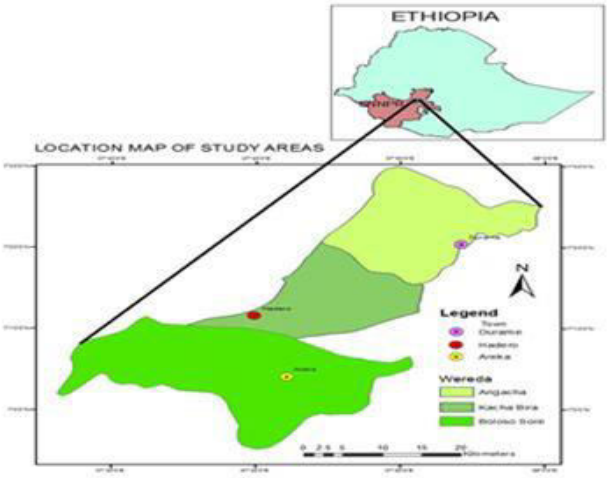

Fig 1. Map of Ethiopia with SNNP Region highlighted in purple color and Sampling site highlighted in different color of yellow, brown and Green in South western Ethiopia.

Fig 2. Diseased rhizome sample were collected form study site of Durame, Hadero and Areka.

Isolation of the Test Pathogens

The bacteria were isolated from the plant rhizomes within 2 days of collecting the samples, as follows. For isolating the bacteria from the plant rhizomes samples (3 g each) of rhizome were surface-disinfected with 1% sodium hypochlorite for 2 min washed three times with sterile water, and 0.1 mL from the last washing is plated onto Nutrient Agar (NA) to confirm that the pieces were completely sterile from outside: if no growth is seen after incubation at 28 0C for 48 hr, samples were assumed to be sterile. The rhizome samples were directly plated on Nutrient Agar medium (NA), incubated at 28 0C for 48 hr, and pure cultures obtained from single colonies were selected and purified. Pure culture of *R. solanacearum* were transferred to nutrient agar media slants and stored at 4 0C for further investigation (Aneja (2005) and Dingera and Sinclair (1993). Designation of bacterial wilt disease of ginger was carried out using eight isolates; Addis Ababa University R. *solanacearum* isolates AAURS-03, AAURS-04, AAURS-05, AAURS-07, AAURS-08, AAURS-018, AAURS-026 and AAURS-034.

Isolation and Identification of Ralstonia solanacearum

Diseased ginger plant parts (rhizomes) and soil samples were collected from the ginger growing areas of Ethiopia. Field diagnosis of diseased plant samples were done by critically observing the bacterial wilt symptoms. *Ralstonia solanacearum* was isolated on Nutrient Agar (NA) medium.The vascular portion of infected rhizome sections were cut into small pieces, washed and sterilized by the same procedure as described above and were directly placed on Nutrient Agar medium, incubated at 28°C for 48 hr . After isolation, *R. solanacearum* isolates were purified by streaking a single colony of each isolate on Tetrazolium Chloride (TZC) medium as described Kelman (1954). The pathogenicity test was performed on three month old healthy ginger seedlings by stem inoculation method. A single colony of *R. solanacearum* showing virulent, fluidal, irregular and creamy white with pink at the center was selected and adjusted to 3.2 x 10^8 cfu ml-1 for inoculation. Pure culture of *R. solanacearum* were maintained on nutrient agar medium slants and maintained at 4°C for further studies.

Identification of Virulent/Avirulent Isolates of *R. solanacearum*

The virulent and avirulent isolates of *R. solanacearum* were differentiated by Kelman (1954). Tetrazolium Chloride (TZC) agar medium containing 0.005% 2, 3, 5-tripenyl tetrazolium chloride (TTC). In this test, virulent isolates produce pink or light red color colonies or colonies with characteristic red centre and whitish margin and avirulent isolates produce smaller, off-white and non-fluidal or dry on TZC medium after 24 hours of incubation (Kelman ,1954, Champoiseau,2008, Rahman *et al*., 2010).

Biochemical Characterization

Catalase Test

The presence of catalase activity in the isolates was checked by flooding a drop of 3% H_2O_2 on pure colonies of 24 hr cultures over the slide, immediate effervescence of gas bubble was recorded as a positive result (Harisha, 2007).

Oxidation Fermentation Test

The oxidation and fermentation of a carbohydrate by isolates were determined on oxidation -fermentation medium using the method described by Aneja (2005). The medium contains: 2.0 g of peptone, 1.0 g of yeast extract, 5.0 g of NaCl, 0.2 g of K2HPO4, 0.08 g of bromothymole blue per 1 liter of distilled water and pH was adjusted to 7.1. The medium was freshly prepared and 5ml of broth medium was dispensed in each test tube. After sterilization, the medium was cooled to about 350C with tap water to avoid dissolution of oxygen in the medium to which 10% (w/v) of sterilized glucose was added. Two test tubes inoculated by stabbing with straight wire loop. One of the tubes were sealed the surface with a thin layer of paraffin oil to create anaerobic condition whereas the other tube was incubated aerobically. Acid production associated with color change from green to yellow in the aerobic tube was indicative of oxidation whereas the color change from green to yellow in the both tubes showed fermentation (Aneja, 2005).

Fluorescent Pigment Production

The ability of bacterial isolates to produce yellow- green fluorescent pigments or *Flourescein siderophore* was tested by culturing the bacterial isolates on KB medium. Twenty four hours old culture was inoculated into the medium and incubated at 28^0C for 48 hr. The fluorescent pigments were observed for the fluorescence under 366 nm UV light after 48hr incubation (Stieglitz and Weimer, 1985).

Indole Production Test

Indole production from isolates were determined by using 1% tryptone broth and incubated for 48 hr at 28^0C. One (1) ml of kovac‟s reagent (diethylamide benzaldehyde) was added to each test tube and agitated gently to extract the indole. The development of bright-pink color on the top layer indicated indole production (Aneja, 2005).

Starch Hydrolysis

The ability of isolate to metabolize was tested by using starch minimal medium containing 0.5% of solute starch, 0.3% of Mg SO4, 0.02% of $CaCl_2$, 1% of K_2HMPO_4 and 20 g of agar per liter of distilled water. Clear zone was detected around colonies after flooding with Lugol‟s iodine is indicative of starch hydrolysis (Grimont *et al.,* 1977).

Levan Production from Sucrose

Single colonies of bacteria were streaked on nutrient agar plates supplemented with 5% sucrose. The plates were then incubated at 28°C for 48 hr. Separate, discrete colonies were observed for the Levan production with naked eye (Schaad, 1980).

Lipase activity on Tween 80 agar

The constituents of the medium, 10.0g peptone, 5.0 g NaCl, 0.1g $CaCl_2 \cdot 2H2O$, 10.0gTween-80 (Sigma), 20.0g Agar per litre, were dissolved in distilled water and heated, at 100°C with periodic swirling to dissolve the agar. The pH was adjusted to 6.0. The molten medium was dispensed in 100 ml quantities in 200 ml screw-capped bottles. Using a wire loop bacterial inoculums were radially streaked across separate halves of Petri plates and incubated at 28°C for 7 days. The plates were examined daily for the presence of a dense precipitate around the bacterial growth (Aneja, 2005).

Grouping of R. solanacearum into biovars

The isolates of *R. solanacearum* were differentiated into biovars based on their ability to utilize disaccharides (lactose, maltose) and sugar alcohols (manitol, sorbitol) as described previously by Hayward (1964) and He et al. (1983). Ten ml of each 10% lactose, maltose, mannitol, and sorbitol was added in to mineral medium $NH_4H_2PO_4$ 1.0 g, KCl 0.2 g, $MgSO4 \cdot 7H2O$ 0.2 g, Difco bacto peptone 1.0 g, Agar 3.0 g and Bromothymol blue10-20 ml per liter) containing 1% sugar. The disaccharides were sterilized by filtration using 0.22 mm micron Millipore membrane and the hexose alcohol was 121^0C for 15 minutes. Bottles of semi-solid basal medium were melted in water bath and cooled to70°C. Media was then allowed to solidify at room temperature. Control was kept by adding 10ml sterilized distilled water instead of sugar solution into the basal medium. Tewenty (20) ml of the melted medium was dispensed into the wells of microtitre plate. Inoculum for each group of isolate was prepared by several loop full of bacteria from 24 hours old cultures to sterilized distilled water to make suspension containing about 10^8 CFU/ml. Then 100 µl of bacterial suspension was added to the wells of microtitre plate incubated at 28°C. The plates were then examined after 2,7 and 14 days of inoculation for the presence of indicator change from olivaceous green to orange color on the surface of medium (Schaad *et al.,* 2001).

Preparation of Inoculum

Selected *R. solanacearum* isolate was cultured in nutrient broth at 28 ± 2^0c on orbital shaker. Twenty four hours old culture was taken and transferred into 100ml of the medium and incubated on orbital shaker at 120 rpm for 48hrs. Then the cultures were diluted with sterilized distilled water to 10^8 cells/ml. Plants were inoculated at eighth leaf stage by stem puncture and leaf infection pin pricks.

The bacterial suspension was inoculated in to the two leaves of each test plant (Stromberg et al., 2004).

Data Analysis
The experimental data was analyzed by using one way analysis of variance and comparison of means, at 0.05 level was made by LSD t- test. Mean and standard deviation and standard error of the mean were analyzed by using SPSS (version 20.0). ANOVA was performed for means comparison at ($p<0.05$).

Results
Characterization and Identification of Aggressive Isolates of R. solanacearum from Ginger
Among 50 bacterial isolates isolated from ginger rhizome, only eight isolates were selected based on the morphological, physiological, cultural, biochemical and pathogenicity studies that were carried out in the laboratory All the isolates were grown on TZC plates at 28 $\pm 2^0$C for 48hr The virulent (colonies with pink or light red color or characteristic red center and whitish margin) and avirulent (smaller, off-white and non-fluidal colonies) isolates of *R. solanacearum* were identified in TZC medium containing 0.005% TTC. All isolated 50 isolates from ginger rhizomes had relatively close colony appearance on TZC medium resembling to *R. solanacearum.* Eight isolates were showed typical symptoms of wilting, where as the remaining 42 were non pathogenic and could be rated as saprophytic although they had similar colony appearance with *R.solanacearum*. Based on the morphological studies it was found that all the isolates were gram-negative, motility, rod shaped, pink color, non-capsulated and non-spore forming (Table 3).

Table 1. Collections and Isolation of *Ralstonia solanacearum* isolates from ginger rhizomes

Strains of R. Solanacearum	Source of Innoculation	Geographic Area/Zone/Region	Pathogenecity to Ginger	Biovar	Race
Type03	Soil	Wolaita, Areka, Boloso sore, SNNPR	Pathogenic	III	4
Type04	Ginger	Wolaita, Areka, Boloso sore, SNNPR	Pathogenic	III	4
Type05	Soil	Kambata, Hadero, Kacha Bira, SNNPR	Weakly Pathogenic	III	4
Tpe02-s	Ginger	Kambata, Durame, Angacha, SNNPR	Highly Pathogenic	III	4
Type sp-s	Ginger	Kambata, Hadero, Kacha Bira, SNNPR	Pathogenic	III	4
Type m-t	Ginger	Kambata, Durame, Angacha, SNNPR	Pathogenic	III	4
Type07	Ginger	Kambata, Durame, Angacha, SNNPR	Pathogenic	III	4
Type08	Ginger	Kambata, Hadero, Kacha Bira, SNNPR	Pathogenic	III	4

The threads of bacterial ooze exuding from the infected xylem vascular bundles were observed from wilted rhizome. (Fig. 3).

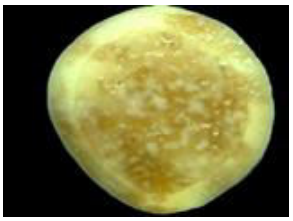

Fig 3. Bacterial ooze from an infected ginger rhizome.

Culture Media Based Identification of R. solanascearum.
The culture identification was based on the bacterial is fluidal, presents irregular shape and white with pink centered colonies on tetrazolium chloride (TZC) media, which is similar with the description of *R. solanascearum* (Fig.4).

A B

Fig 4. Morphology of *R. solanascearum* on TZC and nutrient agar medium: A) on TZC medium and (B) on nutrient agar medium

Identification of Virulent/Avirulent isolates of R. solanacearum
In this test, virulent isolates produce pink or light red color colonies or colonies with characteristic red centre and whitish margin and avirulent isolates produce smaller, off-white and non-fluidal or dry on TZC medium after 24 hours of incubation.

Fig 5. Pink centered virulent colonies of *R. solanacearum* on TZC agar medium

Biochemical Characterization of R. solanacearum

On the basis of all tests performed, data in the Table 3 shows morphological and biochemical characteristics of bacterial isolates. The isolates which were positive for catalase test, indol production test and oxidation of glucose, Hydrolysis of Tween 80, while they gave negative response in levan production from sucrose and remained non-fluorescent,were assumed as *R.solanacearum.* However, some isolates tested in this study showed differential behavior for starch hydrolysis.

Catalase

All eight isolates were catalase positive, release gas upon addition of hydrogen peroxide (Table 3).

Levan production from sucrose

The tested isolates were negative for levan production, and levan formation results from the enzymatic activity of levan sucrase on sucrose (Table 3).

Starch Hydrolysis

The tested isolates give different response for starch hydrolysis, seven isolates were negative for starch hydrolysis, but the only one isolates (AAURS -026) showed that clear zone surrounding the bacterial growth was observed when the plates were flooded with lugol‟s iodine solution for starch hydrolysis (Table 2).

Production of Fluorescent Pigment

All tested isolates were found non-fluorescent. None of fluorescent cultures produced brown diffusible pigments which are the characteristic of *Ralstonia solanacearum* isolates, whereas presence of green, diffusible fluorescent pigment was evident in fluorescent isolates but did not produce fluorescent pigment on Kings B medium (Table 2)

Indole Production Test

The tested isolates showed the development of bright- pink color on the top layer mass of bacterial growth so all isolates were positive for Indole Production Test (Table 2)

Table 2 . Biochemical and physiological characteristics of virulent bacterial isolates from wilted ginger for confirmation of *Ralstonia solanacearum.*

Isolates	Gram Stain	Motility	Shape	Color	catalase	Oxidation of glucose	Hydrolysis of Tween80	Levan Production	Indole Production Test	Starch Hydrolysis
Type03	Gm-	+	r	Pink	+	+	+	-	+	+
Type04	Gm-	+	r	Pink	+	+	+	-	+	+
Type05	Gm-	+	r	Pink	+	+	+	-	+	+
Tpe02-s	Gm-	+	r	Pink	+	+	+	-	+	+
Type sp-s	Gm-	+	r	Pink	+	+	+	-	+	+
Type m-t	Gm-	+	r	Pink	+	+	+	-	+	+
Type07	Gm-	+	r	Pink	+	+	+	-	+	+
Type08	Gm-	+	r	Pink	+	+	+	-	+	+

Source: (Researcher, 2017); ***Key****: All tests were conducted in three replicates; '+' indicates positive reaction; '-' indicates negative reaction; 'r'= rod shaped; Gm- '+' indicates Gram positive reaction and 'Gm-' indicates Gram negative reaction*

Oxidation-fermentation of glucose

All tested isolates were found positive for oxidation of glucose there was a gradual pH change at the surface of the open tubes resulting in color change from green to yellow, no pH change was observed in sealed tubes (Table 2)

Growth at 35 and 40^{o}C

It was seen that all isolates grow at 35oc and all of them failed to grow at 40 ^{o}C (Table 3)

NaCl tolerance

In all the isolates, heave growth appeared in 1 % NaCl, medium with weak growth in 2 % NaCl. As the characteristic of R. *solanacearum*, none grow at 3,4,5,6,7,8,9,% NaCl medium (Table 2).

Discussion

Similarly Laha *et al.* (1992) have stated that the fluorescent pigments produced by *Pseudomonas fluorescens* were appropriate Fe3+ and were considered as siderophores which inhibits the large number of phytopathogenic bacteria and fungi. In this study it was found that biological controls, (Trichoderma,1 isolate and *Pseudomonas fluorescens*) were found relatively higher antagonize pathogen under in vitro conditions on the growth of the pathogen. Thus the main increase in inhibitory activity of pseudomonas strains in nutrient agar medium might be siderophore production that inhibited *R. solanacearum* growth. This is in agreement with other observations that the inhibition zone due to *P. fluorescens* was significantly higher (38.7 mm) followed by combination of *P. fluorescens* and *T. harzianum* (31.8mm) (Bora *et al*.,2016). Ciampi-Panno et al. (1996) reported *P. fluorescens* to be the primary candidates for biological control of bacterial wilt pathogen because of their own ability to synthesize a wide range of secondary metabolites, many of which have antibacterial and fungal activity. The fluorescent pseudomonads could inhibit the growth of plant pathogens by producing a range of metabolites like antibiotics, siderophores and other substances such as cyanide (Loper and Buyer, 1991). The general mechanism of antagonistic activity of *Trichoderma* spp. has been reported to be antibiosis, lysis, competition and mycoparasitism (Henock, 2004; Papavizas and Lumsden, 1980).

Conclusion

The biocmecial characterization revealed that the disease that threat ginger in SNNP region is caused by *Ralstonia solanacearum* biovar III race 4 and the diseases was found distributed in major ginger growing areas of region. The syptomology and pathogenicity test confirmed that *R. solanacearum* causing bacterial wilt of ginger. All isolates of *R. solanacearum* were found virulent producing pink or light red color or characteristic red center and whitish margin on TZC medium after 48 hours of incubation. The members of family Psudomonadaceae such as genus Pseudomonas and *Ralstonia* are oxidative in nature as compared to members of family Enterobacteriaceae like Erwinia, which are fermentative bacteria. There was a gradual pH change at the surface of the open tubes resulting in color change from green to yellow, no pH change was observed in sealed tubes indicating that *R. solancearum*, which was aerobic bacterium, is responsible for such type of change in pH. All the hypersensitive positive isolates were found positive for oxidation of glucose. *Ralstonia solanacearum* isolates varied in utilization pattern of different sugars.

Recommendations

From the findings of the research, it the government is recommended to initiate a large scale assessment of ginger wilt disease biochemical characterization. Investors were recommended to enhance their investment for it is a very good source of income and food security in several regions of the country. Universities and research institutions shall embark on additional researches that focus on production, post-harvest technology, processing, marketing and determination of quality of ginger across the southern regions of the country. More researches should be done on ecology and diversity of *R. solanacearum* and other fungal diseases of ginger in the country. In this work, some fungal genera have showed organ specificity in the development of diseases symptoms; which need further studies to answer the question why. Further intensive characterization of these diseases and controlling methods of these bacterial genera requires more researches to be done on a larger scale.

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