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Lactobacillus paracasei subsp. *tolerans* a Novel Bacteriocin Producing Bacteria for Control of Tomato Bacterial Wilt

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The bacteriocin production by Lactic acid bacteria (LAB) isolated from soil, dairy products, was studied using the culture medium (MRS). The bacteriocin produced by LAB inhibits the *Ralstonia solanacearum* strains. Bacterial wilt caused by *R. solanacearum* is one of the production constraints of tomato. The aim of the study is to evaluate the potential of bacteriocin from *Lactobacillus paracasei* subsp. *tolerans* to suppress the development of wilt pathogen in tomato. *R. solanacearum* was isolated from infected tomato plants on triphenyl tetrazolium chloride (TZC) medium. The antibacterial compound from the culture supernatant was found to be proteinaceous in nature and it identified as bacteriocin. The bacteriocin from *Lactobacillus paracasei* subsp. *tolerans* was found to be heat–stable (121ºC for 15 min) and active over a wide pH range of 4.0–10.0. It showed stability (60%) for 30 days at room temperature (30–32 ºC). Addition of surfactants up to 1% to crude bacteriocin showed increase in antibacterial activity where as metal ions in low concentration (0.5–1mg/l) decreased the activity. Seed treatment with bacteriocin significantly improved the quality of seed germination and seedling vigour. The disease incidence was significantly reduced by about 63 % in plants raised from seed treatment and soil drench method. The present work demonstrates for the first time the ability of bacteriocin to act as plant growth promoting and biocontrol agent against *R. solanacearum invivo*.

Key words: Bacteriocin, Bacterial wilt, *Lactobacillus paracasei* subsp. *tolerans*, *Ralstonia solanacearum*, Tomato.

Bacterial wilt caused by *R. solanacearum* is deemed to be one of the most important plant diseases in tropical agriculture (Milling *et al.*, 2011). It has a large host range of more than 200 species in 50 families (Aliye *et al.*, 2008). The disease also affects other economically important crops such as potato, eggplant, chilly and non *Solanaceous* crops such as banana and groundnut in India (Anuratha *et al.*, 1990). In India, a study showed 10 to 100 % incidence of bacterial wilt during the summer (Kishun, 1985). The pathogen infects roots of susceptible plants, usually through wounds (Pradhanang *et al.*, 2005) and Colonizes within the xylem preventing the water movement into upper portion of the plant tissue (Kelman, 1998). *Ralstonia solanacearum* is a Gram negative, rod shaped, strictly aerobic bacterium, 0.5–0.7 x 1.5–2.0 µm in size, with a single polar flagellum. Individual bacterial colonies are usually visible after 36 to 48 hrs of growth at 30±2 ºC and colonies of the normal or virulent type are white or cream colored with pink centered, irregularly shaped, highly fluidal and opaque. Occasionally colonies of the mutant or non virulent type appear uniformly round, smaller and butyrous or dry. A Kelman's selective nutrient triphenyl tetrazolium chloride

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(TZC) medium can differentiate the two colony types on this medium (Kelman, 1954).

Management of bacterial wilt in tomato caused by *R. solanacearum* has been difficult and it still threatens commercial tomato production. Chemicals may be an effective method in controlling many bacterial diseases, but these chemicals potentially cause negative impact on plant growth or yield. The use of resistant or tolerant varieties, cultural practices, chemical control and biological control are commonly employed methods to control bacterial wilt disease (Dalal *et al.,* 1999). Biological control of soil borne pathogen is gaining much attention in recent years due to the effect of many chemicals used to control the disease (Haas and Defago, 2005). Biological control preserves environmental quality by reducing the dependency on chemical input and maintaining sustainable management practices (Barea and Jeffries, 1995). Lactic Acid Bacteria are known to improve human and animal health (probiotics) and recognized as safe (Stiles, 1996).

Interestingly, Lactic Acid Bacteria (LAB) cultures or their supernatants have been used as biological control agents on plant diseases in chilli, tomato and cucumber caused by the fungi *Colletotrichum capsici* (El-Mabrok *et al.,* 2012), *Fusarium oxysporum* and *Pythium ultimum*, respectively (Lutz *et al.,* 2012). Several species of LABs have been documented as producers of bioactive metabolites that act against a wide spectrum of undesirable microorganisms such as fungi, oomycetes and other bacteria (Axel *et al.,* 2012). Several members of the lactic acid bacteria are known to produce antibacterial substances. The antibacterial effect has been ascribed to the production of antibiotics or antibiotic like substances such as acidophilin, lactocidin, lactolin, nisin etc and various inhibitory substances such as lactic, acetic, probionic acids, and bacteriocins (Ouwehand *et al.,* 2003). Bacteriocin is the most potent of all the antimicrobial compounds produced by Lactic Acid Bacteria. Bacteriocins are ribosomal synthesized peptides which are generally only active against closely related bacterial species (Tagg *et al.,* 1976).

The potential application of bacteriocins of lactic acid bacteria as food preservatives requires in depth knowledge of how they exert their bactericidal effect. Most bacteriocins whose primary mode of action is known act at the plasma membrane. It has been proposed that these peptides form portion complexes that traverse the phospholipid bilayer. Bacteriocins show a bactericidal mode of action against closely related species (Tagg *et al.,* 1976). These substances are of particular interest as they are proteinaceous and may thus be degraded during digestion in humans and other animals. Most bacteriocins from lactic acid bacteria exert their antibacterial effect by permeabilizing the target cell membrane, whereby the cells lose their viability (Moll, *et al.,* 1998). However, information on the occurrence of lactobacilli on living plants is scarce, and no information is available on the interactions of plant–associated lactic acid bacteria with phytopathogenic bacteria.

We introduced bacteriocin from LAB for the first time as an efficient biocontrol compound, through a study for protection of tomato plants against *R. solanacearum*, to improve seed germination, shoot and root length as well as improving plant fresh weight and healthy. The main objective of the present study was analyze the nature of the antibacterial compound (bacteriocin) against bacterial wilt of tomato production by the *L paracasei* subsp. *tolerans* and control in green house studies against *R. solanacearum.*

MATERIALS AND METHODS

Isolation and identification of *R. solanacearum*

The disease plant material and soil samples were collected from the different agro climatic zones of Karnataka and other parts of India. The collected rhizosphere soil and surface sterilized plant tissues were plated onto SMSA (Wenneker *et al.,* 1999) and TZC (Kelman, 1954) media by serial dilution and direct plating methods respectively. The plates were incubated at 28 ± 2 °C for 24–48 h. The suspected colonies were observated for colony characteristics, biochemical, physiological, hypersensitive and pathogenicity tests for conûrmation of the identity of the pathogen and biovar characterization was carried out according to Hayward (Vanitha *et al.,* 2009; Hayward, 1964). The identification of the selected strains were further confirmed by molecular methods based on 16s rRNA sequencing for *R. solanacearum*. NCBI-BLAST search was performed and the top hit

sequences were multiple aligned and phylogenetic tree was constructed using CLUSTAL X2 2.1 (Windows version) software by Neighbor Joining (NJ) analysis with 1,000 bootstrap replications based on the algorithm (Waterman, 1986). The sequences were deposited to NCBI database.

Isolation and identification of bacteriocin producing lactic acid bacteria

Lactic acid bacteria were isolated from dairy products by standard spread plate technique on MRS (de man Rogosa and Sharpe) agar medium. Plates were incubated at 35 °C for 24h. Identification was done by studying the morphological and biochemical characterization (based on carbohydrate fermentation profile, catalase, gelatinase, arginine hydrolysis, motility test). MRS agar without yeast extract was used for the study of carbohydrate fermentation with phenol red as indicator. Identification of the isolate was further confirmed by VITEK 2 system (Biomeriux, USA) (Malini *et al.,* 2012).

Screening of bacteriocin production from lactic acid bacteria against *R. solanacearum*

The isolated LAB was screened for the antagonistic activity against *R. solanacearum* by agar overlay method. Lactic acid bacteria was spot inoculated on MRS agar, incubated at 35 °C for 22–24 h. Pathogenic *R. solanacearum* (approximately 10^8 CFU mL^{-1}) were inoculated into Tryptic soy soft agar (TSA) and overlaid on LAB culture. Plates were incubated at 30 °C for 22 h and zone of inhibition was measured. Organism which showed maximum inhibition was used for further study (Alvarado *et al.,* 2006, Malini *et al.,* 2012).

Preparation of culture supernatants

The selected LAB was subcultured twice at 35 °C in MRS broth (pH–6.0) without Tween 80. Inoculum, (2% v/v) from overnight culture was added to MRS broth, incubated at 35 ºC for 18–20 h. After incubation, cells were removed from the growth medium by centrifugation (10,000×g for 15 min, 4 °C). The cell free supernatant was adjusted to pH 6.0 with NaOH and used as crude antibacterial compound for further study (Ogunbanwo *et al.,* 2003).

Screening of bacteriocin activity against *R. solanacearum*

A well diffusion assay procedure was used Schillinger and Lucke (1989). Crude bacteriocin was serially diluted with sterile distilled water. Aliquot from each dilution was placed in wells of 5mm (diameter) in plates seeded with *R. solanacearum* pathogens. Plates were kept at 4 °C for 8h for diffusion of bacteriocin into the media. Plates were then incubated at 30 °C for 18–22 h and checked for zone of inhibition. The activity was defined as the reciprocal of the highest dilution showing inhibition of the indicator lawn and was expressed as Arbitrary Units per ml (AU mL^{-1}) (Rammelsberg and Radler, 1990).

Characterization of Bacteriocin from lactic acid bacteria

The cell free supernatant was characterized and tested for antibacterial activity by agar well diffusion method against *R. solanacearum*. The bacteriocin samples from *Lactobacillus* species were characterized for stability to proteolytic enzyme (trypsin), temperature, pH, storage and metal ion. After the treatment the samples were tested for bacteriocin activity by agar well diffusion method. *R. solanacearum* was spread on Nutrient soft agar before boring the well. Aliquots of these treated samples were loaded into the well; plates were allowed for diffusion process at 4 °C for 8h then incubated at 30 °C for 24 h. Zone of inhibition against *R. solanacearum* were measured (Ogunbanwo *et al.,* 2003).

Effect of bacteriocin on tomato seed germination and seedling vigor index

The effect of *R. solanacearum* and Crude bacteriocin on seed germination and vigor of seedlings was evaluated under laboratory conditions. Three different wilt susceptible tomato cultivars (Arka Megali, Arka Saurabha and Arka Vikas) were procured from Indian Institute of Horticultural Research (IIHR), Bangalore, India. The germination tests for fresh *R. solanacearum* and crude bacteriocin suspension were carried out according to the paper towel method (ISTA, 2005). The vigor index was calculated by using the formula VI = (mean root length + mean shoot length) x Germination percentage (Abdul Baki and Anderson, 1973). The experiment was conducted with four replicates of hundred seeds each and the entire experiment was repeated thrice.

***In vivo* experimental design under greenhouse conditions**

The efficacy of bacteriocin to control bacterial wilt of tomato in the greenhouse was

tested. Seeds soaked overnight in culture supernatants and non soaked seeds were sown in seedling trays 98 eyes with sterilized soil and coconut pith compost for seed treatment method and soil drench method respectively. Twenty days old seedlings were transplanted as five per pot with sterilized potting soil (soil, sand and coconut pith compost). For seed treatment method, seedlings from bacteriocin treated seedlings were transplanted into pots infested with and without *R. solanacearum*. For soil drench method, seedlings raised from non soaked seedlings were transplanted into pots infested with and without *R. solanacearum* with additional supplementation of bacteriocin at a rate of 50 mL cell free supernatant per pot. Seedlings were maintained in a greenhouse at 24–28 °C and 75–90 % relative humidity, uninoculated plants served as the control (Neelu Singh *et al.*, 2012).

Plant growth promotion assessment

The samples of bacteriocins were tested for their ability to promote plant growth in separate experiment. At the end of the experiment (30 days after transplanting), plants including the roots were harvested from the pots and fresh weight, dry weight, mean shoot length, mean root length and disease incidence were measured to determine the effects of bacteriocin treatments on plant growth. Treated plants were counted and uprooted separately and their weights recorded to measure growth promotion, compared with the untreated control (Lim and Kim, 1997). Wilt incidence was recorded after crude bacteriocin treatment using the formula

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Statistical analysis

All data from the laboratory and greenhouse experiments were analyzed. Analysis of variance (ANOVA) was performed SPSS software (version 16). Significant effects of treatments were determined by the magnitude of F values (Pd”0.05). Treatment means were analyzed using Scheffe post hoc test.

RESULTS

Isolation and identification *R. solanacearum*

Around hundred strains of *R. solanacearum* were isolated, identified and stored as pure cultures in TZC slants at 4°C for further studies. Ten isolates of *R. solanacearum* were

Table 1. Effect of heat stability of bacteriocin against *R. solanacearum*

Time in min	Temperature						
R. solanacearum	Zone of inhibition in mm						
	15ºC	30ºC	45ºC	60ºC	75ºC	90ºC	100ºC
15	12.30± 0.66^{a}	12.50± 0.86^{a}	11.50± 0.57^{a}	9.90± 0.66^{b}	8.33± 0.33^{a}	7.733± 0.66^{a}	6.61± 0.15^{b}
30	12.13± 0.21^{a}	12.0± 0.57^{a}	11.10± 0.6^{a}	8.86± 0.52^{b}	7.16± 0.15^{a}	6.50± 0.57^{a}	5.73± 0.28^{b}
45	11.10± 1.15^{a}	11.10± 1.16^{a}	10.73± 0.88^{a}	8.26± 0.66^{a}	7.0± 0.21^{a}	6.10± 0.66^{a}	5.20± 0.17^{a}
60	11.0± 0.88^{a}	10.0± 0.66^{a}	9.00± 0.66^{a}	7.00± 0.15^{a}	6.90± 0.33^{a}	5.97± 0.17^{a}	3.900.05^{a}

Scheffe post hoc test: Means sharing different alphabetical (a, b, c) superscripts in a column significantly different (Pd”0.05). Means of the groups homogenous subsets are displaced.

Table 2. Effect of seed treatment with bacteriocin on seed germination and seedling vigour of tomato under laboratory conditions.

Treatments	Germination (%)	MRL (cm)	MSL (cm)	Fresh weight	Dry weight	VI
Control	90.66± 3.43^{d}	5.40±0.15^{c}	7.36± 0.15^{c}	1.08± 0.057^{b}	0.28± 0.066^{b}	1143.43± 14.43^{d}
Treated with Rs	34.0± 0.88^{a}	3.73±0.066^{a}	5.20± 0.33^{a}	0.45± 0.021^{a}	0.18± 0.011^{a}	303.56± 6.92^{a}
Treated with Bacteriocin	93.00± 3.46^{c}	6.36±0.33^{b}	8.53± 0.66^{b}	1.36± 0.057^{c}	0.38± 0.025^{c}	1376.30± 16.16^{c}

MRL - Mean Root Length; MSL - Mean Shoot Length; VI - Vigour Index; Rs- *R. solanacearum*. Scheffe post hoc test: Means sharing different alphabetical (a, b, c) superscripts in a column significantly different (Pd”0.05).

tested by pathogenicity assay under greenhouse conditions and causing wilt symptoms in tomato plants. The *R. solanacearum* identified according to based on Biochemical, morphological and physiological characteristics (Narasimha Murthy *et al.*, 2012) and the amplified PCR products were sequenced and a phylogenetic tree was constructed by the blast analysis and multiple sequence alignment data (Fig. 1). The sequences of highly virulent strains of *R.solanacearum* were deposited in NCBI GenBank with GenBank accession numbers KF924739– KF924748.

Isolation and Identification bacteriocin producing bacteria

Out of 30 samples, 35 lactic acid bacteria were isolated and identification of LAB based on their morphological and microscopically characteristics, they were found to be rods. The selected Lactobacillus was identified up to species level as *Lactobacillus paracasei* by VITEK 2 system. The identification was further corroborated with studies on its 16S rDNA gene sequencing carried out by Accugenix, USA. The isolate was confirmed as *Lactobacillus paracasei* subsp. *tolerans* (Malini *et al.*, 2012).

Screening of crude bacteriocin for antibacterial activity

All 35 LAB isolates were screened for bacteriocin production, among them one isolate was showed highest zone of inhibition against ten *R. solanacearum*. *Lactobacillus paracasei* subsp. *tolerans* showed zone of inhibition that is 22–24 mm in diameter (Fig. 2).

Characterization of Bacteriocin

Characterization of bacteriocin culture free supernatant of *Lactobacillus paracasei*

Table 3. Effect of bacteriocin from *Lactobacillus paracasei* subsp. *tolerans* on bacterial wilt disease in wilt susceptible tomato variety by seed treatment method.

Treatments	Seed treatment					
	Plant Height(cm)	MSL (cm)	MRL (cm)	MTFW (gm)	Dry Weight (gm)	DI (%)
Control	23.36± 1.51^{d}	12.1± 0.88^{c}	8.86± 0.57^{d}	5.86± 0.57^{c}	1.30^{d}	0.00^{a}
Treated with Rs	15.43± 1.66^{a}	6.33± 0.28^{a}	4.66± 0.33^{a}	3.33± 0.057^{a}	0.56± 0.11^{a}	86.44± 3.43^{c}
Treated with bacteriocin	29.70± 1.86^{c}	14.25± 1.12^{b}	9.83± 0.66^{c}	7.86± 0.25^{c}	1.53± 0.066^{c}	0.00^{a}
Rs + bacteriocin Treatments	20.76± 1.57^{b}	9.90± 0.33^{b}	6.90± 0.15^{b}	4.75± 0.33^{b}	0.83± 0.033^{b}	32.81± 1.52^{b}

MSL: Mean Shoot Length, MRL: Mean Root Length, MTFW: Mean Total Fresh Weight, DI: Disease Incidence of tomato plants of susceptible variety treated by bacteriocin and infested with *R. solanacearum* (Rs) by seed treatment method. Scheffe post hoc test: Means sharing different alphabetical (a, b, c) superscripts in a column significantly different ($P<0.05$).

Table 4. Effect of bacteriocin from *Lactobacillus paracasei* subsp. *tolerans* on bacterial wilt disease in wilt susceptible tomato variety by Soil drench method.

Treatments	Soil drench					
	Plant Height(cm)	MSL (cm)	MRL (cm)	MTFW (gm)	Dry Weight (gm)	DI (%)
Control	26.13± 1.52^{d}	14.90± 0.66^{c}	8.80± 0.33^{d}	8.80± 0.25^{d}	1.33± 0.033^{c}	0.00^{a}
Treated with Rs	15.86± 0.88^{a}	7.0± 0.57^{a}	4.33± 0.25^{a}	3.66± 0.17^{a}	0.58± 0.01^{a}	86.44± 3.46^{c}
Treated with bacteriocin	28.73± 1.15^{c}	14.86± 1.2^{c}	10.06± 0.57^{c}	9.86± 0.66^{c}	1.56± 0.057^{c}	0.00^{a}
Rs + bacteriocin Treatments	20.33± 1.33^{b}	10.33± 0.25^{b}	6.66± 0.25^{b}	6.66± 0.33^{b}	0.89± 0.033^{b}	31.60± 1.86^{b}

MSL: Mean Shoot Length, MRL: Mean Root Length, MTFW: Mean Total Fresh Weight, DI: Disease Incidence of tomato plants of susceptible variety treated by bacteriocin and infested with *R. solanacearum* (Rs) by soil drench method. Scheffe post hoc test: Means sharing different alphabetical (a, b, c) superscripts in a column significantly different ($P<0.05$).

subsp. *paracasei* was found to be sensitive to trypsin indicating that the inhibitory substance is proteinaceous in nature (Fig. 3). The inhibitory activity was unaffected by heating up to 100 °C for 60min and 70% of activity retained after heat treatment at 121°C for 15min (Table. 1). Bacteriocin produced by the selected *Lactobacillus paracasei* subsp. *tolerans* was stable at broader pH range of

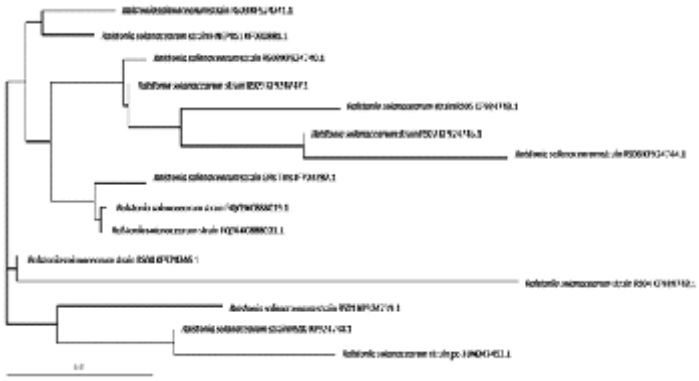

Fig. 1. Phylogenetic relationships of *R. solanacearum* isolates inferred by Neighbor-Joining (NJ) bootstrap tree analysis of 16S rRNA sequences

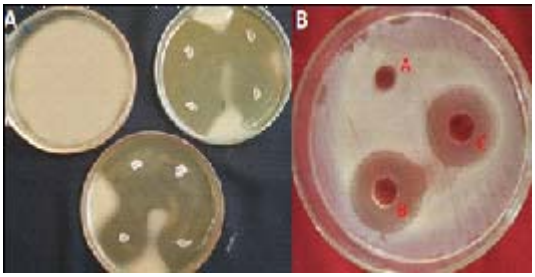

Fig. 2. Zone of Inhibition by **A** *Lactobacillus paracasei* subsp. *tolerans* and **B** Bacteriocin against *R. solanacearum*

Fig. 3. Effect of Trypsin on stability of bacteriocin activity; A Control, B Bacteriocin treated with trypsin.

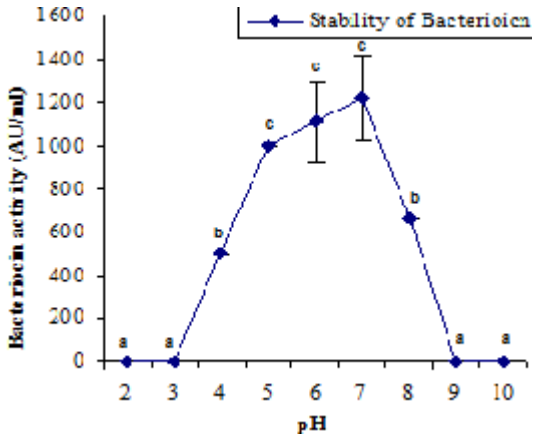

Fig. 4. Effect of pH on stability of Bacteriocin. Scheffe post hoc test: Means sharing different alphabetical (a, b, c) superscripts are significantly different (P<0.05)

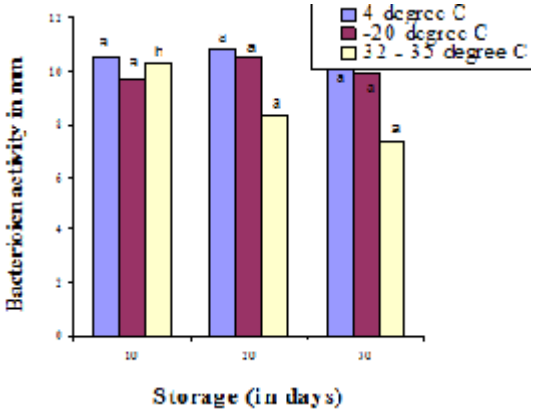

Fig. 5. Effect of storage of Bacteriocin. Scheffe post hoc test: Means sharing different alphabetical (a, b, c) superscripts are significantly different (P<0.05)

4.0–10.0 (Fig. 4). Surfactants tested did not have profound effect on bacteriocin activity except Tween 20 and 80, which decreased the activity. Bacteriocin was found more stable at –20 °C and 4 °C for 30days. Activity decreased at 32 °C after 20 days in storage (Fig. 5). However, increase in the concentration of metal ion tested had reciprocal effect on activity of bacteriocin (Fig. 6) (Malini *et al.*, 2012).

Effect of bacteriocin on tomato seed germination and seedling vigor index

There was an improvement in seed germination and seedling vigor upon bacteriocin seed treatment whereas seed germination of tomato seeds upon *R. solanacearum* inoculation showed reduction (Fig. 7). The bacteriocin treated, enhanced the vigor index when compared to control. The maximum germination was recorded in bacteriocin treated seeds, as tabulated in Table 2.

Plant growth promotion assessment

The efficacy of bacteriocin for the control of bacterial wilt in tomato plants was evaluated under greenhouse conditions. Results of these experiments showed that bacterial compounds (bacteriocins) have been stimulated plant growth promotion and indicated that tomato plants treated with bacteriocin significantly increases compared to the control (Fig. 8 and 9). However, the plant growth characteristics significantly differed in response to bacteriocin by seed treatment and soil drench methods, the fresh weight, shoot length,

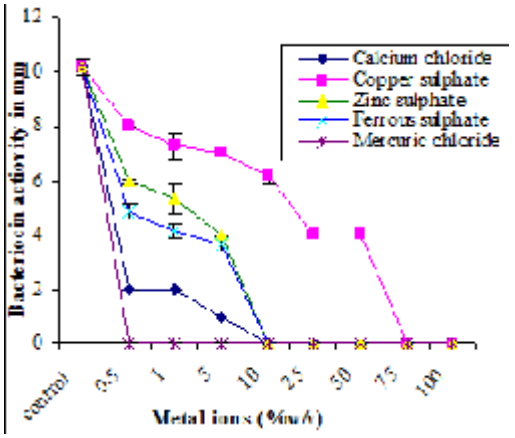

Fig. 6. Effect of metal ion on stability of Bacteriocin. Scheffe post hoc test: Means are significantly different (P<0.05)

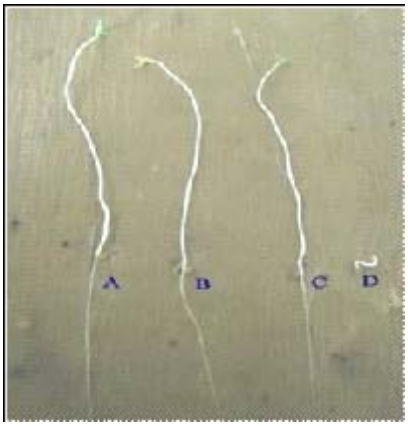

Fig. 7. Seed germination of tomato seeds, A Bacteriocin treated, B & C controls, D *R. solanacearum* treated

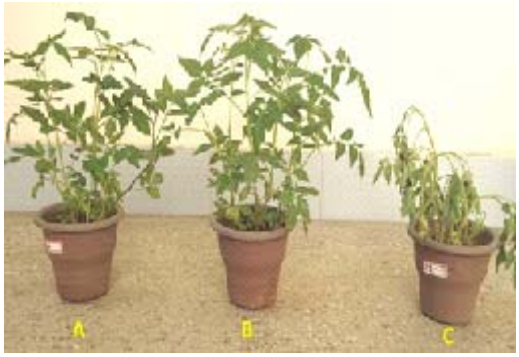

Fig. 8. Study of Plant growth promoting effect of bacteriocins on tomato plant compared with control, A Control, B cell free supernatant of lactic acid bacteria treatment, C *R. solanacearum* treated

Fig. 9. Root growth promotion of tomato plants, A Pathogen treated, B Control and C bacteriocin treated

root length, dry weight and disease incidence were tabulated as in (Table 3 and 4). Results of this experiments showed that bacteriocin exhibited the highest wilt disease reduction percentage in treated tomato plants by seed treatment and soil drench methods 55.59% and 56.24% respectively.

DISCUSSION

In the present study, bacteriocin from *Lactobacillus paracasei* subsp. *tolerans* strain has shown good antagonism against *R. solanacearum*, exhibited plant growth promotion and reduced disease severity. Bioprotection of tomato plants by LAB is a finding reinforced not only by their inhibition capacity but also by their persistence in the soil under hard conditions. Bacteriocins that inhibit plant pathogenic bacteria have been reported from bacteria associated with plants. However, until now there has been no report of a bacteriocin directly increasing plant growth and bacteria–produced compounds may increase germination and plant growth as illustrated by lipo–chitoligosaccharides (LCOs), or Nod factors (Prithiviraj *et al.*, 2003). The Results of *in vitro* antagonism studies revealed that LAB showed high inhibition of *R. solanacearum*. The crude bacteriocin from LAB strain also showed high inhibitory activity against the pathogen. Possible applications of bacteriocinogenic strains in agriculture include their use in biological control of soil borne or phyllosphere inhabiting bacterial plant pathogens on heterologous production of the bacteriocin trifolitoxin by an avirulent agrobacterium strain which effectively enhanced biological control of *Agrobacterium vitis* crown gall. LABs have been isolated from soil (Chen *et al.,* 2005), vegetables surfaces (Trias *et al.,* 2008) and the rhizosphere, suggesting that they may have the ability to colonize plant roots.

The bacteriocin treatment under laboratory conditions, increased seed germination by 59%. In the *in vivo* pot trials, bacteriocin of *Lactobacillus paracasei* subsp. *tolerans* revealed highly effective antagonism against the tomato wilt pathogen *R. solanacearum*. The seed and soil drench treatments of bacteriocin under greenhouse conditions reduced about 55.59 % and 56.24 % disease incidence respectively. A possible mechanism for increasing plant growth by LAB may be due to the efficiency in nutrient transfer from soil to the roots and plants as a result of increasing the number of roots and bioprotection of rhizosphere area by LAB. Another mechanism for increasing plant growth is caused by the antibacterial metabolites (antibiotic) produced by LAB against *R. solanacearum.* Plants are able to produce a broad range of secondary metabolites capable of improving their resistance to insect and pathogen attack. In many cases these metabolites are only synthesized when the plants are exposed to compounds that indicate the presence of the pathogen. Understanding these responses and how to manipulate them has the potential to be an important tool for sustainable crop production in the future.

In addition, treatment of soil with LAB may trigger ISR which develops when plants successfully activate their defense mechanism, in presence of a pathogen infection, resulting in an enhanced synthesis of plant defense chemicals which supports plant growth. In the present study, the obvious elongation in both shoot and root lengths and the increasing number of secondary roots of tomato plants may confirm the capability of LAB to trigger the ISR. Our results could confirm efficacy of *Lactobacillus paracasei* subsp. *tolerans* as Plant Growth Promoting Bacteria (PGPB); bioprotection of tomato seeds and the soil drench with LAB support plant growth, especially that a single application by *Lactobacillus paracasei* subsp. *tolerans* was used. Rhizosphere competence of biological control agents comprises effective root colonization combined with the ability to survive and proliferate along growing plant roots over a considerable time period in presence of the indigenous micro flora (Kochian *et al.,* 2005). During the past 50 years, many studies are reported about bacterial and fungal plant diseases as well as the application of different microorganisms as biocontrol agents. However, the effectiveness of the interaction is yet to be proved under field conditions where limiting factors such as rain, fluctuations in temperature and relative humidity, targeting of biocontrol agents to the effective site, the economical feasibility of control procedures and a greater variety of competitive microorganisms will play a role.

CONCLUSION

The findings of the present study suggest that LABs contain great effectiveness for increasing growth of tomato plant. However, field experiments that examine the mechanisms behind this growth promoting effects are required. Moreover, our results show that LABs can improve plant growth, producing the bacteriocin and suppress bacterial wilt in tomato. The use of chemicals and fungicides in agriculture as well as the environmental pollution would be avoided by *Lactobacillus paracasei* subsp. *tolerans* as a promising PGPB and biocontrol agent. Future research should confirm the mechanism of inhibition, assay for lytic enzymes, and determination of inhibitor substances other than antibiotics for application of *Lactobacillus paracasei* subsp. *tolerans* in biocontrol as a viable alternative method to manage plant diseases.

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