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Research Article

Efficay of a Probiotic *Lactobacillus* as a Biocontrol Agent and Plant Growth promoting Bacteria by controlling *Xanthomonas campestris* infection in Chilli plant

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Abstract

Leaf spot of chilli plant is caused by bacteria belonging to several species in the genus *Xanthomonas* especially *Xanthomonas campestris.* Infected chilli plant were collected from local area of Coimbatore and were processed for isolation, identification and characterisation of *Xanthomonas. Lactobacillus* sp, a probiotics bacteria found mainly associated with milk products was isolated from raw cow milk sample and were subjected to morphological and biochemical characteristics. The cultures supernatant of isolated *Lactobacillus* sp obtained from the bacteriocin producer strain were tested for the antibacterial activity against isolates of *Xanthomonas campestris* by agar well diffusion assay. The chilli seeds treated seeds with *Lactobacillus* sp showed inhibitory activity against *Xanthomona campestris* strain which determined the *in vitro* efficacy of LAB as biocontrol and Plant Growth Promoting Bacteria.

Key words: chilli plant, *Xanthomonas campestris, Lactobacillus* sp- biocontrol agent.

INTRODUCTION

According to human history more than 7000 species of plants were found to be a definite food for the survival and development of human populationsthrough out the globe inspite of pests, disease, climate change and other environmental hazards.

Chilli under *Solonaceae* family is one of the important spice/ vegetable/ cash crop grown in India. It is an ancient essential ingredient of Indian home as it provides colour, flavor and aroma. Chilli is a good source of vitamin and have small quantity of protein, fats and carbohydrate. Chilli extracts are used in wide range of medicines against tonsilities, diphtheria, loss of apetite, rheumatism, sore throat, swelling and hardened tumors.

India being a largest producer, consumer and exporter of chilli in the world (1) is facing a major negativity in growth, development and cultivation because of bacterial (2) and fungal infections (3). The bacterial disease caused by *X. campesteris* causing leaf spot with lesions covering 80% of the leaf area which persist atleast 4 months and 10 months in seeds has reduced the development of the plant. Many physical and chemical methods were tried on the plant to control the disease

Physical control includes tillage to control pests (4). On other hand tillage is energy expensive (5) and contributes soil erosion. Open field burning contributes air pollution. The use of biological control agents has been suggested as an alternative way of controlling plant diseases (6). Biological control using antagonistic *Pseudomonas fluorescens* (bacteria) for seed treatment and as well as spray treatment were found to be effective against

C.capsici (7). *Trichoderma* species (fungus) are able to effectively control *C.capsici* infection in chilli (8).

Experts in the *Lactobacillus* Pafi Techno Resources Corporation in Cebu, Philippines extensively made a scientific study on Lactoplant as the viable replacement of the old conventional method of farming.

Lactoplant is an effective growth enhancer that could boost production in a maximum level, harmless to living being and restores the fertility of the soil for life. Probiotic microorganisms in Lactoplant are extremely important to every agricultural system because it will substantially provide much fertility of the soil.

In our present study probiotics Lactic acid Bacteria (LAB) was isolated from the natural sources like raw cow milk and were applied to determine their efficiency to control the infections caused by *Xanthomonas* sp in chilli plant. This study will reduce the continous use of chemical fertilizer and bacteriocides. The use of biocontrol agent might promote rapid plant growth.

In our present studies the following objectives was undertaken;

- *Xanthomonas campestris* from infected chilli leaf was isolated and characterized
- LAB from raw cow milk was isolated, identified and characterized
- Determination of *In vitro* Antagonistic activity against *Xanthomonas campestris* using LAB
- *In vitro* and *In vivo* access of the efficacy of LAB to act as Biocontrol, Plant growth promoting bacteria

MATERIALS AND METHODS

COLLECTION OF SAMPLE

Infected chilli plant sample (*Capsicum annum* L) were collected from the local area in Coimbatore, Tamilnadu, due their wide cultivation in Coimbatore and botanical name was confirmed from TNAU. Two varieties of chilli seeds (CO1, K2) were collected from TNAU. Immediately after collection, the sample were stored for further study.

Raw cow milk sample were collected from the local area in Coimbatore, Tamilnadu due to their wide acceptance among the consumers of Coimbatore. Immediately after collection, the sample were stored aseptically in low temperature (4^0C), to protect it from contamination and deterioration.

MEDIA

The bacteria *Xanthomonas campestris* was isolated from infected chillli plant leaves by using special media nutrient agar with 5% glucose media and nutrient broth with 5% glucose (9).

The bacteria *Lactobacillus* sp was isolated from raw cow milk sample by using MRS broth and MRS agar media (10).

ISOLATION AND IDENTIFICATION OF BACTERIA

Isolation of *Xanthomonas campestris*

Infected chilli leaves were collected from the local area of Coimbatore, washed the sample under running tap water and treated with 10% Sodium hypochloride. Dissected out a small portion (1×1×3mm) of infected plant material and mounted in a crop of sterile water on a clean, flamed microscope slide. Examined slide under microscope at 40x magnification for microbial presence of bacteria. A loop full of suspension was inoculated on nutrient agar with 5%glucose medium and streaked (zig-zag) out. Incubated at 27-30^0C for 2-5 days. Sub cultured from a single well-separated colony on to richer media, and stored in slant at 5^0C.

Identification of *Xanthomonas campestris*

Identification of *Xanthomonas campestris* based on the colony morphology, and biochemical and nutritional tests described (11; 12) : Gram's stain; oxidative / fermentative metabolism; catalase, oxidase activities; nitrate reduction; hydrogen sulfide production; starch, gelatin and casein hydrolysis; growth on nutrient agar with 5% glucose (13); indole production from Tryptone; citrate utilization (9).

Isolation of *Lactobacillus* sp

Raw cow milk sample were collected from the local area of Coimbatore under aseptic conditions, processed within three hours and used for further studies.

Milk sample was serially diluted and plated on to a De Man RogosaSharpe (MRS) medium for *Lactobacillus* isolation and incubated at 37^0C for 48-72 hrs. Well isolated colonies with typical characteristics namely pure white, small (2-3 mm diameter) with entire margins were picked from each plate and transferred to MRS broth. Around five colonies were picked and were designated as LAB1, LAB2, LAB3, LAB4 and LAB5.

Identification of *Lactobacillus* sp

Further identification of the Lactobacilli was performed according to their colony morphological, cultural, physiological and biochemical characteristics (14; 15) : Gram reaction, production of catalase, carbohydrate fermentation patterns, growth at 15^0C and 45^0C in a *Lactobacilli* De Mann Rogosa and Sharpe (MRS) broth as described by Bergey's Manual of Systematic Bacteriology (15), methyl red and Voges-Proskauer test in MR-VP

medium, nitrate reduction in nitrate broth, indole production in Tryptone broth. Purified cultures were maintained at-20^{0}C in MRS broth with 10% glycerol and enriched in MRS broth incubated at 37^{0}C for 24hrs. The isolates were named as *Lactobacillus* sp.

DETECTION OF ANTAGONISTIC ACTIVITY

Agar spot assay test

Lactic acid bacterial isolates were cultured in 5ml of MRS broth at 30^0 C for 16 hrs. Aliquots (2µl) of the culture were spotted onto agar plates containing 10ml of MRS medium. After 18 hrs at 30^{0}C, the plates were overlaid with 5ml of the appropriate soft agar (1% agar) inoculated with the cell suspension of the indicator strain *Lactobacillus* sp at a final concentration of 10 ^{5} CFU/ml (16). The plates were incubated at 37^{0}C for 24 to 72 hrs, depending on the growth of the indicator strain and the appearance of inhibitory zones were observed. Inhibition was scored positive if the zone was wider than 2mm in diameter.

Agar- well diffusion method

The isolates (LAB1, LAB2, LAB3, LAB4 and LAB5) that were selected as potential bacteriocin producers were grown in MRS broth at 37^{0}C for 48hrs. Cells were separated by centrifugation at 5000 rpm for 10 min at room temperature. Around 6mm diameter wells were made on *X. campestris* strain inoculated agar with 5% glucose media and each well was inoculated with 100µl of culture supernatant of bacteriocin producing *Lactobacillus* strains after neutralization with NaOH (17). Inhibitory activity was performed against Gram positive (LAB) and Gram negative organism (*Xanthomonas campestris*).

***IN VITRO* AND *IN VIVO* EFFICACY OF *LACTOBACILLUS* sp AS BIOCONTROL, PLANTGROWTH PROMOTING BACTERIA**

Chilli plant seeds experiment

Seeds from two varieties of chilli plants such as CO1 and K2, were placed in two replicates of 50 seeds. Each 25 seeds per plate were kept at 4^{0}C before use. First the surfaces of the seeds were disinfected with ethanol (70%) for a minute and rinsed with distilled water four times to minimize microorganism development at the early stages of germination (18). The treatments included immersion of seeds for 10 minutes in LAB1, LAB2, LAB3, LAB4 and LAB5 prepared on MRS broth with two replicates of 25 seeds each per microbial isolates. After immersion, the seeds were dried under laminar flow and then placed in petridishes after the inoculation of *Xanthomona campestriss* broth (19).

***IN VITRO* AND *IN VIVO* GERMINATION DESIGN**

***In vitro* trials**

Experiments were performed under *in vitro* condition. First the LAB treated infected chilli seeds from each variety was inoculated in the MS medium in aseptic under *in vitro* condition. Non- treated infected chilli seeds were inoculated as control. After 30 days, expression of healthy seedling were observed.

***In vivo* trials**

In vivo trials were performed in pot (30cm diameter) filled with unsterilized natural soil. First the LAB treated infected chilli seeds from each variety was inoculated in different pots, at *in vivo* condition. Non treated infected chilli seeds were used as control. After 30 days, expression of healthy seedling were noted (20).

Germination and Statistical method

After one month's growth, chilli plants were harvested under both *in vitro* and *in vivo* condition. To reveal the effect of LAB on the growth characteristics, each plant was measured for shoot length and root length. Statistical analysis data were analyzed using SPSS for windows (SPSS Inc.) by means of a one-way ANOVA and subsequently differences between treatments and control were determined using least significant differences LSD at α0.05 (20).

RESULTS

Isolation and identification of bacteria

Isolation of *Xanthomonas campestris*

Infected chilli plant sample were collected from the local area in Coimbatore and were processed for isolation of *Xanthomonas campestris*. Among the peocesseed samples colonies with typical characteristics namely smooth, round (1.5cm-2cm) with entire margins (Figure 1. a) were picked and were subjected to morphological and biochemical characteristics.

Identification of *Xanthomonas campestris*

Isolate reacted negatively to gram staining under a light microscope, are generally short rods (Figure 1.b). *Xanthomonas campestris* were able to utilize citrate, gelatin is liquefied, indole is produced, catalase is positive, nitrate is reduced, starch and casein is hydrolysed, oxidase is positive. *Xanthamonas campestris* can able to grow on nutrient agar with 5% glucose (Table 1). Based on the above characters it was concluded that all the isolates were identified as *Xanthomonas campestris.*

Isolation of *Lactobacillus* sp

Raw cow milk sample were collected from the local area of Coimbatore and were processed for isolation of Lactobacilli. Among the processed samples colonies with typical characteristics namely pure white, small (2-3mm diameter) with entire margins were picked (Figure 2.a) and subjected to morphological and biochemical characteristics.

Identification of *Lactobacillus* sp

Five isolates described as LAB1, LAB2, LAB3, LAB4 and LAB5 reacted positively to gram staining under a light microscope. Lactobacilli are generally long rods, some times they are short rods (Figure 2.b). *Lactobacillus* sp do not possess flagella and do not create endospores, nitrates are not reduced, gelatin is not liquefied. Indole is not produced, acidic and non-acid end products are produced and catalase negative (Table 2).

Detection of antagonistic activity of *Lactobacillus* sp against against *Xanthomonas campestris*

Agar spot assay

The culture supernatants obtained from *Lactobacillus* sp tested for antibacterial activity against the same group of *Lactobacilli*. This has shown clear zone of inhibition against the indictor organism and they were selected as potential bacteriocin producers (Figure 3.a). All the isolates were able to inhibit the indicator organism.

Agar-well diffusion Assay

The cultures (LAB1, LAB2, LAB3, LAB4, LAB5) supernatant obtained from the bacteriocin producer strains was tested for antibacterial activity against isolates of gram negative bacteria, *Xanthomonas campestris*. Bacteriocins obtained from the isolates showed inhibitory activity against all the tested strains in the form of zone of inhibition (mm in diameter). The antibacterial activity of LAB1 and LAB2 showed strong zone of inhibition (5mm in diameter) against *Xanthomonas campestris*. LAB3 was able to inhibit *Xanthomonas campestris* showing 16mm in diameter. LAB4 showed that inhibitory activity at a range of 16.5mm in diameter. LAB5 strongly inhibited the organism showing zone of inhibition 26mm than all other isolates (Table 3 and Figure.3.b).

***In vitro* and *In vivo* efficacy of *Lactobacillus* sp as Biocontrol, Plant growth promoting bacteria**

Chilli plant seeds experiment

Seeds of two varieties of chilli plants such as CO1 and K2 were treated with strains of *Lactobacillus* (LAB1, LAB2, LAB3, LAB4, and LAB5). Treated seeds are showed activity against the strain of *Xanthomonas campestris*. CO1 seeds were strongly inhibited when compared to K2. LAB5 inhibited the seeds CO1 (8.8 mm in diameter) and K2 (7.6 mm in diameter) at a very stronger rate when compared to other isolates. LAB4 showed inhibition 8.2 mm in CO1 and 7.2 mm in K2 against *Xanthomonas campestris*. LAB3 inhibited K2 at 7.2 mm in diameter and 6.8 mm in CO1 (Table 4 and Figure 4).

***In vitro* and *In vivo* germination trail**

***In vitro* trails**

In vitro trails were performed in MS medium at *in vitro* condition using LAB treated seeds CO1, K2. LAB treated seed revealed their ability to enhance plant growth when compared with control which was processed after one month. Plant growth characteristics significantly differed in response to different LAB strains. Seeds not treated with LAB (control) showed 8.25cm shoot length and 5.5cm root length. LAB5 treated seeds germinated than other seeds and their shoot length was 8.125cm and root length was 8.25cm. But no rapid increase in K2 variety when compared with control. Treated plantlet doesn't show any symptoms of disease (Table 5, Figure 5 and 7).

***In vivo* trails**

In vivo trails were performed on pot at *in vivo* condition using LAB treated seeds such as CO1, K2. LAB applied as seed treatment show their ability to enhance plant growth compare with control. LAB5 treated seeds showed better germination when compared to the other LAB treated seeds. The shoot length was 6.6cm and the root length was 8.66cm found to be strongest enhancement than other treated seeds. K2 variety doesn't show any obvious increase. Treated plantlet doesn't show any symptoms of disease (Table 6, Figure 6 and 8).

These result reveal the capability of LAB to be considered as Plant Growth Promoting Bacteria and Biocontrol agent.

DISCUSSION

Lactic Acid Bacteria is widely distributed in the nature and occurring naturally as indigenous microflora in milk and dairy products, that play an important role in every agricultural system.

Isolation and identification of bacteria

Isolation and identification of *Xanthomonas campestris*

Infected chilli leaves were collected from the local area of Coimbatore, and processed for isolation of *Xanthomonas campestris*.

Isolate reacted negatively to gram staining under a light microscope, are generally short rods (Figure 1

b). *Xanthomonas campestris* were able to utilize citrate, gelatin is liquefied, indole is produced, catalase is positive, nitrate is reduced, starch and casein is hydrolysed, oxidase is positive. *Xanthamonas campestris* can able to grow on nutrient agar with 5% glucose.

The isolate was classified as *Xanthomonas campestris*, based on their morphological and biochemical characters (11), (Table 1). Isolate of *Xanthomonas campestris* were gram negative (21). Reports were been found for isolates of *Xanthomonas campestris* grow on Nutrient agar with 5% Glucose (22). *Xanthomonas campestris*showed gelatin liquefaction, Nitrate reduction (23), starch hydrolysis, Casein hydrolysis, Indole production, and Hydrogen sulphide production (9), oxidase and catalase characters are shown.

Isolation and identification of *Lactobacillus* sp

Raw cow milk sample was collected from the local area of Coimbatore, and processed for isolation of LAB (Figure 2.a). From the tested samples five bacterial cultures were isolated to draw conclusion about the resident lactobacilli. The microbial colonies were counted in the samples by standard plate count. *Lactobacillus bulgaricus* and *Lactobacillus casei* isolated were isolated from yoghurt, different kinds of cheese and a traditional food named 'tarhana' (a fermented food made of a mixture of cereal, yoghurt and thyme) (24).

All isolates of *Lactobacillus* sp were able to grow at 15^{o}C and 24% of the isolates were not able to grow at 45^{o}C. Lactobacilli are generally isolated on rich media such as MRS, which is routinely used for the isolation and counting of Lactobacilli for most fermented food products (25). Classification of *Lactobacillus* sp isolates from temperate regions according to their morphology, physiology and molecular characteristics (15). Few strains were able to utilize citrate and were found to be non motile, catalase, indole, VP negative, nitrates are not reduced and gelatin was not liquefied. MRS agar was suitable for bacteriocin assay of lactobacilli (26). The morphological characters of lactobacilli from raw milk, nature whey starter and cheese (27).

LAB isolated from rainbow trout of west Azarbaijan, Iran were Gram positive, catalase positive bacilli, were able to grow at 15^{o}C and 45^{o}C (28). The identified showed 80% or more similarly to the MTCC type cultures and showed variations in their sugar fermentation pattern.

18.75% of isolates from raw and fermented products like milk, curd, idli batter and pickle are *Lactobacilli* (29). The dairy products in Sudan rich source of *Lactic acid bacteria* (30).

Detection of antagonistic activity against *Xanthomonas campestris* using *Lactobacillus* sp Agar Spot Assay

In this study *in vitro* assay was carried to characterize the antimicrobial potential of the culture supernatant to inhibit some pathogenic bacteria. The LAB isolate was screened for bacteriocin producers by Agar spot assay test (16). Isolates inhibiting the indicator organism by clear zone of inhibition were selected as bacteriocin producers (Figure 3.a). Total inhibition diameter was calculated for each LAB strain as the sum of the inhibition diameter against the indicator strain. The antibacterial activities were done by an agar spot in which only 14.3% of strains made known to produce bacteriocin(31).

Agar Well Diffusion Assay

The inhibitory spectrum of the cell free supernatant fluid against Gram negative pathogens was widely varied. The antibacterial activity of LAB1 and LAB2 showed strong zone of inhibition (5mm in diameter) against *Xanthomonas campestris*. LAB3 was able to inhibit *X. campestris* showing 16mm in diameter. LAB4 showed that inhibitory activity at a range of 16.5mm in diameter. LAB5 strongly inhibited the organism showing zone of inhibition 26mm than all other isolates (Table 3 and Figure 3.b).

LAB isolated from fresh fruits and vegetables effective against the phytopathogenic and spoilage bacteria and fungi, *Xanthomonas campestris, Erwinia carotovora, Monilinia laxa* and *Botrytis cinerea* on apple fruits (32).

Isolates of LAB5 and LAB1 showed higher antibacterial activity under *in vitro* agar well diffusion method.

Bacteriocins form the pores in the membrane of sensitive cells and deplete the trans membrane potential and/or the pH gradient, resulting in the leakage of cellular materials (33; 34). The inhibitory effect was assumed to be bacteriocin and not due to H_2O_2 since there was no oxidizing effect on bacterial cells which destroy the molecular structure of cell protenis (35).

***In vitro and In vivo* Efficacy of *Lactobacillus* sp as Biocontrol, Plant Growth Promoting Bacteria**

In vitro trials were performed in MS medium at *in vitro* condition using LAB treated seeds CO1, K2. LAB treated seed revealed their ability to enhance plant growth when compared with control which was processed after one month. Seeds not treated with LAB (control) showed 8.25cm shoot length and 5.5cm root length. LAB5 treated seeds germinated than other seeds and their shoot length was 8.125cm and root length was 8.25cm. But no rapid increase in K2 variety when compared with control. Treated

plantlet doesn't show any symptoms of disease (Table 5, Figure 5 & 7).

In vivo trails were performed on pot at *in vivo* condition using LAB treated seeds such as CO1, K2. LAB applied as seed treatment show their ability to enhance plant growth compare with control. LAB5 treated seeds showed better germination when compared to the other LAB treated seeds. The shoot length was 6.6cm and the root length was 8.66cm found to be strongest enhancement than other treated seeds (Table 6, Figure 6 & 8).

LAB isolated from fresh fruits and vegetables were reported to be effective against the phytopathogenic and spoilage bacteria and fungi, *Xanthomonas campestris, Erwinia carotovora, Monilinia laxa* and *Botrytis cinerea* on apple fruits (32).

LAB with antifungal activity are well documented in food, meat and milk products as biopreservatives (36) while less attention has been paid to exploit the antifungal activity of LAB for biocontrol of phytopathogenic fungi.

The supension of *Lactobacillus plantarum* delayed the growth of *Aspergillus flavus, Fusirium graminearum, Rhizopus stolonifer* and *Botrytis cinerea* on cucumber (37). The inhibitory activity of *Lactobacillus plantarum* in both the cells and supernatant against fungal *C. gloeosporioides* is in agreement with previous studies (38; 39; 40).

Recently, it was found that tomato seeds treated with lactic acid bacteria reduced the growth of *Fusarium oxysporium* andimproved the growth of roots (41).

Table 1
Biochemical characterization of *Xanthomonas campestris* isolated from infected chilli leaf

S no	Isolate	Morphology	Gram reaction	Growth on nutrient agar with 5% glucose	Gelatin liquefaction	Starch hydrolysis	Casein hydrolysis	Nitrate reduction	Indole production	Oxidase	Catalase	Citrate utilisation	Species identified
1.	Organism isolated from infected leaf	Rods	-	+	+	+	+	+	+	+	+	+	*Xanthomonas capestris*

Table 2
Biochemical characterization of *Lactobacillus* sp isolated from raw cow milk

s.no	Isolates	Gram reaction	Morphology	Indole	MR	VP	Citrase	Catalase	Reduction	Gelatin	Species identified
1	LAB1	+	Rod	-	-	-	-	-	-	-	**Lactobacillus sp**
2	LAB2	+	Rod	-	-	-	-	-	-	-	
3	LAB3	+	Rod	-	-	-	-	-	-	-	
4	LAB4	+	Rod	-	-	-	-	-	-	-	
5	LAB5	+	Rod	-	-	-	-	-	-	-	

Table 3
Antibacterial activity of LAB against *Xanthomonas campestris*

S. NO	Isolate from chilli plant	Zones of inhibition (mm in diameter)				
		Isolates of *Lactobacillus* sp				
		LAB 1	LAB 2	LAB 3	LAB 4	LAB 5
1	*X. campestris*	15mm	15mm	16mm	16.5mm	26mm

Table 4
Chilli plant seeds experiment

S. No	Isolates of *Lactobacillus* sp	Zone of inhibition	
		CO1	K2
1	LAB1	5.8mm	5.4mm
2	LAB2	5.4mm	6.4mm
3	LAB3	6.8mm	7.2mm
4	LAB4	8.2mm	7.2mm
5	LAB5	8.8mm	7.6mm

Table 5
Growth and Statistical analysis- ANNOVA
In vitro germination

Treatments	Shoot length	Root length
Control	8.25±1.08	5.5±0.55
LAB3	6.5±1.5	6.83±0.25
LAB4	7±0.95	5.8±0.83
LAB5	8.125±1.05	8.25±0.25

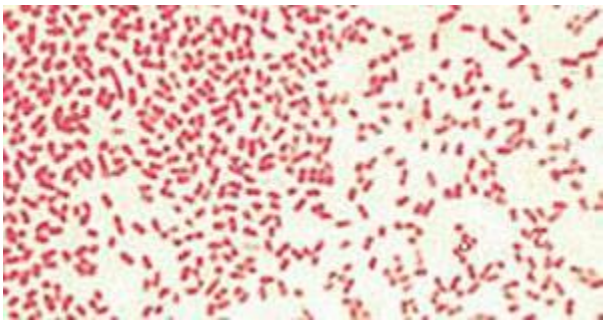
Results are mean values of three replicates determination ±SD

Table 6
In vivo germination

Treatments	Shoot length	Root length
Control	11± 17.	6±0.083
LAB3	3.88±1.	56±0.25
LAB4	6.60±0.93	7.5±0.25
LAB5	6.66±1.05	8.66±0.25

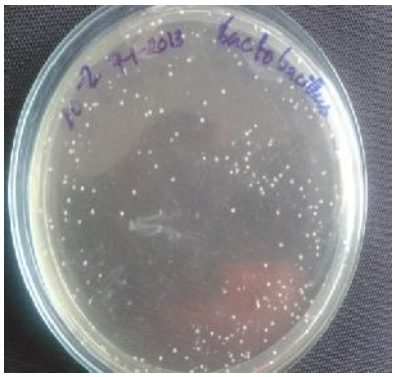
Results are mean values of three replicates determination ±SD

a. *Xanthomonas campestris* Colonies on Nutrient-Agar with 5% Glucose

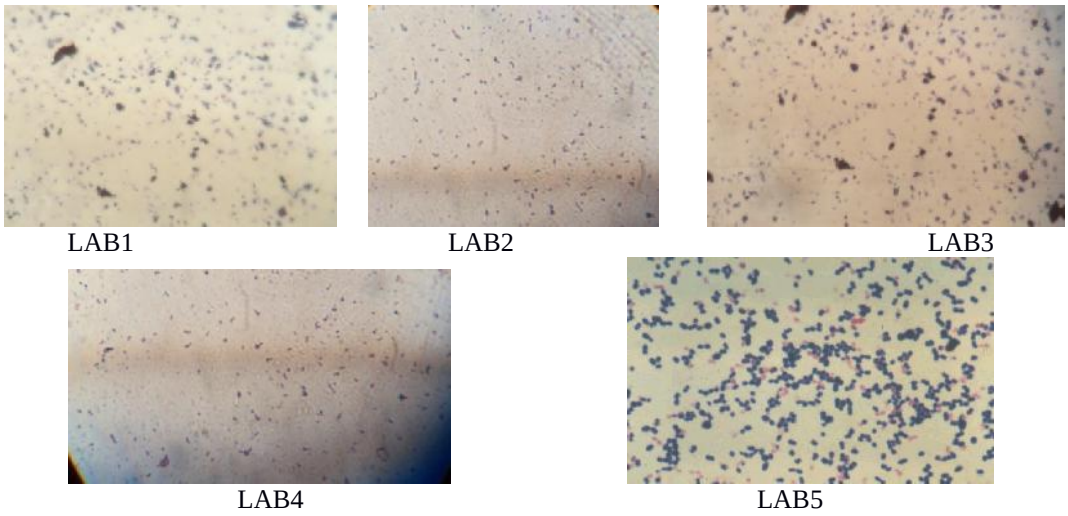


b. Gram staining of *Xanthomonas campestris*

Figure 1
Identification of *Xanthomonas campestris* from infected chilli leaf

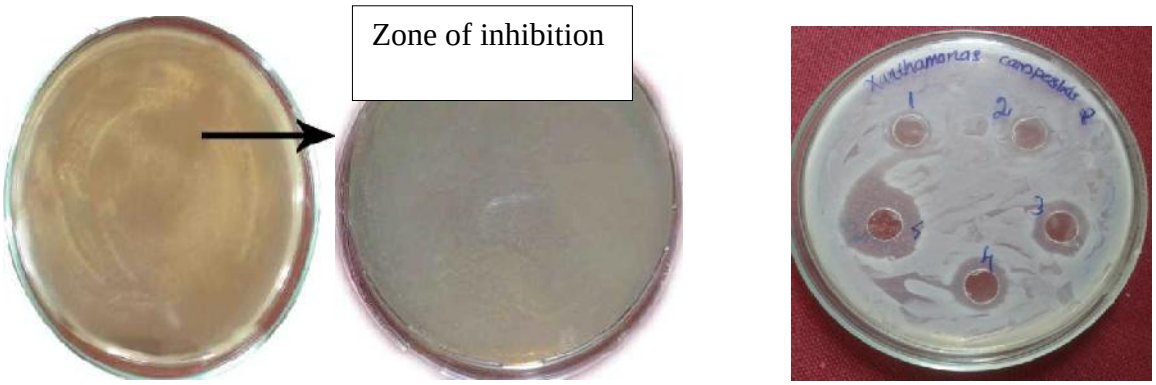


a. *Lactobacillus* sp colonies on MRS Agar media



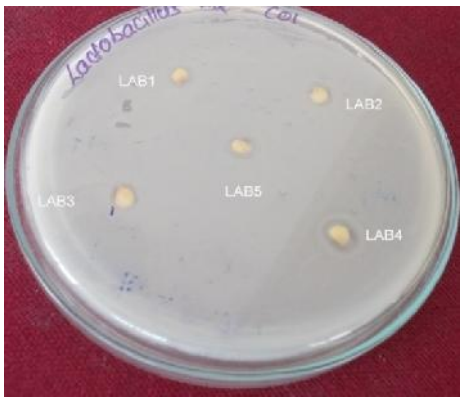
b. Gram staining of *Lactobacillus* sp

Figure 2
Identification of *Lactobacillus* sp from raw cow milk

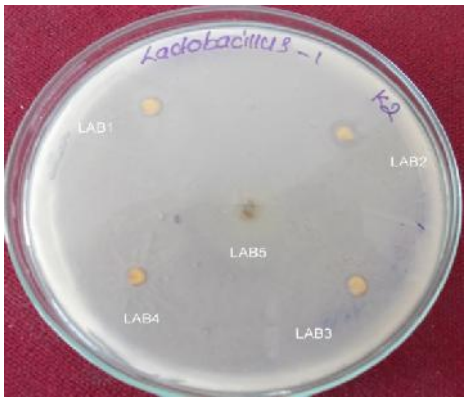


a. Agar spot assay method **b. Agar-well assay method**

Figure 3
Antagonistic activity of *Lactobacillus* sp against *Xanthomonas campestris*



4.1 Activity of LAB treated chilli seed CO1 against *Xanthomonas campestris* LAB treated CO1 seeds



4.1 Activity of LAB treated chilli seed CO1 against *Xanthomonas campestris* LAB treated CO1 seeds

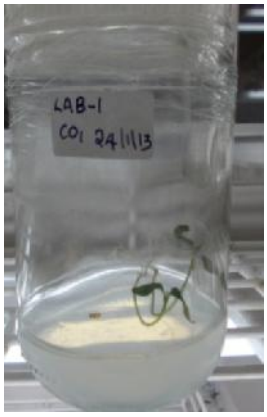
Figure 4

***In vitro* and *in vivo* efficacy of LAB act as Biocontrol, Plant growth promoting Chilli plant seeds treatment**

Control CO1

1. LAB3 treated CO1

2. LAB4 treated CO1



3. LAB5 treated CO1

Figure 5

***In vitro* germination of chilli seed CO1**

control

1.LAB3 treated CO1

2.LAB4 treated CO1

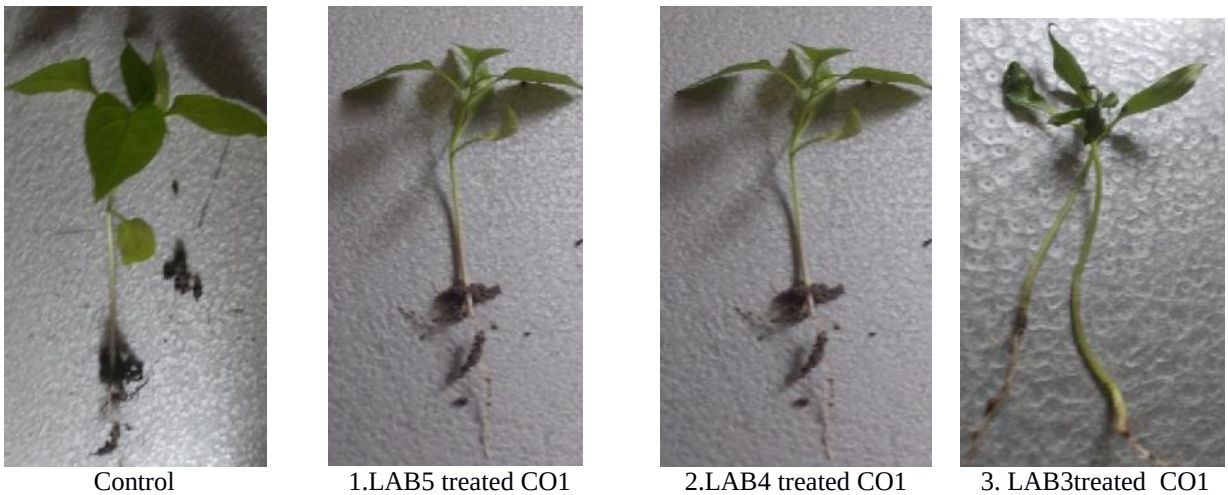
3. LAB5 treated CO1

Figure 6

***In vivo* germination of treated and control CO1 chilli seeds**

Control 1. LAB3 treated CO1 2. LAB4 treated CO1 3.LAB5 treated CO1

Figure 7
***IN VITRO* GERMINATION - SHOOT AND ROOT LENGTH**
CO1 variety of chilli plantlet



Control 1.LAB5 treated CO1 2.LAB4 treated CO1 3. LAB3treated CO1

Figure 8
***IN VIVO* GERMINATION- SHOOT AND ROOT LENGTH**
CO1 variety of chilli plantlet

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