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# Antagonistic potential and plant growth promoting traits of *Achromobacter Xylosoxidans* isolated from tannery sludge sample

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**ABSTRACT**

*In the present study, the bacterium Achromobacter xylosoxidans was isolated from tannery effluent sludge sample in Dindugal, Tamil Nadu, India and screened for its bio-control efficacy against selected plant pathogens viz., Alternaria solani, Curvularia lunata and Fusarium oxysporum under in vitro. It was observed that maximum percent inhibition of mycelial growth of C. lunata (95%), followed by A. solani (85%) and F. oxysporum (80%). An attempt was also made for testing its plant growth promoting traits such as production of Indole acetic acid (IAA), phosphate solubilization efficacy, Hydrogen cyanide (HCN), ammonia and catalase production. Significant amount of plant growth promoting traits were noticed. Further studies on mechanism of bio-control against plant pathogens and plant growth promoting activities of Achromobacter xylosoxidans need to be conformed under in-vivo condition.*

**Key words**: Antagonism, *Achromobacter xylosoxidans, Alternaria solani, Curvularia lunata, Fusarium oxysporum*

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## INTRODUCTION

Plant diseases can significantly diminish the growth and yield or reduce the usefulness of a plant or plant product. Healthy or normal plants develop, and function to the maximum of their genetic potential. Diseases may interfere with absorption and translocation of water and nutrients from soil to various parts of the plant, reduce photosynthetic efficiency of plant parts or translocation of photosynthetic products through the plant and their storage, or may interfere with flowering or fruiting and seed formation and reducing growth, yield and economic or aesthetic value of a plant or plant product. Pathogenic microorganisms, particularly plant pathogenic fungi, affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide [1].

Man's attempts at controlling plant disease go back atleast to 700 B.C. At present chemical fungicides and pesticides are applied in order to bring control over infections. But these usages of chemicals have not only killed the targeted ones but also our useful, friendly microbes, thus destroying the soil fertility and environment. The recent development in organic farming, has bought with it, the usage of bio-fertilizers, bio-pesticides or microbial pesticides.

Bio-control involves disease suppressive microorganisms to improve plant health. Control of plant pathogens in biological ways is gaining prime importance as an eco-friendly agricultural and forestry practices. Inhibition of plant diseases with chemicals is being limited as the result of increasing public concern related to the danger for livelihoods and entering of resistance strains to environment. Thus, developing microbial control agents is another way to manage plant diseases is of high priority. Various plant associated bacteria can positively impact plant health and functioning in a variety of ways [2].

The plant growth promoting rhizobacteria (PGPR) and biological control agents (BCAs) impart beneficial effects on plants through direct and indirect mechanisms. The PGPR directly serve the plants by supplying nutrients through fixation of atmospheric nitrogen ($N_2$), phosphorous (P) solubilization, segregation of iron (Fe) by siderophores, phytohormone synthesis (e.g. indole-3-acetic acid- IAA) and lowering the hosts ethylene level due to ACC deaminase activity. The BCAs support plant health via the suppression of plant pathogens, due to competition for nutrients and space; synthesis of antimicrobial compounds; parasitism or induction of systemic resistance in host plants [3].

Podile and Kishore [4] identified bacteria of diverse genera as PGPR, of which *Bacillus* and *Pseudomonas* species are predominant. In both managed and natural ecosystem, beneficial plant associated bacteria play a key role in supporting and increasing plant health and growth. Several rhizobacterial strains of *Pseudomonas* spp, *Burkholderia* spp, *Enterobacer* spp, *Alcaligenes* spp, *Bacilus* spp, etc are widely used to increase plant growth and act as bio-control agents against plant pathogens [5].

Many earlier studies revealed the beneficial functions of *Achromobacter* sp. including stimulation of ionic transport to promote plant growth [6], production of enzyme (glutaryl-3-deacetoxy-7-aminocephalosporanic acid acylase) which is essential in the production of antibiotic cephalosporin [7] and its inhibitory activity against aflotoxin produced by *Aspergillus* spp,[8]. Hence, in the present study, an attempt was made to isolate and identify the beneficial bacteria from tannery effluent sludge samples collected from Tannery Effluent Treatment Plant in Dindugal, Tamil Nadu and test its antagonistic potential against selected plant pathogens and its plant growth promoting potential under *in vitro.*

## MATERIALS AND METHODS

### Sample collection

Tannery effluent sludge samples were collected from Tannery Effluent Treatment plant at Dindugal, Tamil Nadu in zib lock polythene covers, sealed tightly and immediately transported to laboratory. The samples were kept in refrigerator at 4ºC until further use.

### Isolation of bacterial strains from tannery sludge sample

Serial dilution and plating techniques as described by [9] was adopted for enumerating the population of bacterial isolates from the tannery sludge samples in laboratory. Based upon morphology and 16S rDNA sequence the bacterial isolate was identified as *Achromobacter xylosoxidans.* The sequence of this isolate with all the required information was submitted to European Molecular Biology Laboratory (EMBL) and accession number was obtained.

### Fungal pathogen used for study

The major fungal pathogens used in present study were *Alternaria solani*, *Curvularia lunata* and *Fusarium oxysporum.* The pathogens were taken from germplasm of Pathology Laboratory of Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, India. All the cultures of the pathogens were grown in PDA medium and incubated at 28$^{o}$ C for 5 days and stored for further use.

### *In vitro* antagonism test

Dual culture method was adopted to determine antagonistic potential of the bacterial isolate, *Achromobacter xylosoxidans* as described by [10]. The effect of potential bacterial antagonist on mycelial growth of selected fungal pathogens *viz., A. solani, C. lunata* and *F. oxysporum* was studied. One loop full of bacterial culture was streaked on opposite side of the PDA plate and a 6 mm five day old fungal culture plug was kept at the center of the PDA plate and incubated at 28°C for 5 to 7 days. Inhibition was scored by measuring the reduction of fungal mycelial growth after 3, 5 and 7$^{th}$ day intervals. Percentage of inhibition was calculated using the formula as under:

Percent of Inhibition= C – T/ T x 100
Where, C is the mycelial growth of fungi in control plate and T is the mycelial growth of fungi in treatment.

### Study on plant growth promoting traits

### Screening of bacterial isolates for its Indole-3- acetic acid (IAA) production

The ability of bacterial isolate to produce IAA was determined qualitatively and on Nutrient broth amended with tryptophan (0.1 g/l). The culture was incubated in dark at 30$^{o}$C for 7 days, and then, the culture was centrifuged at 3000 rpm for 30 minutes. Two ml of the supernatant was mixed with 2 drops of orthophosphoric acid, 4 ml of Salkowski's reagent (50 ml, 35% perchloric acid; 1 ml 0.5 M $FeCl_3$), and the mixture was allowed to stand for 1 hour in dark as described by [11] and the formation of pink colour indicates the production of IAA.

**Evaluation of phosphate solubilizing ability of bacterial isolates**

The isolates were screened for phosphate solubilization as per methodology described by [12]. Pikovskaya agar medium was used for point inoculation and incubate it at 30°C for 3-4 day. These isolates were stabbed in triplicate using sterile toothpicks. The halo zone around the colony was presumptive confirmation of phosphate solubilization and was measured after 7 days of incubation.

**Analysis of Hydrogen cyanide production in bacterial isolates**

According to [13] hydrogen cyanide (HCN) production of bacterial isolates was evaluated. Bacterial isolate was streaked on King's B agar medium amended with glycine. Whatman No.1 filter paper soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed in the lid of each petri plate. The plates were then sealed air-tight with parafilm and incubated at 30°C for 48 h. A colour change of the filter paper from deep yellow to reddish-brown colour was considered as an indication of HCN production.

**Screening of ammonia and catalase production**

The bacterial isolate was tested for the production of ammonia as described by [14]. Overnight grown bacterial culture was inoculated in 10 ml peptone broth and incubated at 30°C for 48 h in incubator shaker. After incubation 0.5 ml of Nessler's reagent was added. The development of faint yellow to dark brown color indicated the production of ammonia.

Catalase test was performed by 3% hydrogen peroxide, it was added to 48 h old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The effervescence indicated catalase activity.

## RESULTS AND DISCUSSION

**Antagonistic effect of *Achromobacter xylosoxidans* against fungal pathogen**

Fungal pathogens are responsible for severe damage on many economically important plant species. Three fungal plant pathogens *viz., A. solani*, *C. lunata* and *F. oxysporum* were used in the present study. The bacterial isolate, *Achromobacter xylosoxidans* (LK391696) was tested for its bio-control efficacy against these selected fungal plant pathogens under *in vitro*. The results of the study revealed that the growth of all fungal pathogens was inhibited by the bacterium, *A. xylosoxidans* but variation in percent inhibition. It was found that 95% of inhibition of *C. lunta*, 85% of *A. solani* and 80% of *F. oxysporum (*Figs. 1 and 2). The findings are in accordance with the research work carried out by other researchers. The fungal wilt caused by *Fusarium oxysporum* shows 23.8 to 72.1% of disease reduction in nursery condition by dipping the roots of plant in *Achromobacter xylosoxidans* culture [15]. The siderophore produced by *Achromobacter* sp. can induce bio-control efficacy by the determinant of Induced Systemic Resistance (ISR) in plants [16]. The biocontrol nature of a bacterial isolate, *A. xylosoxidans* was due to the production of chitinase enzyme was reported by [17].

**Figure 1: Percentage of inhibition of plant pathogenic fungal mycelium**

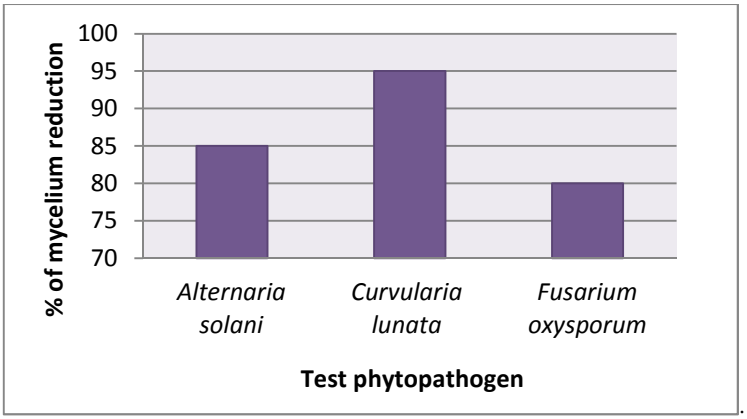
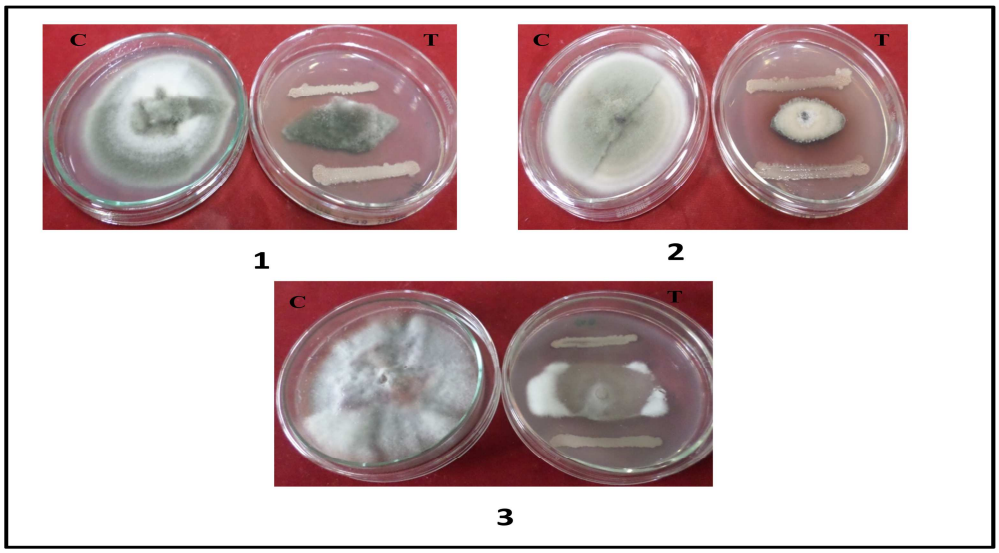


**Figure 2: Effect of *Achromobacter xylosoxidans* on the growth of plant pathogenic fungi**




***C*- *Control*, *T*- *Test***

*Mycelium reduction of **1**- Alternaria solani, **2**- Curvularia lunata **3**- Fusarium oxysporum with Achromobacter xylosoxidans as antagonistic bacteria*

**Plant growth promoting traits**

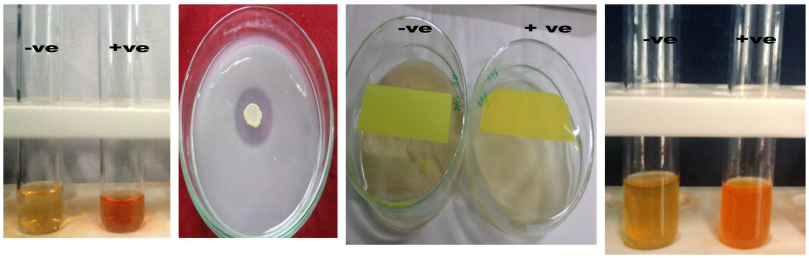
The bacterial isolate, *A. xylosoxidans* (LK391696) was tested for its plant growth promoting activity under *in vitro*. Data on IAA production, Phosphate solubilization efficacy, and production of Ammonia, HCN and Catalase were reported and presented in Table 1 and Figure 3. All the plant growth promoting traits were found to be positive for the tested organism, and the findings of the experiments are corroborated with the findings made by other researchers. Jha and Kumar, [18] isolated *A. xylosoxidans* as plant growth promoting endophytic bacteria from wheat and identified as diazotrophic bacteria which showed considerable level of nitrogenase activity, IAA production and P solubilization ability. The five of the six pathways for auxin biosynthesis in bacteria rely on tryptophan as the main IAA precursor was described by [19-20]. On the other hand, phosphate solubilizing bacteria could convert insoluble phosphates into available forms for plant via the process of acidification, chelation, exchange reactions and production of gluconic acid [21-22].

**Table1: Plant growth promoting traits of *Achromobacter xylosoxidans* (LK391696)**

| Sl.No. | *Achromobacter xylosoxidans* | |
|---|---|---|
| | Plant Growth Promoting activity | Result |
| 1 | IAA production | +++ |
| 2 | Phosphate solubilization | +++ |
| 3 | Hydrogen cyanide production | ++ |
| 4 | Ammonia production | +++ |
| 5 | Catalase production | +++ |

*IAA = Indole acetic acid, +++ = highly presence, ++ = moderately present*

**Figure 3: Plant growth promoting activity of *Achromobacter xylosoxidans*(LK391696)**




*1- IAA production, 2- Phosphate solubilization, 3- Hydrogen cyanide production, 4- Ammonia production*

## CONCLUSION

In the present investigation, bacterial isolate of *Achromobacter xylosoxidans* was isolated from tannery effluent sludge samples and tested their bio-control efficacy against selected plant pathogens and it shows promising antagonistic ability. Further studies are needed to inoculate the bacterial isolate in nurseries and field conditions in order to determine its potential bio-control efficacy.

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