# New report of endophytic bacterium Achromobacter xylosoxidans from root tissue of Musa paradisiaca

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**Short Report**

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# Abstract

Banana plant (*Musa paradisiaca*) worldwide cultivated crop harbors many endophytic bacteria. Endophytic bacterium (MRH 11) isolated from root tissue of *M. paradisiaca* was identified as *Achromobacter xylosoxidans* (ON955872) which showed positive response in IAA production, phosphate solubilization, catalase production. *A. xylosoxidans* also showed *in vitro* antagonism against *Curvularia lunata* causing leaf spot disease of banana.

# Full Text

Endophytic bacteria are those that live inside plant tissues without producing any visible symptoms of infection or having an adverse effect on their hosts (Schulz and Boyle 2006). Endophytic bacteria are very crucial to their host plants and benefit them in a variety of ways, including (i) aiding in plant nutrient uptake (Prieto et al. 2017), (ii) protecting host plants against numerous pests and pathogens (Firdous et al. 2019, White et al. 2019,Verma et al. 2018).Banana plants (Musa paradisiaca) root samples were collected in various places in the District of Uttar Dinajpur. Using a GPS location app on a mobile device, location data of this banana fields were also recorded at the same time. The root samples were cut into small pieces (1 cm) and transferred into Nutrient Agar (NA) slant after surface sterilization with 0.1% $HgCl_2$( 2 min ), followed by 70% ethanol ( 2 min). A bacterium (MRH-11) was isolated from root pieces. The scanning electron microscopic image of bacterial isolate (MRH 11) was taken(Fig. 1A) and 16S rDNA fragment sequence was found to be *Achromobacter xylosoxidans* and was deposited to GenBank with accession number ON955872. The evolutionary history of *Achromobacter xylosoxidans* was inferred by using the Neighbor-Joining method (Saitou and Nei 1987).The phylogenetic tree is shown inFig. 2. This evolutionary analysis was conducted by using MEGA11 (Tamura K et al. 2021). This analysis involved 23 nucleotide sequences and atotal of 1588 positions in the final dataset. The bacterium showed phosphate-solubilising activity by producing a clear zone on the Pikovskaya's (PKV) medium(Fig. 1B). *A. xylosoxidans* was also positive in IAA production and catalase production (Table 1). IAA production, phosphate solubilisation, hydrogen cyanide production, ammonia production, catalase production by *A.xylosoxidans*has also been reported (Devi and Mohan, 2015).

A fungus (MLP-01) was isolated from the infected leaf part of banana plant onto potato dextrose agar plate ( an antibiotic Monocef is added in the media ) . The infected leaf had lesions which were yellowish brown in coloured and scattered in leaf surface. This disease of leaf spot was observed in 20 plants out of 30 in a field located at Sarai, Raiganj. The leaf spot disease in banana from Sarai, Raiganj was first reported by Chowhan and Chakraborty (2022) and Koch's postulate was successfully established for the pathogen causing leaf spot disease in banana. 18S rDNA fragment sequence of strain MLP-01 was deposited on GenBank with accession number ON246070. The fungus was morphologically identified as *Curvularia lunata* by Agharkar Research Institute, Pune, India with Accession number NFCCI 5361. The fungal colonies on malt-extract agar (MEA) at 25±2$^{\circ}$C after 15 days, were fast growing, dark brown in colour, reverse oblivaceous brown, stroma were not observed. Mycelia of the fungus were olivaceous black or brown, condiophores mononematous and macronematous, conidia without protuberant hilum, 3 septate, smooth walled curved and asymmetrical, dark brown in colour, 18-30   9-15 μm.

*A.xylosoxidans* showed *in vitro* antagonism against *C. lunata* (Chowhan and Chakraborty 2022) causing leaf spot disease of banana from Raiganj, Uttar Dinajpur. *A.xylosoxidans* also inhibited the growth of the tested pathogen markedly (Fig.1C). The tested fungus was inhibited (83.5%) by *A.xylosoxidans* (Table 2). Similar antimicrobial activity of *A.xylosoxidans*-an endophytic bacterium of banana against banana soft rot pathogen *Pectobacterium carotovorum* subsp*. carotovorum* was reported by Ragavi et al. (2019). The scanning electron microscopic image of the transition zone between mycelia of *C. lunata* and cell of *A.xylosoxidans* during *in vitro* study was analyzed (Fig.1 D). These findings can help to boost growth of banana plant and to develop strategies to manage leaf spot disease.

# Declarations


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### Conflict of Interest

Authors declare that there are no conflict of interest .

### Compliance with Ethical Standards

#### *Disclosure of potential conflicts of interest*

The authors declare that they have no conflicts of interest.

#### *Research involving human participants and/or animals*

This article does not contain any studies with human participants or animals performed by any of the authors.

#### *Informed consent*

No informed consent is required.

### Data Availability Statement

No datasets were generated or analyzed during the current study.

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# Tables

**Table 1:** *In vitro* Plant growth promoting traits of *Achromobacter xylosoxidans* (ON955872)

| **PGP activities** | *(Achromobacter xylosoxidans*) |
|---|---|
| IAA production | **+** |
| HCN production | **-** |
| Catalase production | **+** |
| Phosphate solubilisation | **+** |

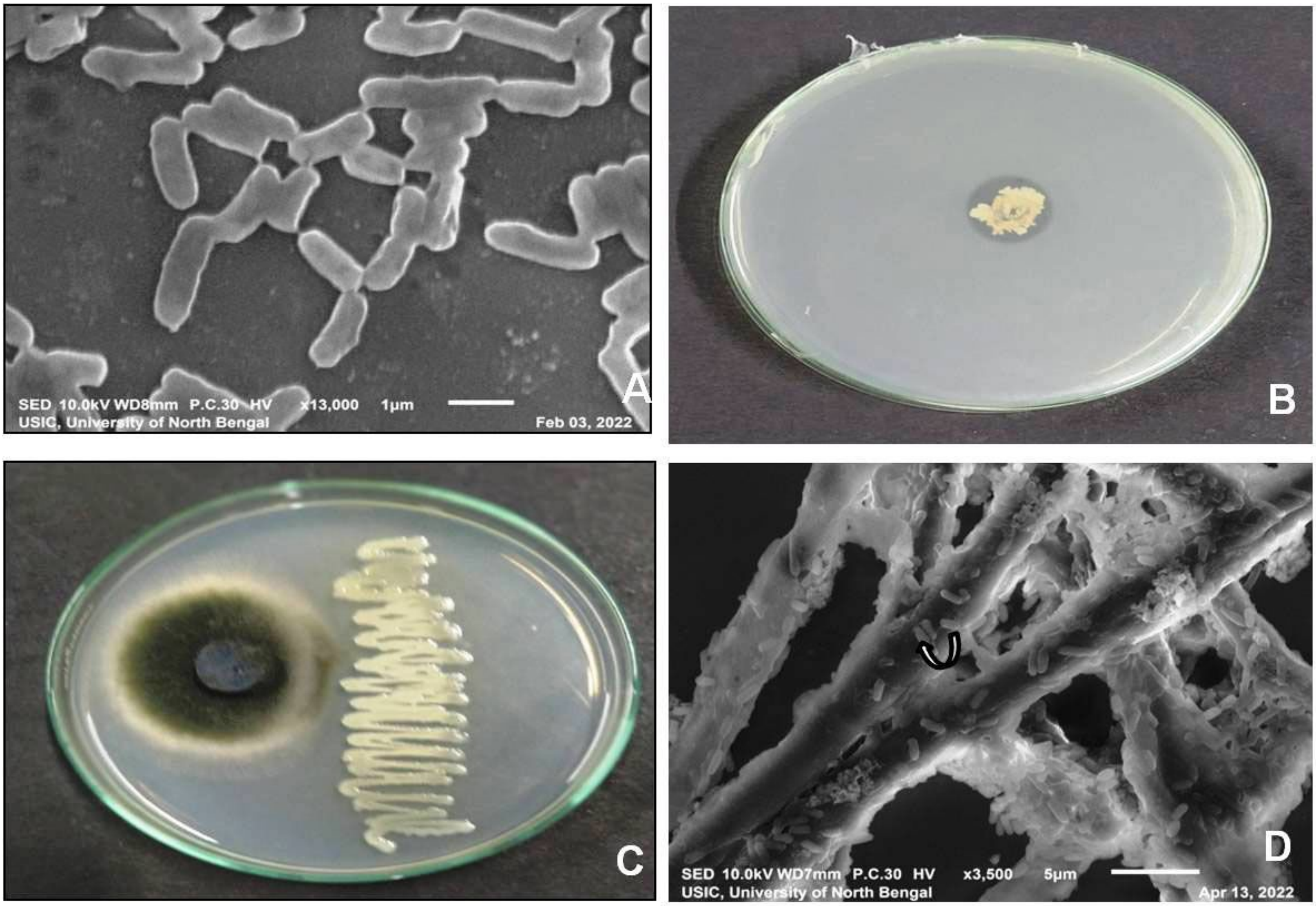
***+= activity present; - = activity absent***

**Table 2.** *In vitro* antagonistic tests of *Achromobacter xylosoxidans* (ON955872) against *Curvularia lunata* (ON246070)

| Test fungi | Paired with | Dia. of fungal growth (cm)* | Zone of inhibition (cm) | % of inhibition |
|---|---|---|---|---|
| *Curvularia lunata* (ON246070) | - | 8.8±1.2 | - | - |
| | *Achromobacter xylosoxidans* (ON955872) | 1.5±0.09 | 2.0±0.24 | 83.5±2.65 |

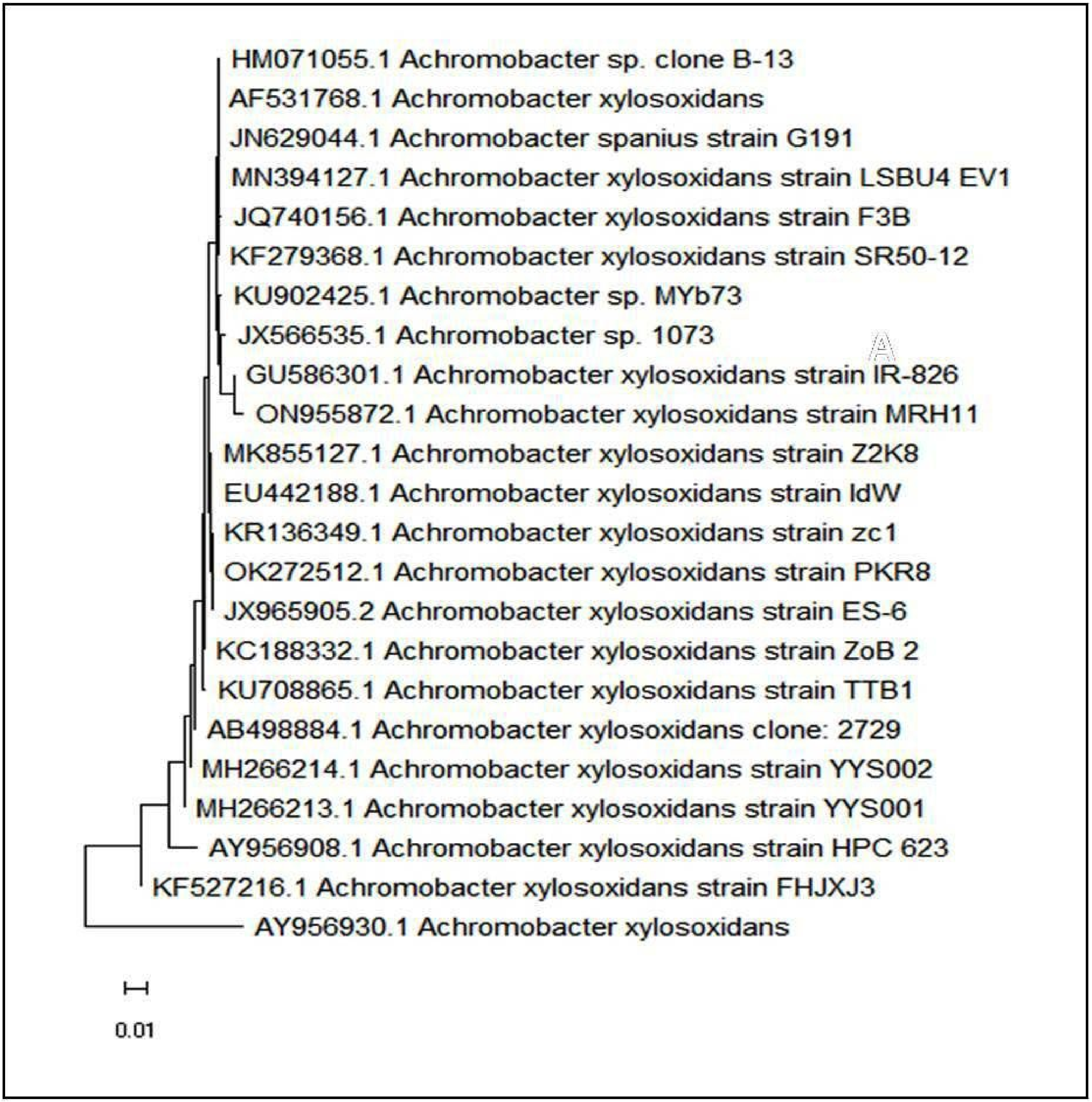
***After 7 days; ±= SE***

# Figures




### Figure 1

Scanning Electron Microscopic view of *Achromobacter xylosoxidans* (A); *In vitro* PGP activities of *A. xylosoxidans*- phosphate solubilization positive (B); Antagonistic plate (C) and Interaction (transition) zone between mycelia of *C. lunata* and cell of *A. xylosoxidans* under scanning electron micrograph (D)




## Figure 2

Molecular identification of the bacterial isolate (MRH 11) and phylogenetic analysis of *Achromobacter xylosoxidans*(ON955872) was performed with other ex types of *Achromobacter xylosoxidans* by Neighbour Joining (NJ) method