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# Identification Of A New Strain Of Xanthomonas Translucens Pv. Unduolsa Parasitizing On Wheat Crop

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**ABSTRACT**

Current study aimed to detect the spreading and virulence of leaf streak disease on wheat in some sites of Najaf province during 2020 season. Pathogenicity test, morphological, chemo-biological and molecular identification of the pathogenic bacteria was conducted. Results of field survey and the spreading of the disease were varied as the percentage of infection in was estimated between 24 and 49%, disease severity between 23.14% and 46.13%. The outcome of pathogenicity test indicated that among 47 isolates tested, 19 showed same symptoms of leaf streak disease and varied in their pathogenicity as the isolate 3 was exceeded other Xanthomonas translucens isolates and reached 46.13%. Identification tests were confirmed that the 19 isolates grown on the specific medium XTS were pale yellow with a dark brown pigment, gram negative and can grow on NA medium as the colonies appeared yellow and glossy opaque. The chemo biological tests showed that isolates were positive to KOH solubility, Aesculin hydrolysis, Catalase, Gelatin and Motility tests and negative to Hydrolysis of starch and Oxidase tests. The identification of bacteria was confirmed by PCR and based on 16S rRNA when new strain of X. translucens was registered in GenBank (NCBI) under the accession number OK287049. Results of current study were beneficial to highlight the spread of bacterial leaf streak disease on wheat in Iraq.

**Key words:** Xanthomonas translucens, wheat, disease severity, leaf streak.

## INTRODUCTION

Bacterial plant pathogens pose a serious threat to both agriculture and human health worldwide; in addition to the emergence of new pathogens and continuous evolution of existing species and the pathogens of humans and animals are often the main focuses of research due to their adverse impact on public health. However, it is also necessary to study the bacterial plant pathogens that threaten agricultural production (Schaad et al 2001; Sheppard et al 2013). In recent years, agriculture has suffered great economic losses and reduced crop production due to outbreaks of diseases caused by bacterial plant pathogens (Borkar and Yumlemban 2016). The mechanism of entry of bacteria to plant host is occurred through stomata or wounds and their spread by raindrops, contact with plants or by insects which cause secondary infection during the season. Also, seeds can be contaminated with bacteria at the end of the season and then return to the soil with the residues (Prescott et al 2020). The infection can develop and the leaves become infected, where they appear in the form of water spots and then become dead which similar to the symptoms of bacterial blight, and the seeds wrinkle at their bases and often fail to germinate. Infection with the bacteria leads to the delay of spikes maturity or it becomes not productive especially if they are infected before the flowering

stage and sticky secretions appear on spikes in humid atmospheres as transparent droplets, the bacteria can survive in plant residues and soil tolerate wide ranges of temperature and humidity (Pesce et al 2017). The seeds of infected plants play a major role in spreading the pathogen and considered a source of primary infection. The straw produced from harvesting the infected wheat turns black as the disease is called streaked leaves that are initially reddish in color and then become dark or black during different environmental conditions such as availability of moisture or vertical and spray irrigation in tropical areas or warm areas during the day and cold at night (Vauterin et al 1995; Khan et al 2021). The virulence factors of bacteria that use the third type of secretory system T3SS are responsible for the influences inside plant cells, as the factors determining the virulence of pathogenic bacteria can be genetic or structural which enables them to cause the disease. The relationship between the pathogen and plant host is a dynamic movement, as each tries to change the activities of the other and result in the infection process depends on the pathogen's virulence which is a measure of the degree of damage causes to the host and the degree of its resistance or sensitivity that depends on the effectiveness of its defense mechanisms (Sharif 2012; Endrawati et al 2021).

Thus, this study aimed to detect the bacteria that cause leaf streak disease on wheat X. translucens isolated from seeds and identified new strains of this pathogen in Iraq.

## MATERIALS AND METHODS

### The detection of bacterial leaf streak disease on wheat

A field survey was conducted in three regions of Najaf province including Meshkab, Kamassy and Abasia which commonly grow wheat during 2020 season and the percentage of infection was estimated in those regions, while the severity of disease was estimated according to Duveller et al (2002). Samples of seeds were collected from seeds stores randomly and put in plastic bags with labels then kept in the refrigerator at $4^0C \pm 1$ until used.

### X. translucens bacteria

The bacteria was isolated from seeds by making a solution contains 250ml of NaCl and 0.02% of Tween 20 then 120g of wheat seeds was added, all contents were mixed very well for 5 minutes then left for 1 minute. Sterile tubes containing 9ml of sterile distilled water was prepared and 1ml of water that taken from washed seeds was added then a series of dilutions was made. 1ml of last dilution was added to Petri plates contains NA medium with slowly movement then incubated at $30^0C$ for three days. The single colonies were purified on Petri plates containing specific medium XTS using streak method to ensure the growth of the pathogenic bacteria (Duveller et al 2002).

The bacteria was isolated from wheat leaves by sterile the surface of wheat leaves samples by using 10% of commercial sodium hypochlorite (1% free chlorine) for 1 minute. Samples were washed with sterile water then filter paper was used to get rid of water. Leaves were put on glass slides and a few drops of water were added then leaves were crashed and left until the appearance of bacteria in water drops. Fresh plates containing Nutrient Agar were inoculated with water drops contaminated with the pathogenic bacteria then incubated after that, single colonies were purified on Petri plates containing specific medium XTS using streak method (Fostr and Schaad 1985; Surai et al 2019).

### Pathogenicity test

14 days old wheat plants (Adana cultivar) were inoculated with $5.4*10^6$ CFU/ml of bacterial inoculation by injection the top surface of leaves for many times with sterile needle then the bacterial inoculation of 19 isolates was sprayed using small sprayer with three replicates for each isolate and control treatment (sterile water only) then the results were collected after 10 days of inoculation (Duveller et al 2002; Sharif 2012).

**Chemo-biological tests**

KOH solubility, Aesculin hydrolysis, Catalase, Gelatin, Motility tests, Hydrolysis of starch and Oxidase chemo-biological tests were conducted to identify the pathogenic bacteria (Azhar et al 2013).

**Molecular identification of X. translucens bacteria**

DNA of X. translucens 3 bacteria was extracted using Favour Prep TM Genomic DNA Mini Kit prepared by Intron company (Korea) and the gen **rRNAS16** was multiplied using '3-AGAGTTTGATCCTGGCTCA -5' primer as forward and '3-GGTTACCTTGTTACGACTT -5' as reverse. PCR products were obtained using gel electrophoreses (1% agarose gel) at 100v for 60min then it visualized by UV and sent to Macrogen (Korea) to do the sequencing.

**RESULTS**

**The detection of bacterial leaf streak disease on wheat**

The field survey showed that the bacterial leaf streak disease on wheat was spreading in 13 sites of the tree regions studied (Meshkab, Kamassy and Abasia) when symptoms appeared on leaves and the infection percentage rated between 24 to 49%, as Kamassy region was recorded the highest infection rate followed by Meshkab (39%), while the lowest percentage of infection was recorded in Abasia (24%) and these infection rates causes an economic losses on wheat crop (Table 1).

**Table 1.** Severity of infection of X.translucens pv.unduolsa on wheat leaves in studied sites.

| Region | Infection rate |
|---|---|
| Kamassy | 27% -49% |
| Meshkab | 26% - 39% |
| Abasia | 24% - 32% |

**Pathogenicity test**

The pathogenicity indicated that among 47 isolates tested (obtained from seeds), 19 showed symptoms of leaf streak disease on wheat (Adana 99 cultivar), while, the other 28 isolates were nonpathogenic on wheat (Table 2). Symptoms of disease were appeared on leaves after 10 days of inoculation with$5.4*10^6$ CFU/ml of bacterial suspension. There were significant differences between studied isolates in term of disease severity on inoculated wheat plants as it reached 43.75 to 46.13% respectively in the most of isolates and the lowest disease severity was recorded in X. translucens 9 isolate which amounted 23.14%. The rest of isolates were graded in their ability to cause infection through the size of symptoms on leaves compared to the control treatment (Fig 1).

**Table 2.** The pathogenicity of X.translucens pv.unduolsa stains isolated from seeds and leaves of wheat after 10 days of inoculation.

| Isolate number | Disease severity % | Isolate number | Disease severity % |
|---|---|---|---|
| Control | 0.00 | X.t 22 | 34.27 |
| X.t 1 | 30.41 | X.t 24 | 36.32 |
| X.t 3 | 46.22 | X.t 28 | 43.26 |
| X.t 5 | 33.04 | X.t 29 | 33.68 |
| X.t 6 | 42.22 | X.t 30 | 36.55 |
| X.t 9 | 37.22 | X.t 32 | 38.35 |
| X.t 10 | 25.30 | X.t 35 | 30.65 |
| X.t11 | 40.15 | X.t 38 | 35.42 |
| X.t 13 | 39.05 | X.t 40 | 35.35 |
| X.t 17 | 22.46 | X.t 44 | 27.22 |
| **L.S.D (0.05) = 0.82** | | | |

**Fig 1.** Symptoms of bacterial leaf streak disease on wheat leaves.

**Microscopic and chemo-biological tests**

Results of microscopic test of the 19 bacterial isolates showed bacilli bacterial cells gram negative (Fig 2), while, the chemo-biological tests showed that isolates reacted positively in KOH solubility test, Aesculin hydrolysis, Catalase, Gelatin and Motility test, negatively in Hydrolysis of starch and Oxidase test (Fig 3).

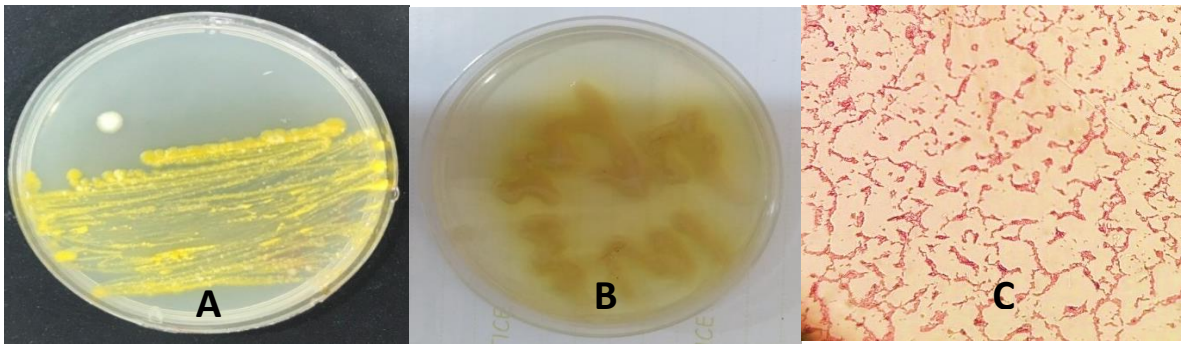


**Fig 2.** Morphological and microscopic characteristics of bacterial cells and colonies of Xanthomonas.

A- The growth of Xanthomonas on XTS medium after 48h.

B- The growth of Xanthomonas on Nutrient Agar medium after 48h.

C- Microscopic characteristics of bacterial cells of Xanthomonas under X1000.

**Table 3.** Chemo-biological tests of the 19 isolates of Xanthomonas translucens bacteria.

| Motility | Hydrolysis of starch | Aesculin hydrolysis | KOH solubility | Hydrolysis of Gelatin | Catalase | Oxidase | Gram stain |
|---|---|---|---|---|---|---|---|
| + | - | + | + | + | + | - | - |

*(+) = Positive (-) = Negative

**Molecular identification of X. translucens bacteria**

X. translucens 3 bacteria was identified molecularly based on S16 (rRNA) when the results showed the appearance of a bundle of 800 base pairs (Fig 3) which confirmed that this sequence belongs to X. translucens. The analysis of DNA sequence showed that a new strain of X. translucens was obtained and registered in GenBank (NCBI) under the accession number OK287049.

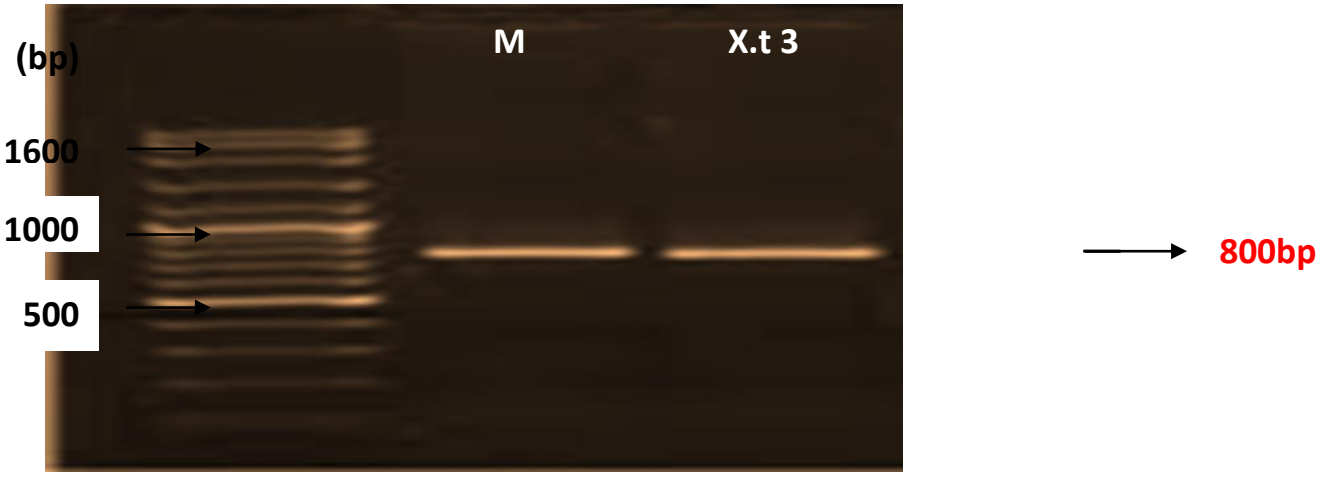


**Fig 3.** Gel electrophoreses (1% agarose gel) at 100v for 60min for S16 (rRNA) of X. translucens 3.

M= 100 bp DNA ladder marker.

X.t3= S16 (rRNA) of X. translucens 3.

**DISCUSSION**

Current study highlighted the infection of wheat by bacterial leaf streak disease in Iraq due to the lack of knowledge about this pathogen, in addition to wide spread of disease in the three regions in Najaf province particularly Kamassy. The development of disease on wheat leaves occurred due to the temperature and moisture at wheat growing season which leads to fill plant tissues with water that considered essential to the development of disease (Duveiller et al 2002). The variation in the severity of infection may be attributed to the repeating cultivation for successive years and the contamination of seeds by bacteria which considered primary infection source (Pesce et al 2017; Prescott et al 2020).

X.translucens pv.unduolsa bacteria reproduce at the interfaces of leaf tissues and produce enzymes to break down cell walls 96 hours after inoculation, and the bacteria are often active on leaves and cause tissue death which weakening their role in food production by inhibiting photosynthesis that depends on chloroplasts. The bacteria cause elongated, narrow yellow sores or watery-looking streaks form and yellow secretions emerge from exposure to periods of wetness and high humidity. The secretions may collect in the form of drops or form a membrane that dries out later and turns into crusts (Schaad 1980; Surai et al 2019). Results of chemo-biological tests on the 19 isolates of X. translucens showed that the bacteria have certain characteristics that are consistent with previous studies (Schaad 1985; Azhar 2013). The results of the molecular diagnosis were confirmed using PCR and S16 (rRNA) (Loy et al 2002; Harris et al 2004).

**CONCLUSIO**

Bacterial leaf streak disease on wheat is considered an important pathogen in field and cause economic losses. This pathogen is transferred by seeds and develops quickly at wet conditions. Current study highlighted the infection of wheat by this disease in Iraq when a series of chemo-biological tests followed by molecular tests to confirm the results which are beneficial to solve challenges in differentiation between species.

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