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Research Article

Presence of Flagellation in Plant Pathogenic Strain of *Klebsiella pneumoniae* - A New Report

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Abstract

Klebsiella pneumoniae strain Borkar (NCBI accession number: KY941097.1), a new bacterial plant pathogen responsible for root bark necrosis and wilting in pomegranate plants was studied for its colony morphology, cell morphology and flagellation under transmission electron microscopy.

The bacterial colonies of *Klebsiella pneumoniae* strain Borkar were creamy white, circular, raised, fluidal with 2 to 3 mm diameter on nutrient agar media at 72 h of growth. In nutrient broth media the bacterial culture turned into a frothing culture after three days of growth. The bacterial cell size of the pathogen varied from 1.76-2.50 ×0.94-1.08 µm and has minute cilli all over the cell surface. Transmission electron microscopy of the bacterial cells indicated that the bacteria possess sub-polar flagellum measuring 3.44 to 4.33 µm in length. This is the first report of flagellated plant pathogenic Klebsiella pneumoniae.

Generally, *Klebsiella pneumoniae* is a non-motile, non-flagellated human pathogen. However, Our results along with the results of Liu., et al. indicated that *Klebsiella pneumoniae* has now evolved itself as soil-borne plant pathogen. But the genus *Klebsiella* is not yet included as a genus of plant pathogenic bacteria. Therefore, we propose the inclusion of the genus *Klebsiella* as a plant pathogenic bacterial genus. Further, our results confirm that plant pathogenic *Klebsiella pneumoniae* pathogenic on pomegranate has a sub-polar flagellation. Our results on flagellation in plant pathogenic *Klebsiella pneumonia* are supportive to the results of flagellation in human pathogenic klebsiella pneumonia as reported by A.C.Lima. Based on these results, it is proposed to redefine the genus *Klebsiella* as the bacterial genus of motile and Flagellated bacteria also.

Keywords: *Klebsiella pneumoniae*; Pomegranate; Root Bark Necrosis Disease; Flagellation

Introduction

Klebsiella pneumoniae is non-flagellated, non-motile, gram-negative, encapsulated, lactose fermenting rod shaped bacterium of the family Enterobacteriaceae. It is an important opportunistic human pathogen causing pneumonia, nosocomial infection, urinary tract infection, and infection of the respiratory tract [1]. *Klebsiella pneumoniae* is also reported to be soil inhabiting bacteria [2], associated with the plant root system, probably as a nitrogen fixer in maize, poa and wheat [3,4]. As a soil dwelling bacterium, it was reported to be a plant pathogen on onion bulbs causing internal tissues decay in Guangdong province, China [5]. Subsequently, it was reported on maize plant causing top rot disease of maize [6] from Yunnan province, China. Ajayasree and Borkar [7] reported the infection of *Klebsiella pneumoniae* strain borkar on pomegranate plants causing root bark necrosis and wilt disease from pomegranate orchards of Mahatma Phule Agriculture University, Rahuri in Maharashtra state, India.

The bacterium of *Klebsiella pneumoniae* has been considered to be a non-flagellated, and this phenotypical character is considered as important criteria to differentiate this genus from others within Enterobacteriaceae family [8]. However, the presence of polar flagella in *Klebsiella pneumoniae*, isolated from a patient with neonatal sepsis was reported by Alejandro carabarin-Lima., *et al.* [9]. In many bacterial species the presence of flagellum is reported to be involved in bacterial pathogenicity [10-12]. To date, plant pathogenic *Klebsiella pneumoniae* is not reported to have flagellation. Therefore, the bacterial cells of plant pathogenic *Klebsiella pneumoniae* strain Borkar was studied under transmission electron microscopy for its cellular morphology including flagellation.

Materials and methods

Colony morphology of *Klebsiella pneumoniae* strain Borkar

The bacterial culture of *Klebsiella pneumoniae* strain borkar (NCBI Accession number: KY 941097.1) causing root bark necrosis and wilt in pomegranate plants [7] available in our laboratory was used to study the colony morphology of the bacterium. To study the bacterial colony morphology, a 24 h young loop-full bacterial growth was suspended in 5 ml distilled sterile water and subsequent dilutions were made up to 10^{-6}. An aliquot of 0.5 ml of each dilution was plated on sterile nutrient agar medium. The individual colonies appeared on the nutrient agar plates were studied for its colony morphology at 72 h of growth. The colony morphology characters were recorded by following routine descriptive procedures of the bacteria [13].

Similarly, the *Klebsiella pneumoniae* culture grown in nutrient broth was observed for its growth pattern in broth culture. The experimentation was repeated five times for the consistency of the results.

Bacterial cell morphology of *Klebsiella pneumoniae* strain Borkar by transmission electron microscopy

A standard protocol of grid preparation was used for transmission electron microscopy. For this, the bacterial suspension was prepared by adding 10 ml phosphate buffer [(A) 0.2 M dibasic sodium phosphate Na_2HPO_4; 28.39 g in 1L distilled water; (B) 0.2 M monobasic sodium phosphate $NaH_2PO_4.2H_2O$; 31.21g in 1L distilled water. Mix buffer (A) 36 ml and (B) 14 ml] to a nutrient agar slant containing 18 h young bacterial culture and the bacterial cell suspension was obtained. A drop of this bacterial suspension was placed on a formvar grid and allowed to dry. When the suspension has partly dried the grid was washed three times by touching the surface with a drop of distilled water. Excess water was removed by touching the grid with filter paper. A small drop of 2 per cent uranyl acetate was then applied to the grid to stain it. After 10 seconds the excess stain was removed by touching the edge with filter paper. The grid was allowed to dry at room temperature and viewed under TEM (JEOL model) at 15000 to 20000X for bacterial cell and at 40000X for flagellation.

The reading for the bacterial cell size and flagellation was repeated at least ten times by fresh grid preparations and observation under TEM for the confirmation of flagellation.

Results

Colony morphology of *Klebsiella pneumoniae* strain Borkar

The bacterial colonies of *Klebsiella pneumoniae* strain borkar were creamy white, circular, raised, fluidal with 2 to 3 mm diameter (Figure 1) on nutrient agar media at 72 h of growth. In nutrient agar media the bacterial culture turned into a frothing culture (Figure 2) after 3 days of growth. Similarly, in nutrient broth the bacterium formed a frothing culture after 3 days of growth.

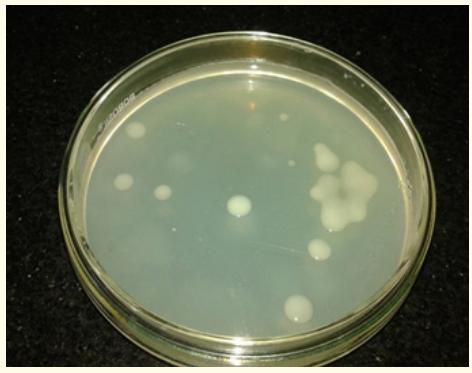

Figure 1: Colonies of *Klebsiella pneumonie* strain Borkar on nutrient agar medium.

Figure 2: Frothing of *Klebsiella pneumoniae* strain Borkar culture on nutrient agar slant.

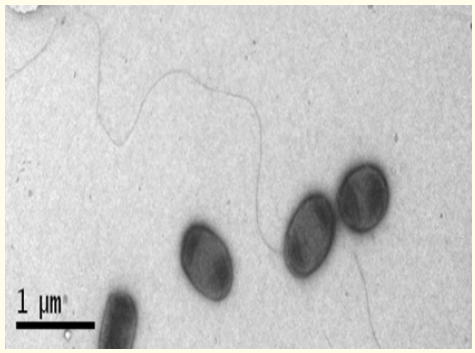

Figure 3: Transmission Electron microscopic image of Klebsiella pneumoniae strain Borkar showing sub-polar flagellum.

Bacterial cell morphology of *Klebsiella pneumoniae* strain Borkar under transmission electron microscope

The bacterial cells of *Klebsiella pneumoniae* strain Borkar under transmission electron microscope appeared rod-shaped measuring 1.76-2.50 ×0.94-1.08 µm. The bacterial cell has minute pili all over the cell surface. The bacterial cell has sub-polar flagella measuring 3.4-4.33 µm in length (Figure 3).

Discussion

Bacterial flagella, is an important cell organelle used in bacterial movement, and is used in the identification of bacterial genus [14]. Until recently, *Klebsiella pneumoniae*, which is an important human pathogen, was known to be a non-flagellated, non-motile bacterium of the family *Enterobacteriaceae* [15]. This phenotypical character is considered to be an important criterion to differentiate this genus from others within the Enterobacteriaceae family [8]. However, recently evidence of polar flagella in *Klebsiella pneumoniae*, isolated from a patient with neonatal sepsis, was reported by Alejandro carabarin-Lima., *et al.* [9] indicating that the *Klebsiella pneumoniae* pathogenic to human possess the flagellation. This has increased the curiosity of flagellation in plant pathogenic *Klebsiella pneumoniae*. To date, the known plant pathogenic *Klebsiella pneumoniae* infecting onion [5] and maize plant [6] is not studied for its flagellation and therefore it is not known if plant pathogenic *Klebsiella pneumoniae* possess the flagellation or not. Our transmission electron microscopy results indicated that the bacterial cells of plant pathogenic *Klebsiella pneumoniae* strain Borkar was flagellated and motile rods. This is the first report of flagellation in plant pathogenic *Klebsiella pneumoniae*.

The *Klebsiella pneumonia* causing top rot of maize in China was found pathogenic in mice [6]; however, the Indian strain of *Klebsiella pneumoniae* causing root bark necrosis and wilt in pomegranate is found non-pathogenic on Indian bird chicken, thereby indicating it to be plant host specific [16].

The bacterial cell rods of plant pathogenic *Klebsiella pneumoniae* strain Borkar measured 1.76-2.50×0.94-1.08 µm whereas the bacterial cell rods of human pathogenic *Klebsiella pneumoniae* measures 2×5 µm [17] indicating the variation in the cell size of plant pathogenic and human pathogenic *Klebsiella pneumoniae*. The flagella in plant pathogenic *Klebsiella pneumoniae* strain Borkar measures 3.44 to 4.33 µm in length, a characteristic not reported yet in any plant pathogenic *Klebsiella* species. Haiko and Wikstrom [18] reported bacterial flagellum as potential virulence factor besides its function as adhesion to host cell. Presence of flagellation in soil-borne *Klebsiella pneumoniae* might have been contributed to make them a plant pathogenic strain.

Conclusion

Our results along with results of Liu., *et al.* [5] indicated that *Klebsiella pneumoniae* has now evolved itself as soil-borne plant pathogen. However, the genus *Klebsiella* is not yet included as a genus of plant pathogenic bacteria. Therefore, we propose the inclu-

sion of the genus *Klebsiella* as a plant pathogenic bacterial genus. Further, our results confirm that plant pathogenic *Klebsiella pneumoniae* pathogenic on pomegranate has a sub-polar flagellation. Our results on flagellation in plant pathogenic *Klebsiella pneumonia* are supportive to the results of flagellation in human pathogenic *klebsiella pneumonia* [9]. Based on these results, it is proposed to redefine the genus *Klebsiella* as the bacterial genus of motile and Flagellated bacteria also.

Conflict of Interests

There is no conflict of interest by the authors.

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