Biochemical characteristics of plant pathogenic Klebsiella pneumoniae causing root bark necrosis and wilt in pomegranate

Abstract

A plant pathogenic Klebsiella pneumoniae causing root bark necrosis and wilt in pomegranate was designated as Klebsiella pneumoniae strain Borkar by NCBI (accession number: KY984973.1) on the basis of 16s rRNA partial gene sequence submitted. This strain was studied for its biochemical characteristics as it was a new bacterial pathogen causing plant disease and its biochemical characteristics was compared with the known biochemical characteristics of other Klebsiella pneumoniae sub species and Klebsiella species.

The bacterium was positive for ONPG, lysine utilization, urease, vogus paskeur reaction, methyl red test, PYR, esculin hydrolysis, sorbitol, trehalose, malabiose, salcin, mannose, sucrose, glucose utilization, malonate, arabinose, rhamnose and mannitol utilization tests; whereas it was negative for ornithine utilization, Indole, β-glucoronidase, α- galactosidase, β- xylidosiade, cellubiose, maltose, Raffinose, Lactose, H₂S and Adonitol.

The plant pathogenic Klebsiella pneumoniae strain Borkar differed from Klebsiella pneumoniae in respect of sugar utilization. The plant pathogenic Klebsiella pneumoniae does not utilize α-galactosidase, β-xylidosiade, cellubiose, maltose, Raffinose, Lactose whereas Klebsiella pneumoniae utilizes these sugars.

Similarly Plant pathogenic Klebsiella pneumoniae strain Borkar differed from Klebsiella oxycocta, Klebsiella pneumoniae subsp. pneumoniae, Klebsiella rhizoscleronatis, Klebsiella ozaenae and Klebsiella terigena in one or the other known biochemical characteristics.

This opportunistic soil borne bacteria has emerged as a new soil borne plant pathogen particularly on pomegranate crop. Barring one example of its infection on maize this bacterial species is new one to cause infection in the plant. The genus Klebsiella is not yet included as a genus of plant pathogenic bacteria. Therefore we propose to include the genus Klebsiella as a plant pathogen and its biochemical characteristics are described in this paper.

Keywords: biochemical tests, klebsiella sp, plant pathogenic klebsiella pneumoniae, pomegranate pathogen

Introduction

Biochemical characterization is an important tool to differentiate the bacterial genus into species and subspecies. The individual bacterial species and subspecies are specific in the utilization or non-utilization of particular chemical molecules. Klebsiella pneumoniae is generally a serious human pathogen causing respiratory tract infection, loss of breathing leading to pneumonia and death of patient. This bacterial pathogen is also known to cause infection in animals particularly in dogs and pre-weaned pigs and in birds also. Immuno deficient animals, especially those with severe immune-deficiencies or defect in immune system, develop clinical disease symptoms associated with Klebsiella infection.

In 2016 the bacterium Klebsiella pneumoniae was reported to infect maize plant in China causing bacterial top rot disease of maize in Yunnan province. In subsequent year, its involvement as plant pathogen was reported. In 2017, it was detected in pomegranate orchard of Mahatma Phule Agricultural University, Rahuri, India. The differentiation system of plant pathogenic Klebsiella pneumoniae species from other Klebsiella pneumoniae subsp is not available in the literature, since Klebsiella pneumoniae is recently reported as a plant pathogen. It is very necessary to characterize this plant pathogenic Klebsiella pneumoniae from other Klebsiella pneumoniae subsp and species. As the differentiation of species and subsp is not available in the literature, since Klebsiella pneumoniae is recently reported as a plant pathogen. It is very necessary to characterize this plant pathogenic Klebsiella pneumoniae strain Borkar, so as to differentiate it from the other known Klebsiella pneumoniae and Klebsiella species.

Materials and methods

Biochemical characterization of plant pathogenic Klebsiella pneumoniae strain borkar

Rapid biochemical identification test kits of Himedia viz. KB002 (HiAssorted™), KB001 (Hi/MVic™), KB003 (Hi25™) and KB016 (Hi24™) were used for biochemical characterization of Klebsiella pneumoniae strain Borkar.

A total 35 biochemical tests included in these identification test kit were used. These tests were ONPG, lysine utilization, ornithine utilization, urease, phenyl alanine deamination, Voges proskauer,
Biochemical characteristics of plant pathogenic Klebsiella pneumoniae causing root bark necrosis and wilt in pomegranate

Methyl red, indole, PVR, β-glucoronidase, α-galactosidase, β-xyllosidase, esculin hydrolysis, sucrose, sorbitol, trehalose, glucose, cellobiose, melibiose, salicin, mannose, raffinose, lactose, TDA, nitrate reduction, H₂S, citrate utilization, malonate, arabinose, xylose, adonitol, rhamnose, saccharose and mannitol. Each biochemical test is a standardized colorimetric identification system utilizing conventional biochemical test and carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On inoculation with the bacterial culture in test kit media and incubation, the test media undergoes changes, due to metabolic activity of bacteria, which are indicated by a colour change in the media that are interpreted visually or after addition of the respective test reagent after incubation period.

The test kit was opened aseptically by peeling off the sealing tape. Each well of the test kit was inoculated with a loop of 24hour old bacterial culture by stab inoculation method and covered with peel off sealing tape. The kits were incubated at 35-37°C temperature for 24hours. The results were interpreted as per the standards given in the result interpretation chart. Addition of reagents wherever necessary was done to obtain the test results after the incubation period. The results are presented in the tabular form.

**Results**

**Metabolic activity and utilization of different substrates by plant pathogenic Klebsiella pneumoniae strain Borkar**

The bacterium was positive for ONPG, lysine utilization, urease, vogus paskeur reaction, methyl red test, PVR, esculin hydrolysis, nitrate reduction, citrate utilization and utilization of sucrose, sorbitol, trehalose, malatoise, salicin, mannose, glucose, malonate, arabinose, rhamnose and manitol as carbohydrate sugar, while it was negative for ornithine utilization, indole, H₂S, β-glucoronidase, α-galactosidase, and β-xyllosidase and did not utilize cellobiose, maltose, raffinose, lactose and adonitol as carbohydrate sugar (Table 1). This plant pathogenic Klebsiella pneumoniae strain Borkar was different in some of the biochemical characters to other Klebsiella pneumoniae and its subspecies (Table 2A‒2f).

| No. | Metabolic activity/ utilization of substrate | Reaction of Klebsiella pneumoniae strain Borkar |
|-----|------------------------------------------|-----------------------------------------------|
| 1   | ONPG                                     | +                                             |
| 2   | Lysine utilization                        | +                                             |
| 3   | Ornithine utilization                     | −                                             |
| 4   | Urease                                   | +                                             |
| 5   | Phenyl alanine deamination                | V/−                                           |
| 6   | VP                                       | +                                             |
| 7   | MR                                       | +                                             |
| 8   | Indole                                   | −                                             |
| 9   | PYR                                      | +                                             |
| 10  | β-glucoronidase                          | −                                             |
| 11  | α-galactosidase                          | −                                             |
| 12  | β-xyllosidase                            | −                                             |

+ = Positive, V = 11-89% positive, − = negative

**Table 2 Variable characteristics of Klebsiella pneumoniae strain Borkar with comparison to other Klebsiella species**

| Sr. No. | Metabolic activity/ utilization of substrate | Klebsiella pneumoniae strain Borkar | Klebsiella pneumoniae strain Borkar |
|---------|---------------------------------------------|-----------------------------------|-----------------------------------|
| 1       | MR                                          | V                                 | +                                 |
| 2       | Maltose                                     | +                                 | −                                 |
| 3       | Raffinose                                   | +                                 | −                                 |
| 4       | Lactose                                     | +                                 | −                                 |

b. Variation in biochemical characteristics of Klebsiella pneumoniae strain Borkar with Klebsiella oxycota

| Sr. No. | Metabolic activity/ utilization of substrate | Klebsiella oxycota |
|---------|---------------------------------------------|---------------------|
| 1       | Indole                                     | +                   |
| 2       | α-galactosidase                            | +                   |
| 3       | β- Xyllosidase                             | +                   |
| 4       | Cellobiose                                 | +                   |
| 5       | Maltose                                    | +                   |
| 6       | Raffinose                                  | +                   |
| 7       | Lactose                                    | +                   |
| 8       | Adonitol                                   | +                   |

**Table 1 Metabolic activity and utilization of different substrates by plant pathogenic Klebsiella pneumoniae strain Borkar**

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c. Variation in biochemical characteristics of *Klebsiella pneumoniae* strain Borkar with *Klebsiella ozaenae*

| Sr. No. | Metabolic activity/ utilization of substrate | Klebsiella ozaenae | Klebsiella pneumoniae strain Borkar |
|---------|--------------------------------------------|-------------------|-----------------------------------|
| 1       | Lysine utilization V/–                        | +                 |                                   |
| 2       | Urease –                                    | +                 |                                   |
| 3       | VP –                                        | +                 |                                   |
| 4       | α-galactosidase –                            | +                 | –                                 |
| 5       | β-Xylosidase V                               | –                 |                                   |
| 6       | Sucrose –                                   | –                 | +                                 |
| 7       | Sorbitol V                                  | +                 |                                   |
| 8       | Cellobiose +                                | –                 |                                   |
| 9       | Maltose +                                   | –                 |                                   |
| 10      | Lactose V                                   | –                 | +                                 |
| 11      | Nitrate reduction V                          | V                 | +                                 |
| 12      | Citrate utilization V                        | V                 | +                                 |
| 13      | Adonitol +                                  | –                 | –                                 |

d. Variation in biochemical characteristics of *Klebsiella pneumoniae* strain Borkar with *Klebsiella pneumoniae* subsp. *pneumoniae*

| Sr. No. | Metabolic activity/ utilization of substrate | Klebsiella pneumoniae subsp. pneumoniae | Klebsiella pneumoniae strain Borkar |
|---------|--------------------------------------------|---------------------------------------------|-----------------------------------|
| 1       | MR V                                       | +                                           |                                   |
| 2       | α-galactosidase +                          | –                                           |                                   |
| 3       | β-Xylosidase +                             | –                                           |                                   |
| 4       | Cellobiose +                               | –                                           |                                   |
| 5       | Maltose +                                  | –                                           |                                   |
| 6       | Raffinose +                                | –                                           |                                   |
| 7       | Lactose +                                  | –                                           |                                   |
| 8       | Adonitol +                                 | –                                           | –                                 |

e. Variation in biochemical characteristics of *Klebsiella pneumoniae* strain Borkar with *Klebsiella rhizoscleronatis*

| Sr. No. | Metabolic activity/ utilization of substrate | Klebsiella rhizoscleronatis | Klebsiella pneumoniae strain Borkar |
|---------|--------------------------------------------|-----------------------------|-----------------------------------|
| 1       | ONPG –                                     | +                           |                                   |
| 2       | Lysine utilization –                        | –                           | +                                 |
| 3       | Urease –                                   | –                           | +                                 |
| 4       | VP –                                       | –                           | +                                 |
| 5       | β-Xylosidase V                             | V                           | –                                 |
| 6       | Esculin hydrolysis V                       | V                           | +                                 |
| 7       | Sucrose V                                  | V                           | +                                 |
| 8       | Cellobiose +                               | +/V                         | –                                 |
| 9       | Maltose +                                  | –                           | –                                 |
| 10      | Raffinose +                                | +                           | –                                 |
| 11      | Citrate utilization –                      | –                           | +                                 |
| 12      | Adonitol +                                 | +                           | –                                 |

f. Variation in biochemical characteristics of *Klebsiella pneumoniae* strain Borkar with *Klebsiella terigena*

| Sr. No. | Metabolic activity/ utilization of substrate | Klebsiella terigena | Klebsiella pneumoniae strain Borkar |
|---------|--------------------------------------------|---------------------|-----------------------------------|
| 1       | Ornithine utilization V                     | –                   | +                                 |
| 2       | Urease –                                   | –                   | +                                 |
| 3       | MR V                                       | V                   | +                                 |
| 4       | Raffinose +                                | +                   | –                                 |
| 5       | Lactose +                                  | –                   | +                                 |

Discussion

The biochemical characteristics in bacteria are an important tool in their identification. *Klebsiella pneumoniae* is a known important human pathogen causing pneumonia, nosocomical infection, urinary tract infection and infection of respiratory tract and fatal to human being. The strain of *Klebsiella pneumoniae* is also reported to infect maize plant in Yunnan province, China and recently in India on pomegranate crop causing wilt. The known biochemical characteristics of *Klebsiella pneumoniae* subsp and *Klebsiella* species was compared with plant pathogenic *Klebsiella pneumoniae* strain Borkar for its biochemical characteristics.

The plant pathogenic *Klebsiella pneumoniae* strain Borkar was positive for ONPG, lysine utilization, urease, VP, MR, PYR, esculin hydrolysis, nitrate reduction, citrate utilization and utilization of sucrose, sorbitol, trehalose, malatose, salicin, mannose, glucose, malonate, arabinose, rhamnose and manitol as carbohydrate sources, while it was negative for ornithine utilization, Indole, H₂S, β-glucuronidase, α-galactocidase, β-xylosidase and did not utilize cellobiose, maltose, raffinose, lactose and adonitol.

Plant pathogenic *Klebsiella pneumoniae* strain Borkar was different from previously reported *Klebsiella pneumoniae* in utilization of at least three sugars viz, maltose, raffinose and lactose. Plant pathogenic *Klebsiella pneumoniae* strain Borkar did not utilize these sugars whereas *Klebsiella pneumoniae* utilizes these.

*Klebsiella pneumoniae* is also reported to be associated with plant root system, probably as nitrogen fixer in maize, poa and wheat, but not yet reported as soil borne plant pathogen. Though Huang et al. reported *Klebsiella pneumoniae* causing bacterial top rot disease of maize; it was not described as soil borne plant pathogen. *Klebsiella pneumoniae* strain Borkar, is a soil borne plant pathogen infecting pomegranate plant by causing root bark necrosis and wilt in the plant. This pathogen under our experimentation did not infect the maize plant causing top rot and therefore, seems to be different strain of *Klebsiella pneumoniae* reported from China. Thus plant pathogenic *Klebsiella pneumoniae* strain Borkar is different from *Klebsiella* species and not reported earlier. The biochemical characteristics of this strain certainly differentiate it from other *Klebsiella pneumoniae* sub spp.

Conclusion

Plant pathogenic *Klebsiella pneumoniae* strain Borkar can be differentiated from other known *Klebsiella pneumoniae* species on the basis of various biochemical tests. This opportunistic soil borne bacteria has emerged as a new soil borne plant pathogen particularly...
on pomegranate crop. Barring one example of its infection on maize this bacterial species is new one to cause infection in the plant. The genus Klebsiella is not yet included as a genus of plant pathogenic bacteria. Therefore we propose to include the genus Klebsiella as a plant pathogen and its biochemical characteristics are described in this paper.

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None.

Conflict of interest

The author declares that there is no conflict of interest

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