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Differentiation tests between the plant pathogenic bacteria *Klebsiella pneumoniae* and *K. variicola*

Abstract

The bacterium *Klebsiella pneumoniae* strain Borkar causing root bark necrosis on pomegranate was isolated and purified on nutrient agar sucrose medium. The plant pathogenic *K. variicola* bacterial culture was obtained from microbial culture collection centre, Pune, India (accession number MCC 2623). Both cultures were subjected for biochemical characterizations by using rapid biochemical identification test kits of Hi-media.

K. pneumoniae strain Borkar was positive for ONPG, lysine utilization, urease, Vogus paskeur reaction, methyl red test, PVB, Esculin hydrolysis, sucrose, sorbitol, trehalose, malatiose, salicin, mannose, glucose utilization, nitrate reduction, citrate utilization, malonate, arabinose, rhamnose and mannitol utilization tests. Whereas, it was negative for ornithine utilization, indole, β-glucoronidase, α-galactosidase, β-xylosidase, cellobiose, lactose, maltose, raffinose, H_2S and adonitol.

K. variicola was positive for ONPG, Urease, Vogus Paskeur reaction, methyl red, Esculin hydrolysis, utilization of sucrose, sorbitol, trehalose, glucose, cellobiose, melibiose, salicin, mannose, maltose, mannitol, rhamnose, arabinose, and nitrate reduction. It was negative for indole, β-gluonidase, α-galactosidase, β-xylosidase and does not utilize raffinose, lactose, adonitol and citrate utilization.

The main difference between plant pathogenic *K. pneumoniae* and *K.variicola,* in respect of metabolic activity/utilization of substrate was for citrate and maltose utilization. *K. pneumoniae* did not utilize both citrate and maltose, while *K.variicola* utilized them. This happens to be main differentiation test for the plant pathogenic *K. pneumoniae* and *K. variicola*.

Keywords: biochemical tests, *klebsiella pneumoniae*, strain Borkar, *klebsiella variicola*

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Introduction

Microbes differ in their metabolic activities and reactions. *Klebsiella pneumoniae* and *K. variicola* are plant pathogenic bacteria among *Klebsiella* genus.[1,2] But, both bacteria were differed slightly in their gene sequence.[3] So identification of both species is difficult at genomic level. To identify bacteria, we must rely heavily on biochemical testing as the types of biochemical reactions of each organism act as a thumbprint for its identification.[4]

Biochemical test reactions of both plant pathogenic bacteria, *i.e. K. pneumoniae* strain Borkar and *K. variicola* is not available in Bergey's manual. Therefore, we felt it necessary to study biochemical reactions of these two genomically closely related bacteria for their comparative studies and differentiation.

Material and methods

Isolation of the plant pathogenic *Klebsiella pneumoniae*

The bacterial wilt and root bark necrosis in pomegranate is caused by *Klebsiella pneumoniae* strain Borkar.[5] The roots of wilted pomegranate plants showing root bark necrosis symptoms were collected from the infected orchard of MPKV Rahuri, India for the isolation of causal agent. The infected samples were washed in tap water to remove the soil particles and left to air dry. The root bark necrosis portion was cut into small pieces with sterile razor blade and disinfested with 0.1% $HgCl_2$ solution for 2-3minutes followed by three washings in sterile water to remove traces of $HgCl_2$ solution for 2-3minutes. These sterile root bark pieces were macerated in sterile pestle mortar containing 5ml sterile water and left for 5minutes for dispersal of bacteria and settlement of sediments. 0.1ml suspension from this macerate was pipetted on sterile nutrient agar medium in Petri-plates and spread over the agar medium. These plates were incubated at 28±2°C for 72hrs in BOD incubator. The bacterial colonies appeared in the plates were selected and purified by single colony isolation method and pure cultures were obtained. The bacterial culture was identified as *K. pneumoniae* by Gene Ombio Technology, Pune, India by using 16s rRNA partial gene sequence. This partial gene sequence was further confirmed by NCBI, USA as a gene sequence of Klebsiella pneumoniae and named the strain as *K. pneumoniae* strain Borkar. These cultures were used for pathogenicity test or to prove Koch's postulates on pomegranate plant. The bacterial cultures were pathogenic and induced wilting symptoms on pomegranate plant in one month in pot culture experiments. This bacterial culture was used for biochemical test reactions.

Collection of *K. variicola*

The plant pathogenic *K. variicola* bacterial culture was obtained from Microbial Culture Collection Centre, Pune, India (accession number MCC 2623). This bacterial culture was used for biochemical test reactions.

Biochemical characterization of genomically closely related *Klebsiella pneumoniae* and *K. variicola*

The pure cultures of both *K. pneumoniae* (causes root bark necrosis and wilt in pomegranate plant) and *K. variicola* (collected from Microbial Culture Collection Centre, Pune, India) were used for the

biochemical characterization test. Rapid biochemical identification test kits of Hi-medium viz. KB001 (HilMVICTM), KB 002 (Hi Assorted TM) and KB016 (Hi24TM), which comprises of various biochemical test were used.

A total of 31 biochemical tests included in these identification kits were used. These tests were ONPG, lysine utilization, ornithine utilization, urease, phenylalanine deamination, Vogus paskeur reaction, methyl red, indole, PYR, β-gluconidase, α-galactosidase, β-Xylosidase, esculin hydrolysis, sucrose, sorbitol, trehalose, glucose, cellobiose, melibiose, salicin, mannose, maltose, raffinose, lactose, citrate utilization, nitrate reduction, H_2S production, adonitol, arabinose, mannitol and rhamnose.

The test kits were open aseptically by peeling off the sealing tape. Each well of the test kit was inoculated with a loop of 24hrs old bacterial cultures by stab inoculation method[6] and covered with peeled off sealing tape. The kits were incubated at 35-37°C temperature for 24hrs.

The tests were based on the principle of pH change and substrate utilization. On inoculation with the bacterial culture in test kit media, the substrate undergo changes, due to metabolic activity of bacteria (Figure 1), which was indicated by colour change in medium that was interpreted visually or after addition of the respective test reagent aseptically after incubation period.[7]

Figure 1 Biochemical identification test kits of Himedia for both *K. variicola* (above) in comparison with *K. pneumoniae* strain Borkar (down).

The results were analysed as per the standards given in the result interpretation chart. Addition of reagents, wherever necessary was done aseptically to obtain the results of tests after incubation period.[8]

Results

Colony characteristics of genomically closely related *K. pneumoniae* and *K. variicola*

The bacterial colonies of *K. pneumoniae* (isolated from root bark necrosis symptoms on nutrient medium), were creamy white, circular, raised, fluidal with 2-3mm growth diameter within 72hrs (Figure 2). The colonies of *K. variicola* were also creamy white, circular, raised, fluidal with 2-3mm growth diameter within 72hrs. There was no difference in the colony characteristics of both bacteriua.

Metabolic activity and utilization of different substrates by *K. pneumoniae* and *K. variicola*

The bacterium *K. variicola* was positive for ONPG, urease, Vogus Paskeur reaction, methyl red, Esculin hydrolysis, nitrate reduction, utilization of sucrose, sorbitol, trehalose, glucose, cellobiose, melibiose, salicin, mannose, maltose, mannitol, rhamnose and arabinose. In addition, *K. variicola* was negative for indole, β-gluconidase, α-galactosidase, β-Xylosidase, citrate utilization and does not utilize raffinose, lactose and adonitol (Table 1).

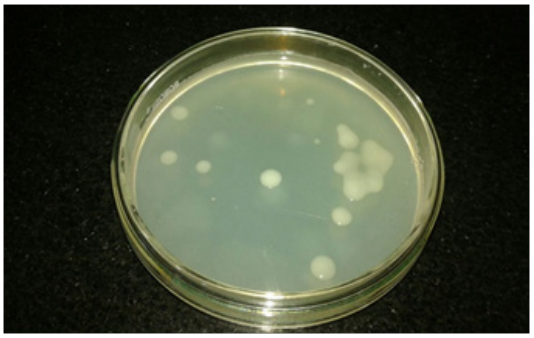

Figure 2 Colony characteristics of genus *Klebsiella (*plant pathogenic bacterium).

Table 1 Metabolic activity and utilization of different substrates by the plant pathogenic bacterium *K. variicola*.

Sr. no	Metabolic activity/ Utilization of substrate	Reaction of *K. variicola*
1	ONPG	+
2	Urease	+
3	Voges paskeur reaction	+
4	Methyl red	+
5	Indole	–
6	β-gluconidase	–
7	α-galctosidase	–
8	β-Xylosidase	–
9	Esculin hydrolysis	+
10	Sucrose	+
11	Sorbitol	+
12	Trehalose	+
13	Glucose	+
14	Cellobiose	+
15	Melibiose	+
16	Salicin	+
17	Mannose	+
18	Maltose	+
19	Raffinose	–
20	Lactose	–
21	Citrate utilization	–
22	Nitrate reduction	+
23	Adonitol	–
24	Arabinose	+
25	Mannitol	+
26	Rhamnose	+

+ = positive and – = Negative

Citation: Ajayasree TS, Borkar SG. Differentiation tests between the plant pathogenic bacteria *Klebsiella pneumoniae* and *K. variicola*. *J Appl Biotechnol Bioeng.* 2018;5(4):237–240. DOI: 10.15406/jabb.2018.05.00144

The bacterium *K. pneumoniae* strain Borkar was positive for ONPG, Lysine utilization, urease, Vogus paskeur reaction, methyl red test, PYR, Esculin hydrolysis, nitrate reduction, citrate utilization and utilization of sucrose, sorbitol, trehalose, maltiose, salicin, mannose, glucose, malonate, arabinose, rhamnose and mannitol as carbohydrate sugar.

K. pneumoniae strain Borkar was negative for ornithine utilization, indole, H_2S production, β-glucoronidase, α-galactosidase, β-xylosidase and does not utilize cellobiose, maltose, raffinose, lactose and adonitol as carbohydrate sugar (Table 2).

The main difference between both bacteria (Table 3) was citrate and maltose utilization. In this respect, *K. pneumoniae* strain Borkar did not utilize both substrates, meanwhile, *K.variicola* utilized them.

Table 2 Metabolic activity and utilization of different substrates by plant pathogenic bacterium *K. pneumoniae* strain Borkar.

Sr. No	Metabolic activity/Utilization of substrate	Reaction of *K. 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-xylosidase	—
13	Esculin hydrolysis	+
14	Sucrose	+
15	Sorbitol	+
16	Trehalose	+
17	Glucose	+
18	Cellobiose	—
19	Melibiose	+
20	Salicin	+
21	Mannose	+
22	Maltose	—
23	Raffinose	—
24	Lactose	—
25	Nitrate reduction	+
26	H_2S	—
27	Citrate utilization	+
28	Malonate	+
29	Arabinose	+
30	Adanitol	—
31	Rhamnose	+
32	Mannitol	+

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

Citation: Ajayasree TS, Borkar SG. Differentiation tests between the plant pathogenic bacteria *Klebsiella pneumoniae* and *K. variicola*. *J Appl Biotechnol Bioeng.* 2018;5(4):237–240. DOI: 10.15406/jabb.2018.05.00144

Table 3 Variable characteristics of *K. pneumoniae* strain Borkar in comparison with *K. variicola.*

Sr. no	Metabolic activity/Utilization of substrate	*K. variicola*	*K. pneumoniae* strain Borkar
1	Citrate utilization	+	–
2	Maltose	+	–

Discussion

The differentiation among species of the same genus is generally, based on the ability of the bacterium to utilize or not a particular substrate. This is mainly due to the metabolic activities of the bacterium. Several biochemical tests and utilization of sugars are used for differentiation among bacterial species of the same genus.[9] Recently genomic variation among the species are also studied and reported, but sometimes there is very negligible genomic variation in gene sequence[3] and therefore biochemical utilization tests are still useful to differentiate among species of the same genus.

The main difference between *K. pneumoniae* and *K. variicola,* in respect of metabolic activity/utilization of substrate was for citrate and maltose utilization. *K. pneumoniae* did not utilize both substrates; meanwhile *K. variicola* utilized both substrate. The difference in utilization of citrate and maltose is the main test for the differentiation of closely related plant pathogenic *K. pneumoniae* strain Borkar and *K. variicola*.

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None.

Conflict of interest

The author declares that there is no conflict of interest.

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